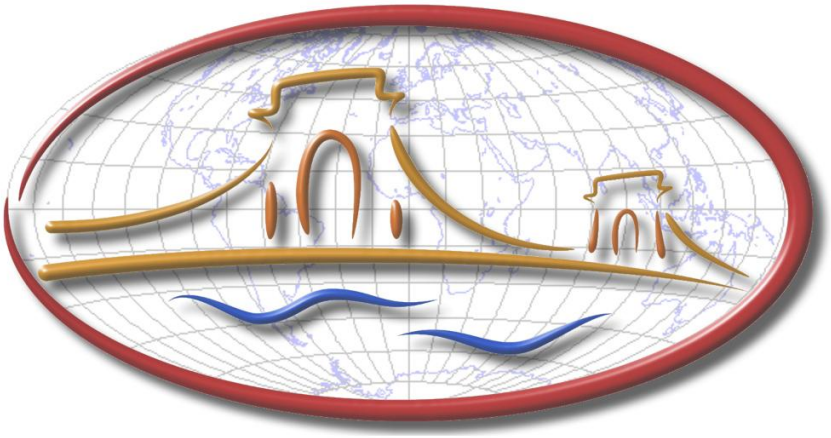
Bridges in Life Sciences 10th Annual Scientific Conference



RECOOP HST ASSOCIATION

April 16-19, 2015

Wroclaw, Poland

Wrocław University of Technology

Centre for Advanced Materials and Nanotechnology

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Editor: Sandor G. Vari, M.D.

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Agenda of the Bridges in Life Sciences 10th Annual Conference, Wroclaw, Poland

Venue: HOTEL VEGA***, ul. Grabiszyńska 251, 53-234 Wrocław, Poland, Tel: +48 71 339 03 67, Fax: +48 71 339 03 68, www.hotelvega.pl

April 16, 2015 (Thursday)

15:00 – 19:00 **Arrival and Registration at the Front Desk**

20:00 – 20:30 **Opening Ceremony**

Welcome
Cezary Madryas, Vice-Rector for Development, Wroclaw University of Technology, Wrocław, Poland

Edward Prunchunas, Senior Vice President for Finance and Chief Financial Officer, Cedars-Sinai Medical Center, Los Angeles, CA, USA
Chairman of the Supervisory Board of the RECOOP HST Association

20:30 – 23:00 **Welcome Buffet Dinner**

April 17, 2015 (Friday)
Conference

8:30 – 8:40 **Conference Guide**
Sandor G. Vari, Director, International Research and Innovation Management Program, Cedars-Sinai Medical Center, Los Angeles, CA, USA & President of RECOOP HST Association

8:40 – 9:10 **Keynote speaker: Road from Budapest to Wroclaw**
Calvin J. Hobel, Miriam Jacobs Chair in Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles, CA, USA

9:10 – 10:15 **Poster Session #1** **Parallel Poster Sessions**

Lifestyle, Metabolic Disorders and Stress

Session Chairs:
Eva Szoko, Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University Budapest, Hungary
Katarina Sebekova, Institute of Molecular BioMedicine, Medical Faculty, Comenius University, Bratislava, Slovak Republic
Natalija Filipović, Laboratory for Neurocardiology, Department of Anatomy, Histology and Embryology, School of Medicine, University of Split, Croatia

Presentations: *5 minutes presentation and 5 minutes discussion*

Modulation of cholesterol content of brain nerve terminals by cyclodextrin-coated maghemite nanoparticles
Arsenii Borysov, Department Neurochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine

Mother's High Fat Diet Has Influence on Metabolic Characteristics and Ovarian Morphology in Rat Offspring
Anđela Marić
Department of Physical Medicine and Rehabilitation, University Hospital Centre Osijek and Department of Anatomy and Neuroscience, Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Croatia

How does maternal smoking influence the early neurobehavioral development of rat pups?
Barbara Mammel, Department of Obstetrics and Gynaecology, Medical School, University of Pécs, Pécs, Hungary

Chemerin blood levels are associated with cross-sectional areas of different compartments of adipose tissue at L5 level
Zvonimir Vrselja, Assistant - Department of Anatomy and Neuroscience, School of Medicine Josip Juraj Strossmayer University of Osijek, Croatia

Assessment of endothelial dysfunction in patients with generalized periodontitis
Yuriy Riznyk, Department of Therapeutic Dentistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Alteration of ObR expression in the Sprague-Dawley rat brain as a consequence of acute and chronic stress
Vedrana Ivic, Laboratory for Neurobiology, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Croatia

Cardiovascular Diseases

Session Chairs:
Attila Borbely, Institute of Cardiology, Medical and Health Science Center, University of Debrecen, Hungary
Ivancica Pavlicevic, Department of Family Medicine, University of Split School of Medicine, Croatia
Jan Pitha, Laboratory for Atherosclerosis Research, Centre for Experimental Research, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic

Presentations: *5 minutes presentation and 5 minutes discussion*

Age- and titin isoform -dependence of oxidative insults on passive force in perinatal rat left ventricular
Beata Bodi, Clinical Physiology Department, Institute of Cardiology, University of Debrecen, Hungary

Arginase-NO-synthase system in patients with ischemic heart disease of different age groups
Anna Besedina, Department of Family Medicine, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Acute TLR2 activation after stroke is modulated by transplanted mesenchymal stem cells
Dora Polšek, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Croatia

Platelets as regulators of plasminogen activation system
Yana Roka-Moiia, Department Enzyme Chemistry and Biochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine

Hyperbaric oxygenation affects the mechanisms of acetylcholine-induced relaxation in healthy and diabetic rats
Martina Mihalj, Department of Physiology and Immunology, School of Medicine, Josip Juraj Strossmayer University of Osijek, Croatia

10:15 – 10:45 Coffee break

10:45 – 13:00 **Plenary Session #1**

Neurodegenerative and Metabolic Diseases

Session Chairs
Konrad Talbot, Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA
Marija Heffer, Department of Medical Biology, School of Medicine, Josip Juraj Strossmayer University of Osijek, Croatia
Tatiana Borisova, Department of Neurochemistry, Palladin Institute of Biochemistry NAS of Ukraine, Kiev, Ukraine

Keynote speaker: (20 minutes)
A proposal to study brain insulin resistance driven by the insulin resistance syndrome as a causal factor in neurodegenerative diseases (NDGDs)
Konrad Talbot, Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Speakers: *12 minutes presentation – 3 minutes discussion*
Changes of VEGF and NPY expression in rat trigeminal ganglion induced by diabetes
Natalija Filipović, Head of Laboratory for Neurocardiology, Department of Anatomy, Histology and Embryology, School of Medicine, University of Split, Croatia

The quantitative assessment of corneal nerve structure in an animal model of type 2 diabetes mellitus
Fanni Palya, Department of Ophthalmology, Faculty of Medicine, Semmelweis University, Budapest

A New Model of Stroke in Type-1 Diabetic Rats
Mihael Mišir, Neurology Clinic, Osijek Clinical Hospital Centre, Faculty of Medicine Josip Juraj Strossmayer, Osijek, Croatia

Prenatal dietary load of Maillard reaction products combined with postnatal Coca-cola drinking affects metabolic status of female Wistar rats
Radana Gurecká, Institute of Molecular BioMedicine, Faculty of Medicine, Comenius University, Slovak Republic

Let's check this one more time.. decision change frequency during recognition as a predictor of working memory capacity
Aneta Brzezicka, Interdisciplinary Center for Applied Cognitive Studies, Warsaw School of Social Sciences and Humanities, Warsaw, Poland

Nociceptin and nocistatin in the myometrium: relaxing effect of two neuropeptides
Robert Gaspar, Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy University of Szeged, Hungary

$GABA_B$ receptor positive allosteric modulator rac-BHFF: Influence on the extracellular level, uptake/release of [^{3}H]GABA, Em and synaptic vesicle acidification in cortical and hyppocampal presynaptic nerve terminals
Tatiana Borisova, Department of Neurochemistry, Palladin Institute of Biochemistry NAS of Ukraine, Kiev, Ukraine

13:00 – 14:00 **Buffett Lunch**

14:00 – 16:00 **Plenary Session # 2**

Cardiovascular Diseases

Session Chairs:
Jan Pitha, Laboratory for Atherosclerosis Research, Centre for Experimental Research, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic
Katarina Sebekova, Institute of Molecular BioMedicine, Medical Faculty, Comenius University, Bratislava, Slovak Republic
Robert Gaspar, Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy University of Szeged, Hungary

Keynote speaker: (20 minutes)
Shared decision making in life style and nutrition for intervention in women with risk factors in cardiovascular health
Ivancica Pavlicevic and Mario Malički, Department of Family Medicine, University of Split School of Medicine, Split, Croatia

Speakers: *12 minutes presentation – 3 minutes discussion*

Estimation of central obesity in Slovak adults: comparison of waist circumference vs. waist-to-height ratio
Melinda Csongova, Institute of Molecular BioMedicine, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

Gender differences in morphological and functional characterization of athlete's heart in a rat model
Dalma Kellermayer, Heart and Vascular Center, Faculty of Medicine, Semmelweis University, Budapest, Hungary

Macrophage phenotype in the adipose tissue of postmenopausal women
Anna Králová, Laboratory for Atherosclerosis Research, Centre for Experimental Research, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic

Characterization of right ventricular function using 3D and speckle tracking echocardiography in patients after heart transplantation
Marton Tokodi, Cardiovascular Imaging Research Group, Heart and Vascular Center, Faculty of Medicine, Semmelweis University, Budapest, Hungary

Sex-specific chronic stress response at the level of adrenal gland modified sexual hormones and leptin receptors
Marta Balog, Department of Medical Biology, J. J. Strossmayer University of Osijek, Faculty of Medicine.

Effect of resveratrol on caspase 3 activation in primary mouse fibroblasts
Zsófia Ulakcsai, Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Budapest, Hungary

Relationship of semicarbazide sensitive amine oxidase/vascular adhesion protein-1 and insulin resistance in lean vs centrally obese male students
Ivana Koborová, Institute of Molecular BioMedicine, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

16:00 – 16:30 **Coffee Break**

16:30 – 18:30 **Poster Session #2**

Nanomediciene and Medical Imaging

Session Chairs:
Srećko Gajović, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Croatia
Artur Podhorodecki, Department of Experimental Physics, Wroclaw University of Technology, Wroclaw, Poland
Tatiana Borisova, Department of Neurochemistry, Palladin Institute of Biochemistry NAS of Ukraine, Kiev, Ukraine

Presentations: *5 minutes presentation and 5 minutes discussion*

Assessment of Immunotoxic effects of TiO_2 Nanoparticles
Silvia Ilavska, Slovak Medical University, Faculty of Medicine, Department of immunology and immunotoxicology, Bratislava, Slovak Republic

Cytotoxicity of maghemite core/polyaniline shell nanoparticles
Beata Zasonska, Department of Polymer Particles, Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Improvement of biocompatibility in laboratory rats of synthetic 4-thiazolidinone derivatives by complexing these drugs with polymeric nanocarrier
Lesya Kobylinska, Department Biochemistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Potentials of C60 Fullerene as a biocompatible platform for drug delivery: in vitro and in vivo studies
Rostyslav Panchuk, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

Ligand attraction with PEG-monooleate as efficient method of inorganic nanocrystals transfer to water
Daria Kociolek, Department of Experimental Physics, Wroclaw University of Technology, Wroclaw, Poland

Application of novel radiopaque ZrO_2-Gd_2O_3 nanocomposite functionalized with hyaluronic acid for repair of bone defects in experimental animals
Rostyslav Stoika, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

18:30 – 19:30 RECOOP HST Association General Assembly

Cedars-Sinai Medical Center, Los Angeles, USA
Edward Prunchunas, CSMC and Sandor G. Vari, CSMC - RECOOP

Josip Juraj Strossmayer University of Osijek, School of Medicine, Osijek, Croatia
Authorized Representative

Josip Juraj Strossmayer University of Osijek, Department of Biology, Osijek, Croatia
Elizabeta Has-Schön

University of Split School of Medicine, Department of Family Medicine, Split, Croatia
Ivančica Pavličević

IKEM - Institute for Clinical and Experimental Medicine, Prague, Czech Republic
Jan Pitha

Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic
Pavel Bostik

University of Debrecen, Hungary
Attila Borbely

University of Pecs, Hungary
Tibor Ertl

University of Szeged, Hungary
Gyorgy Falkay

Wroclaw University of Technology, Institute of Physics, Wroclaw, Poland
Artur Podhorodecki

Carol Davila University of Medicine and Pharmacy, Bucharest, Romania
Iuliana Ceausu

Slovak Medical University, Bratislava, Slovak Republic
Shubhada Bopegamage

Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine
Tatiana Borisova

Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Rostyslav Stoika

Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
Roman Lesyk

Observer:
Srećko Gajović, Editor-in-Chief, Croatian Medical Journal, Schools of Medicine of Universities of Osijek, Rijeka, Spit, and Zagreb, Croatia

18:30 – 19:30 **Breakaway Sessions**

Neurodegenerative Diseases

Konrad Talbot, Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA
Eva Szoko, Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University Budapest, Hungary
Imre Fehervari, Department of Transplantation and Surgery, Semmelweis University, Budapest, Hungary
Linn Defensor, Office of Research Compliance and Quality Improvement Cedars-Sinai Medical Center & RECOOP HST Association Clinical Research Site Management Network (CRSMN) Leader
Katarina Sebekova, Institute of Molecular BioMedicine, Medical Faculty, Comenius University, Bratislava, Slovak Republic
Robert Gaspar, Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy University of Szeged, Hungary
Tamas Tabi, Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Budapest, Hungary
Natalija Filipović, Head of Laboratory for Neurocardiology, Department of Anatomy, Histology and Embryology, School of Medicine, University of Split, Croatia
Oleksandr Korchynskyy, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

Cardiovascular Diseases, Life Style and Stress

Ivancica Pavlicevic and Mario Malički, Department of Family Medicine, University of Split School of Medicine, Split, Croatia
Marta Balog, Department of Medical Biology, J. J. Strossmayer University of Osijek, Faculty of Medicine.
Anna Besedina, Department of Family Medicine, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Anna Králová, Laboratory for Atherosclerosis Research, Centre for Experimental Research, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic

Nanobiotechnology, Imaging and Oncology

Denys Kolybo, Deputy Director, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine
Timea Tokes, 1st Department of Internal Medicine, Oncology Division, Medical Faculty, University of Semmelweis, Budapest, Hungary
Wafa Tawackoli, Research Imaging Core and Micro Molecular Imaging, Biomedical Sciences
Cedars – Sinai Medical Center, Los Angeles, CA, USA
Alena Gabelova, Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovakia
Rostyslav Bilyy, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Rostyslav Panchuk, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

Child Health and Infectious Diseases

William J. Britt, Department of Pediatrics, University of Alabama, Birmingham, Birmingham, AL, USA
Chander P. Arora, Research Project Advisor, International Research & Innovation Management Program, Cedars-Sinai Medical Center & Scientific Manager of the RECOOP HST Association
Danylo Kaminskyy, Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine
Maria Borsanyiova, Enterovirus Laboratory, Medical Faculty, Slovak Medical University, Bratislava, Slovak Republic
Barbara Mammel, Department of Obstetrics and Gynaecology, Medical School, University of Pécs, Pécs, Hungary
Cristian Poalelungi, Obstetrics-Gynecology Department, Hospital "Dr.I.Cantacuzino", "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

19:30 – 22:00 **Buffet Dinner**

April 18, 2015 (Saturday)
Conference

8:30 – 8:45 **RECOOP Leadership Message**
Sandor G. Vari, Director, International Research and Innovation Management Program, Cedars-Sinai Medical Center, Los Angeles, CA, USA & President of RECOOP HST Association

8:45 – 9:00 **Croatian Medical Journal - RECOOP**
Srećko Gajović, Editor-in-Chief, Croatian Medical Journal, Schools of Medicine of Universities of Osijek, Rijeka, Spit, and Zagreb, Croatia

9:00 -10:45 **Poster Session #3**

Infection and Immunology

Session Chairs:
Shubhada Bopegamage, Head, Entervirus Laboratory, Medical Faculty, Slovak Medical University, Bratislava, Slovak Republic
Rostyslav Bilyy, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Pavel Bostik, Associate Dean for Research, Center for Advanced Studies, Faculty of Military Health Sciences, University of Defence, Hradec Kralove, Czech Republic

Presentations: *5 minutes presentation and 5 minutes discussion*

Cervical microbiota in women with preterm prelabor rupture of membranes
Tomas Bestvina, Department of Obstetrics and Gynecology, Charles University in Prague, Faculty of Medicine Hradec Kralove, Czech Republic

Effect of DSS on Bacterial Growth in Gastrointestinal Tract
Jana Hlinková, Institute of Molecular BioMedicine, Medical Faculty, Comenius University, Bratislava, Slovakia

***Ureaplasma* species and *Mycoplasma* hominis in cervical fluid of pregnancies complicated by preterm prelabor rupture of membranes**
Miroslav Gregor, Department of Obstetrics and Gynecology, Charles University in Prague, Faculty of Medicine Hradec Kralove, Czech Republic

Interaction of Recombinant Diphtheria Toxin Molecule Fragments with Resistant to Toxin Cells
Denys Kolybo, Deputy Director, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine

Examination of antibacterial effect of neutrophilic granulocytes derived microvesicles
Viktoria Szeifert, Department of Physiology, Faculty of Pharmacy, Semmelweis University, Budapest, Hungary
10:45 – 11:00 Coffee break

11:00 – 13:00 **Plenary Session #3**

Molecular Biology, Infectious Diseases and Immunology

Session Chairs:
Wafa Tawackoli, Research Imaging Core and Micro Molecular Imaging, Biomedical Sciences
Cedars – Sinai Medical Center, Los Angeles, CA, USA
William J. Britt, Department of Pediatrics, University of Alabama, Birmingham, Birmingham, AL, USA
Shubhada Bopegamage, Head, Entervirus Laboratory, Medical Faculty, Slovak Medical University, Bratislava, Slovak Republic

Keynote Speaker (20 minutes)

Translation of Stem Cell Therapies for Skeletal Disorders
Wafa Tawackoli, Research Imaging Core and Micro Molecular Imaging, Biomedical Sciences
Cedars – Sinai Medical Center, Los Angeles, CA, USA

Speakers: *12 minutes presentation – 3 minutes discussion*

Study of immunoglobulin IgG glycosylation alteration in systemic and organ-specific autoimmune disorders
Iryna Magorivska, Department of Internal Medicine 3, University Hospital Erlangen, Institute of Immunology and Rheumatology, Germany and Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

Importance of dosage and immunization schedule on the adjuvancity of poly (lactide-co-glycolide) particles as antigen carriers for immunization *per os*
Tetyana Chudina, Department of molecular immunology, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine

Thiazolidinone-based design of new antitrypanosomal agents
Danylo Kaminskyy, Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Characterization of the effect of D- and L-limonene on pregnant rat myometrium *in vitro*
Judit Hajagos-Tóth, Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, Hungary

Experimental oral and intraperitoneal infection of Swiss albino mice with two coxsackievirus strains
Shubhada Bopegamage, Head, Entervirus Laboratory, Medical Faculty, Slovak Medical University, Bratislava, Slovak Republic

13:00 – 14:00 **Buffet Lunch**

14:00 – 14:30 **Overview of RECOOP Networks and Projects**
Sandor G. Vari, Director, International Research and Innovation Management Program, Cedars-Sinai Medical Center, Los Angeles, CA, USA & President of RECOOP HST Association

14:30 – 16:00 **Plenary Session #4**

Nanomedicine, Medical Imaging and Oncology

Session Chairs:
Rostyslav Stoika, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Alena Gabelova, Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovakia
Srećko Gajović, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Croatia

Keynote Speaker (20 minutes)
NanoBioTech activity at Institute of Cell Biology, NAS of Ukraine: Achievements in 2014, existing problems, and perspectives within RECOOP-HST Network
Rostyslav Stoika, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

Speakers: *12 minutes presentation – 3 minutes discussion*

Bioluminescent imaging of the mouse brain molecular response after stroke
Srećko Gajović, Croatian Institute for Brain Research, University of Zagreb School of Medicine, Croatia

Response Evaluation after Primary Systemic Therapy of Her2 Positive Breast Cancer – an Observational Cross-Sectional Study
Timea Tokes, 1st Department of Internal Medicine, Oncology Division, Medical Faculty, University of Semmelweis, Budapest, Hungary

Pyrazoline-thiazolidinone hybrids in the design of new anticancer agents
Roman Lesyk, Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

The surface modified magnetic iron oxide nanoparticles; interactions of nanoparticles with cells *in vitro*
Alena Gabelova, Cancer Research Institute, Slovak Academy of Sciences, Bratislava, Slovakia

16:00 – 16:30 Coffee Break

16:30 – 17:45 **Poster Session #4** **Parallel Poster Sessions**

Molecular and Cell Biology

Session Chairs:
Gyorgy Falkay, Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, Hungary
Martina Mihalj, Department of Physiology and Immunology, School of Medicine, Josip Juraj Strossmayer University of Osijek, Croatia
Tamas Tabi, Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Budapest, Hungary

Presentations: *5 minutes presentation and 5 minutes discussion*

Inhibitory κBα is a Direct Bone Morphogenetic Proteins Target Gene and an Essential Mediator of Anti-catabolic and Joint Regenerating Effects of BMPs
Oleksandr Korchynskyy, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

Plasma membrane Ca^{2+}-pump new inhibitor and supressor of myometrium spontaneus relaxation
Iuliia Mazur, Department of Muscle Biochemistry, Palladin Institute of Biochemistry National Academy of Sciences of Ukraine, Kiev, Ukraine

Histopathological Analysis of Subchronic Toxicity on Genetically Modified Maize Pioneer and Monsanto Mon810
Julia Ondrejkova, Department of Toxicology, Slovak Medical University, Bratislava, Slovakia

Effect of perinatal hypoxia on GABA transporter functioning in cortical, hippocampal and thalamic nerve terminals of the developing rat brain
Nataliia Pozdniakova, Department of Neurochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiev, Ukraine

Analysis of NMDA modulators with CE-LIF in different biological samples
Istvan Vincze, Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Budapest, Hungary

Translational Life Sciences

Session Chairs:
Eva Szoko, Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Budapest, Hungary
Chander P. Arora, Research Project Advisor, International Research & Innovation Management Program, Cedars-Sinai Medical Center & Scientific Manager of the RECOOP HST Association
Iuliana Ceausu, Department of Obstetrics and Gynecology of "Dr. I. Cantacuzino" Hospital, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Presentations: *5 minutes presentation and 5 minutes discussion*

Alterations of peritubular capillaries in experimental renal fibrosis
Peter Boor, Institute of Molecular Biomedicine, Bratislava, Slovakia; Bratislava & Institute of Pathology & Department of Nephrology, University Clinic of RWTH Aachen, Germany

Stress monitoring on gastrointestinal smooth muscle by electromyography
Kálmán Szűcs, Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy University of Szeged, Hungary

Risk Assessment of the Genetically Modified Maize Pioneer Mon810
Radka Aláčová, Department of Toxicology, Slovak Medical University, Bratislava, Slovakia

Animal model for the better understanding of bronchopulmonary dysplasia
Tolnai Marina, Department of Obstetrics and Gynecology, Medical School, University of Pécs, Hungary

Changes in superior cervical ganglion of adult rats induced by gonadectomy
Natalija Filipović**,** Laboratory for Neurocardiology, Department of Anatomy, Histology and Embryology, School of Medicine, University of Split, Croatia

Inhibitory effect of original synthetized isoquinoline derivatives for the rat uterus contraction
Dora Domokos, Department of Pharmacodynamics and Biopharmacy Faculty of Pharmacy, University of Szeged, Hungary

Clinical Research

Session Chairs:
Calvin J. Hobel, Miriam Jacobs Chair in Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles, CA, USA
Tibor Ertl, Department of Obstetrics and Gynecology, Vice Dean, Medical School, University of Pécs, Hungary
Imre Fehervari, Department of Transplantation and Surgery, Semmelweis University, Budapest, Hungary

Presentations: *5 minutes presentation and 5 minutes discussion*

Single-neuron novelty responses in the human substantia nigra during recognition memory
Jan Kaminski, Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Evaluation of patient's opinion about herbal medicines and phytotherapy in Ukraine
Khrystyna I. Makukh, Department of Clinical pharmacy, Pharmacotherapy and Medical Standardization, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Disturbance of regulatory mechanisms of spermatozoa in patients with infertility
Roman Fafula, Department of Biophysics, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Computer modeling assistance in planning of endoscopical osteosynthesis of the mandibular condyle
Khrystyna Pohranychna, Department of Surgical Dentistry and Maxillofacial Surgery, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Health Risk and Safety Assessments of Genetically Modified Food and Feed
Maria Bartusova, Department of Toxicology, Slovak Medical University, Bratislava, Slovak Republic

17:45 – 18:00 **Closing Remarks**
Edward Prunchunas, Senior Vice President for Finance and Chief Financial Officer, Cedars-Sinai Medical Center, Los Angeles, CA, USA

Sandor G. Vari, Director, International Research and Innovation Management Program, Cedars-Sinai Medical Center, Los Angeles, CA, USA & President of RECOOP HST Association

18:00 – Transportation to the Boat Tour with dinner

April 19 (Sunday) 2015 Departure

Oral presentation: 12 minutes presentation and 3 minutes discussion.

Poster session: 5 minutes presentation and 5 minutes discussion

Poster size: width 90 cm x height 120 cm.
The posters can be reviewed and discussed throughout the Conference. The posters have to be placed on the assigned Poster Stand on April 16 and shall be removed on April 18 after 18:00.

The 10th Bridges Conference partially sponsored by the International Visegrad Fund Standard Grant 21420033 "Fostering innovative life science research and competitive biotechnologies in the Visegrad Region".

The Association for Regional Cooperation in the (RECOOP) Fields of Health, Science and Technology (HST)

Short Name: **RECOOP HST Association**

RECOOP Short Summary: The Regional Cooperation for Health, Science and Technology (RECOOP HST) Consortium, led by Cedars-Sinai was formed in 2006 and transformed into Association in 2012 includes 17 universities and academic organizations from nine countries in Central and Eastern Europe (Croatia, Czech Republic, Hungary, Poland, Romania, Slovakia and Ukraine), Denmark and USA. The Association is a structured, functional and working research organization.

According to its mission statement: "The RECOOP HST Association explores and enhances LOCAL scientific outputs of the partner organizations, creates critical mass of scientifically sound innovative research at REGIONAL level and exploits the research outcomes at GLOBAL level to improve the prevention and treatment of major public health problems."™

RECOOP builds multinational, multidisciplinary collaborations, and assists, coordinates the research activities of the eighteen research groups at the Cedars-RECOOP Research Centers (CRRC). Implementations of RECOOP's strategic goals enable diverse talents geared towards integration of new knowledge derived from multispecialties to investigate Gender Differences (GD) in Common Mechanism of Diseases (CMD). In the CRRCs, researchers study genetic preconditions and the common mechanisms in molecular biology provide information on up-regulation or activation and down regulation or suppression of genetic codes by risk factors in nutrition, lifestyle (smoking, and alcohol, drug, mental and physical abuse), acute and chronic stress.

The scientific quality of the CRRCs' research reflected partly in the RECOOP's Annual Scientific Review Journals: the Biopolymers & Cell Journal (www.biopolymers.org.ua) 2010-13 Vol. 26;27;28;29, N2 supplementary. In 2014, RECOOP had Annual Scientific Review in the Croatian Medical Journal 2014, June, No.3 (www.cmj.hr).

The RECOOP HST Association organizes annually the Bridges in Life Sciences Conferences to review the scientific progress in the Association. During the Bridges in Life Sciences Annual Meeting the Scientific Advisory Board selects the top ten young scientists.

The top ten young scientists selected during the Bridges in Life Sciences Annual Conferences have the opportunity to **apply for International Visegrad Fund (IVF) Scholarship and receive the RECOOP Young Scientists Matching Fund.** Also for the Bohdan Malaniak CSMC - RECOOP Young Scientists Research Award. 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. CSMC – RECOOP Research Centers (CRRC) are 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 apply for IVF – CSMC - RECOOP Scholarship. The selected young scientists (preferably Ph.D. students) will spend maximum four semesters receiving: €2,300 / semester and the corresponding host universities/institutes will receive €1,500/semester/scholar. The host CRRC will get €1,000 for laboratory expense and consumables from CSMC – RECOOP HST Association.

In 2014 RECOOP announced the Bohdan Malaniak CSMC - RECOOP Young Scientists Research Award for preclinical and clinical studies.

The host and appointing organizations can be basic or translational research organizations nevertheless they should have an assisting clinical organization with clinical practice. The research project and the performed lab tests and measurements must have clinical relevance.

The maximum requested amount is 1,500 USD but it will only be paid if the applicant provides and proves matching found for the same amount. The submission date July 27, 2015, the application should be sent to Sandor G. Vari, MD. Please follow strictly the word counts in the template!

The Select Committee composed from the Presidency of the RECOOP HST Association and four invited RECOOP scietists based on the research areas of the applications. The winner will be announced on August 21, 2015.

The winner in 2014 was Marta Balog – Medical Faculty, Osijek, Croatia: Impact of acute and chronic stress on cardiovascular diseases

Host organization: Department of Pharmacodynamics and Biopharmacy, Faculty of Pharmacy, University of Szeged, Hungary

Appointing organization: Department of Medical Biology, Laboratory of Neurobiology, Faculty of Medicine University Josip Juraj Strossmayer Osijek, Croatia

Assisting organizations with lab tests and measurements have clinical relevance: Institute of Cardiology, Medical and Health Science Center, University of Debrecen, Hungary

RECOOP would like to provide more opportunities for young scietists, therefore agreed with Korányi Frigyes Science Dormitory, Semmelweis University Budapest to select the top abstracts were submitted to the call of Korányi Frigyes Scientific XX Forum of University Semmelweis, Budapest, Hungary.

The Forum was on March 12-13, 2015, and aimed to provide opportunities for young researchers, clinicians, pharmacists and students involved in Students' Scientific Association, while promoting a scientific contact between various disciplines within our university.

The submitted18 English abstracts were reviewed by RECOOP Review Board for the Bridges in Life Sciences 10th Annual Conference, Wroclaw, Poland. This peer –review is considered by RECOOP as a pre-selection, and will be finalized after the outcome of the Koranyi Science Forum 2015. All abstracts were reviewed by minimum 2 RECOOP experts. Those

who had no ECA - Ethical Committee or IACUC – Institutional Animal Care and Use Committee approvals and could not or did not provide the approval were excluded from the final selection.

Only those received RECOOP invitation for the Bridges Conference in Wroclaw who were already selected by RECOOP Review Board and at the same time ranked first or second in their session during the 2015 Koranyi Science Forum.

> Dalma Kellermayer, Medical Student, Heart and Vascular Center, Faculty of Medicine, Semmelweis University, Budapest, Hungary
> Abstract: Gender differences in morphological and functional aspects of athlete's heart in a rat model
>
> Viktoria Szeifert, Pharmacy Student, Faculty of Pharmacy, Department of Physiology, Semmelweis University, Budapest, Hungary
> Abstract: Examination of antibacterial effect of neutrophilic granulocytes derived microvesicles
>
> Marton Tokodi, Medical Student, Cardiovascular Imaging Research Group, Heart and Vascular Center, Faculty of Medicine, Semmelweis University, Budapest, Hungary
> Abstract: Characterization of right ventricular function using 3D and speckle tracking echocardiography in patients after heart transplantation

The Head of the Korányi Frigyes Scientific XX Forum of Organizing Committee Fanni Palya Medical Student, Faculty of Medicine, Semmelweis University, Budapest receieved an honorary invation as an appreciation of her hard work.
Abstract: The quantitative assessment of corneal nerve structure in an animal model of type 2 diabetes mellitus

In 2015 the top three RECOOP Young Scientists ranked during the Bridges in Life Sciences 10th Annual Conference will win a trip to Split to attend the 2015 Summer School of Scientific Communication: Publishing Research for Multidisciplinary Audiences on June 30 – July 3 2015. CSMC – RECOOP will pay their travel and accommodation expenses.

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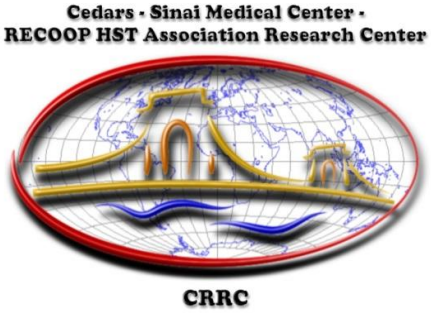

In the RECOOP Research Networks 147 scientists are working in 23 research projects in clinical, basic and translational research studies to investigate biological pathways leading to gender differences in cardiovascular diseases, preterm birth, breast, cervical and brain tumors.

In 2012 the RECOOP HST Association integrated the multidisciplinary, multicenter research studies of the **RECOOP Research Networks** into the RECOOP Life Science Research Platform and formed 18 **CSMC RECOOP Research Centers (CRRC)** from 7 countries (Croatia, Czech Republic, Hungary, Poland, Romania, Slovak Republic, and Ukraine) working on translational and clinical research in the field of Genomics - Proteomics, Epigenetics, Metagenomics, Molecular Biology, Metabolomics and NanoBioTechnology Nano-biotechnology.

The participating CRRCs from the member universities and research organizations implemented research programs for Medical and Ph.D Students.

RECOOP Life Science Research Platform already realized short term (1-2 years) pilot research studies. The Networks continue the translational and clinical research studies in midterm (5 years), and the RECOOP HST Life Science Research Platform shall plan to continue the clinical research studies for minimum 20 - 30 years and follow up the women, men and newborns registered in the Electronic Data Entry Forms (EDEF) started in 2011.

The Association inspires young scientists and clinical researchers for creative thinking, and helps to learn how to make decision on “publish and disclose” or “protect and publish”. RECOOP provides training for young scientists to learn proper and scientifically sound communication of their research results in their manuscripts and presentation. The Association provides practical training on the presentation of data in the manuscript from the “Introduction, Methods, Results and Discussion” to the “Conclusion” sections and guides the young scientists how to organize data in tables and graphs, presenting results of statistical analysis.

The CSMC - RECOOP Research Centers (CRRC)

Common Mechanisms of Diseases

Study pathogenesis and role of transcription factors in adipose tissue inflammation, in Common Mechanisms of Diseases (CMD) their relevance in co-morbidities
Imre Fehervari, MD., PhD., assistant professor, Department of Transplantation and Surgery Semmelweis University, Budapest, Hungary
Tamas Tabi, PhD, Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Hungary
Oleksandr Korchynskyy, Ph.D., Senior Scientist, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

Brain insulin resistance driven by the insulin resistance syndrome as a causal factor in neurodegenerative diseases (NDGDs)
Konrad Talbot, Ph.D., Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, California, U.S.A.
Professor Katarina Sebekova, Institute of Molecular BioMedicine, Medical Faculty, Comenius University, Bratislava, Slovak Republic
Natalija Filipović, PhD, DVM, assistant professor, Head of Laboratory for Neurocardiology School of Medicine, University of Split, Department of Anatomy, Histology and Embryology

Study of gender difference in vascular dementia
Robert Gaspar Pharm. D., Ph.D., Department of Pharmacodynamics and Biopharmacy Faculty of Pharmacy, University of Szeged, Hungary
Konrad Talbot, Ph.D., Department of Neurosurgery, Cedars-Sinai Medical Center, Los Angeles, California, U.S.A.
Natalija Filipović, PhD, DVM, assistant professor, Head of Laboratory for Neurocardiology School of Medicine, University of Split, Department of Anatomy, Histology and Embryology
Srećko Gajović, MD, PhD, Professor of Histology and Embryology, University of Zagreb School of Medicine, Croatia

Obesity, bone density and cardiovascular diseases
Professor Martin Gajdoš, M.D, Ph.D. and Dr. Zora Krivosikova, PhD Medical Faculty, Department of Clinical and Experimental Pharmacotherapy, Slovak Medical University, Bratislava, Slovak Republic
Marija Heffer, MD, PhD, Department of Medical Biology, School of Medicine, Josip Juraj Strossmayer University of Osijek, Croatia
Prof. Eva Szoko, PhD, DSc., Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Budapest, Hungary

Stress, obesity and myocardial contractile dysfunction
Attila Borbely, M.D., Ph.D., Cardiologist, Institute of Cardiology, Medical and Health Science Center, University of Debrecen, Hungary
Professor Marija Heffer MD, PhD, Department of Biology and Neuroscience, School of Medicine University Josip Juraj Strossmayer Osijek
Robert Gaspar Pharm. D., Ph.D., Department of Pharmacodynamics and Biopharmacy Faculty of Pharmacy, University of Szeged, Hungary

Prof. Eva Szoko, PhD, DSc., Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University, Budapest, Hungary

Lifestyle intervention in women's cardiovascular health with different reproductive and risk factors

Jan Pitha M.D., Ph.D., Head of Laboratory for Atherosclerosis Research Department of Cardiology, IKEM - Institute for Clinical and Experimental Medicine, Prague, Czech Republic

Ivancica Pavlicevic, MD,GP,PhD, Assistant Professor, Department of Family Medicine University of Split School of Medicine, Croatia

Sandor G. Vari, MD, Director, International Research and Innovation Management Program Cedars-Sinai Medical Center, Los Angeles, CA, USA & President of the RECOOP HST Association

Study of immunoglobulin IgG glycosylation alteration in systemic and organ-specific autoimmune disorders

Rostyslav Bilyy, PhD., Senior Researcher, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine

András Guttman, Ph.D., D.Sc., MHAS, Research Centre for Molecular Medicine, Horváth Laboratory of Bioseparation Sciences, Medical and Health Science Center, University of Debrecen, Hungary

Martina Mihalj, MD., Department of Physiology and Immunology, School of Medicine Josip Juraj Strossmayer University of Osijek, Croatia

Bioluminescent imaging of nanoparticles for biomedical applications

Srećko Gajović, MD, PhD, Professor of Histology and Embryology, University of Zagreb School of Medicine, Croatia

Wafa Tawackoli, Ph.D., Technical Director, Research Imaging Core and Micro Molecular Imaging, Biomedical Sciences, Cedars – Sinai Medical Center, Los Angeles, CA, USA

Rostyslav Bilyy, PhD., Senior Researcher, Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine

Professor Daniel Horák, Ph.D. Department of Polymer Particles, Institute of Macromolecular Chemistry, Academy of Sciences, Prague, Czech Republic

Artur Podhorodecki, Ph.D. Institute of Physics, Wroclaw University of Technology, Wroclaw, Poland

4-Thiazolidinone derivatives and related heterocyclic systems as prototypes of potential antitrypanosomal drugs

Professor Roman Lesyk, PhD, DSc, Head of the Dept. of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Chander P. Arora, PhD, Research Project Advisor, International Research & Innovation Management Program, Cedars-Sinai Medical Center & Scientific Manager of the RECOOP HST Association

Professor William J. Britt, M.D., University of Alabama- Birmingham, Department of Pediatrics, Birmingham, AL, USA

Cardiovascular Disease and Women's Health

The role of myofilamentary protein changes in diastolic dysfunction of newborns

Zoltan Papp, M.D., Ph.D., D.Sc., Institute of Cardiology, Clinical Physiology Department, University of Debrecen, Hungary

Oxidative stress markers for metabolic diseases
Professor Elizabeta Has-Schön, PhD, Department of Biology, University J.J. Strossmayer, Osijek, Croatia

Role of semicarbazide sensitive amine oxidase (SSAO) in Cardiovascular Diseases (endothelial cells and/or adipocytes)
Professor Éva Szökő, Ph.D., D.Sc., Department of Pharmacodynamics, Faculty of Pharmacy, Semmelweis University

Vitamin D deficiency and effect on neurotransmitters in diabetes, ischemic heart disease and preterm birth
Tatiana Borisova, Department of Neurochemistry, Palladin Institute of Biochemistry NAS of Ukraine

Glycosylation and Bioseparation
András Guttman, Ph.D., D.Sc., MHAS, Research Centre for Molecular Medicine, Horváth Laboratory of Bioseparation Sciences, Medical and Health Science Center, University of Debrecen, Hungary

Mother and Child Health

Enterovirus infection in mother, neonate, infants and follow up in child development
Professor Shubhada Bopegamage, Ph.D., Entervirus Laboratory, Virology Department, Slovak Medical University, Bratislava, Slovak Republic

Screening mothers and newborns for cytomegalovirus infection
William J. Britt, M.D., University of Alabama- Birmingham, Alabama, USA and Iuliana Ceausu, M.D., Ph.D, the Department of Obstetrics and Gynecology of "Dr. I. Cantacuzino" Hospital, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

Fetal sex disparities in Vitamin D levels of smokers and non smokers
Chander P. Arora, PhD, Research Project Advisor, International Research & Innovation Management Program, Cedars-Sinai Medical Center & Scientific Manager of the RECOOP HST Association

NanoBioTechnology and Cancer

Toxicity risk assessment of carbon-containing nanoparticles, ash of burning carbohydrate-based products (used tyres, turf, wood, plants, oil, fuel, garbage plastics)
Tatiana Borisova, PhD, DSc, Head, Department of Neurochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiyv, Ukraine

Iron nanoparticles for monitoring different diseases
Professor Daniel Horák, Ph.D. Department of Polymer Particles, Institute of Macromolecular Chemistry, Academy of Sciences, Prague, Czech Republic
Tatiana Borisova, PhD, DSc, Head, Department of Neurochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kiyv, Ukraine

Biofunctionalization of nanoparticles and targeted drug delivery studies
Professor Rostyslav Stoika, PhD, D.Sc., Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine

Targeted drug delivery for cancer treatment
Professor Roman Bogdanovych Lesyk, Ph.D., D. Sc, Department of Pharmaceutical, Organic and Bioorganic Chemistry, Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

Tunable nanocrystals for biomedical imaging
Artur Podhorodecki, Ph.D. Institute of Physics, Wroclaw University of Technology, Wroclaw, Poland

In vitro and in vivo immune assays for evaluation of immunotoxic effect of nanoparticles
MUDr. Jana Tulinska, PhD. Laboratory of Immunotoxicology, Slovak Medical University, Bratislava, Slovak Republic

The Croatian Medical Journal (April 2015, www.cmj.hr) published now the second RECOOP thematic issue, this time dedicated to the RECOOP Bridges 10th Annual Scientific Conference in Wroclaw, Poland.

CROATIAN MEDICAL JOURNAL

The mission of Croatian Medical Journal (CMJ) was from its very beginning to support the biomedical research in emerging countries. CMJ first aimed to help Croatian research community to publish their high quality papers in a more friendly way, but very soon, the scope was extended to the neighboring countries, Central East Europe, Asia and Africa. Currently, CMJ is orientated to all researchers having low visibility either due to their geographical location or due to their professional status.

The major tool that CMJ is offering to this group of emerging researchers is a specific Author-Helpful Policy. The submitted articles were judged according to their potential and the dedicated team of professionals is there to assist the authors. The team consisted of the members of CMJ editorial group and CMJ associates were engaged to help authors to present their research according to the high standards of international publishing. Such policy had many benefits. The first were for the Journal itself, as it helped to establish a core of knowledge under CMJ umbrella which although offered internationally was indeed a national asset. Croatian experts in statistics, manuscript editing, scientific language, and manuscript production were established. The criteria of manuscript quality subsequently raised and the CMJ Impact Factor increased to its maximum of 1.8, which made it to rank among first 10 general medical journals.

The effects on global community were as well visible. Many young researchers were able to publish their first contributions in CMJ, many other national journals followed the suite and the strength of the previously unrecognized research became obvious and accessible to the global scientific community.

CMJ has published the second thematic issue made by scientists from RECOOP institutions. We can say that there is already a small tradition to offer and share CMJ virtues with the RECOOP scientists and to collaborate successfully.

We at CMJ strongly believe that RECOOP and CMJ share their basic mission. By increasing the across border collaboration in the Central and Eastern Europe the undiscovered opportunities start to shine and bring benefits to the national, regional and global community. Indeed, the scientific community should continuously discover multinational and multidisciplinary collaborations, and should be nourished, developed and properly presented.

The whole venture to create this thematic issue was an excellent international team effort, were many small pieces were arranged together to identify, develop and present the excellence growing under RECOOP umbrella. Therefore, we wish that the published papers in the thematic issues are a clear sign of growing common RECOOP activities, and a long-term establishment of regional excellence, excellence that CMJ wants to be part of.

Prof. Srecko Gajovic
Editor-in-Chief
Croatian Medical Journal

CMJ is an international peer-reviewed Diamond Open Access journal published six times per year. The CMJ uses the Diamond Open Access model. This means that there are NO author processing fees and NO fees to access the published papers. The free access is available on our web page, www.cmj.hr, also on PubMed Central. Impact factor of CMJ for 2013 is 1.373. The RECOOP thematic issue is as well fully accessible at journal web page www.cmj.hr

Abstracts

April 17, 2015

Poster Session #1

Lifestyle, Metabolic Disorders and Stress

Modulation of cholesterol content of brain nerve terminals by cyclodextrin-coated maghemite nanoparticles

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Keywords: maghemite nanoparticles; cholesterol; methyl-β-cyclodextrin; glutamate; uptake; rat brain nerve terminals

Introduction: Recently, we have shown that a reduction of cholesterol content by cholesterol acceptor methyl-β-cyclodextrin (MCD) increased the extracellular glutamate level in brain nerve terminals. A decrease in the level of membrane cholesterol attenuated glutamate transporter reversal. Therefore, lowering cholesterol may be used for neuroprotection in stroke, ischemia, traumatic brain injury that are associated with an increase in glutamate uptake reversal. However, beside these disorders lowering cholesterol may cause harmful consequences decreasing glutamate uptake by nerve terminals. MCD as cholesterol-depleting agent has many restrictions in medical application. The aim of the research was to modulate the cholesterol level in isolated rat brain nerve terminals (synaptosomes) by magnetic nanoparticles (NPs) coated with cholesterol-reducing drug.
Methods: Transmission electron microscopy, preparative biochemistry, radiolabel assay. 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 # 1 from 19/09-2012). 10 animals were used in the study.
Results and discussion: A new simple method for preparation of maghemite nanoparticles surface of which is modified with MCD was developed. The reaction consists in silanization of maghemite with a reagent prepared by acylation of MCD using 3-isocyanatopropyltriethoxysilane. The effectiveness of these NPs to modulate the level of cholesterol in synaptosomes was assessed. NPs at a concentration of 4 mg/ml decreased the level of cholesterol in synaptosomes by 50% that consisted of 0.375 ± 0.02 mmol/ml in control and 0.19 ± 0.01 mmol/ml after application with NPs. 1 mg of NPs was able to extract and kept 0.035 ± 0.002 mmol of membrane cholesterol. A decrease in the level of cholesterol in nerve terminals was accompanied by a reduction of Na^+-dependent L-[^{14}C]glutamate uptake by 20 % and this data was completely correlated with experiments with MCD *per se* without NPs.
Conclusion: NPs for modulation of cholesterol level in nerve terminals were obtained. This new approach allows to extract cholesterol from the cells, and then remove it outside using external magnetic fields.

Support: This work was supported by Grant of Space research program of NAS of Ukraine.
Acknowledgements: We thank for Cedars Sinai Medical Center's International Research and Innovation Management 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).

Mother's High Fat Diet Has Influence on Metabolic Characteristics and Ovarian Morphology in Rat Offspring

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Key words: high fat diet, offspring, epigenetic, ovary

Introduction: Adipose tissue acts as an important endocrine organ. It secretes substances involved in the regulation of energy balance and secretes various pro-inflammatory cytokines. Disturbances in the metabolism of adipose tissue are the basis of the pathogenesis of many diseases. There is important influence of maternal nutrition on the physiological and pathological processes in the offspring which is usually explained as posttranslational modification of the nucleic acids of offspring without changing the genetic sequence.
In this study, we examined the effect of diet of the mother and offspring on metabolic features and histological changes in the ovaries of female offspring.
Methods: Ten female rats were randomly divided in two groups. One group was fed with high content of saturated fatty acid food (HFD) and the other one with standard laboratory chow (CD). Female offspring from both groups were randomly divided in two subgroups after coupling and lactation period, subsequently there were four groups of offspring (n=6 each) with different feeding protocol: a) CD-CD, b) CD-HFD, c) HFD-CD and d) HFD-HFD. At the age of 18 weeks in offspring biochemistry analysis was determined, as a weight measurements and BMI calculating. Afterwards they were sacrificed and ovarian morphology was studied.
Results: Significant changes were observed in triglyceride (the highest in HFD-HFD group, P=0.001) and cholesterol levels, being highest in CD-HFD group (P=0.002). Ovarian histopathological analysis of offspring of CD mothers shows normal histological characteristics, while in the ovaries of the offspring of HFD mothers large cystic follicles were observed.
Discussion: Mother's diet has influence on the regulation of the metabolic and reproductive functioning of female offspring.
Conclusion: Maternal HFD consumption predisposes offspring to increased risk of developing metabolic abnormalities and ovarian disorders.

Acknowledgements
This work was supported by the Ministry of Science, Education and Sports of the Republic of Croatia (grant number: ID: 219-2192376-2092). The authors are grateful to Tomislav Ivankvić for nutritional assistance and to Nikola Bijelić for histological assistance. None of the authors received any personal financial remuneration or funding for research studies from any chemical industrial company.

Experiments were approved by the Ethics Committee of the Faculty of Medicine University of Osijek (March 31, 2014; No. 2158-61-07-14-13).

How does maternal smoking influence the early neurobehavioral development of rat pups?

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Introduction: Exposure to tobacco smoke during perinatal life is known to have deleterious effects. Several studies have reported declined cognitive functioning, decreased locomotor behavior and an increased risk for pshychiatric disorders. However, little is known about the effects of prenatal smoking on the early neurobehavioral development. Our aim was to investigate the influence of maternal smoking during pregnancy on the early physical and neural development of newborn rats.

Methods: Wistar rats were placed into a closed-chamber smoking system for whole-body smoke exposure 2x40 minutes daily from mating until delivery. For the treatment two research cigarettes per occasions were used. Controls did not smoke. After birth, pups were cross-fostered to eliminate maternal influence. Somatic and neurobehavioral development was evaluated daily for 3 weeks. Motor coordination tests were carried out on the 4th week. Weight gain was also registered. The data of prenatally smoke-exposed pups (s+) were compared to that of the controls (s-).

Results: Results show that certain physical reflexes of s+ pups (eyelid and eartwitch reflex) appeared later compared to s-. We also experienced a delay in reflexes indicating the neural maturity - hindlimb grasping, fore-and hindlimb placing reflexes - in the group of s+ pups. We did not show any differences in weight and motor coordination.

Discussion: This study suggests that maternal smoking during pregnancy has early detectable effects on the neurological development of the rat pups which may indicate the future vulnerability of these individuals.

Conclusion: Although it is well-known that smoking during pregnancy has short and long-term effects on the offsprings, but it has not been known if there is an early effect on the neurobehavioral development. In our animal experiment we showed an early delay in some developmental signs. This work may suggest to pay more attention to those individuals who were affected by cigarette smoke during intrauterine life.

This work was supported by PTE-MTA “Lendület” program, the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0001 ‘National Excellence Program, Arimura Foundation, Bolyai Scholarship

Acknowledgement: Experiments were approved by the Institutional Animal Care and Use Committee (IACUC). Approval BA02/2000-5/2011

Chemerin blood levels are associated with cross-sectional areas of different compartments of adipose tissue at L5 level

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Keywords: chemerin, adipose tissue, MRI

Introduction: Increasing obesity prevalence is of great importance since it is associated with low-grade chronic inflammation. Adypocytes which constitute adipose tissue, among other cells, produce a large number of adipocytokines which are responsible for immunometabolic modulation. Chemerinis a multifunctional protein which primarily acts as a chemotactic cytokine. Chemerin is associated with early stages of low-grade chronic inflammation of adipose tissue possibly through regrutation of immune cells to the adipose tissue, angiogenesis, osteoblastogenesis etc. We aim to investigate the relationship between chemerinblood levels and abdominal region adipose tissues.

Methods: Study was approved by local ethical committee. We included 60 volunteers in the study; all participants underwent magnetic resonance (MR) of abdominal region using 2D FLSAH sequence for optimal detection of adipose tissue. MRI data was analyzed using open source *ImageJ* program in order to obtain the adipose tissue surface area. Blood samples were taken from all participants, chemerin serum levels were determined with commercial ELISA kit.

Results: Average participant had BMI of 24.5±4.7 and was 20 [20-30.75] years old. The average area of adipose tissue at L5 level was 281±119 cm^2, subcutaneous adipose tissue 215±91 cm^2 and visceral adipose tissue 65±38 cm^2. Chemerin positively correlated with overall adipose tissue ($r=0.721$, $p<0.001$), subcutaneous ($r=0.694$, $p<0.001$) and visceral adipose tissue ($r=0.588$, $p<0.001$). After age and genderadjustment, overall adipose tissue was the most important predictor of chemerin levels ($R^2=0.749$, $p<0.001$, $\beta=0.27$).

Discussion and Conclusion: Chemerin blood levels had a strong and medium positive correlation with different compartments of adipose tissue. Visceral and subcutaneous adipose tissue might contribute equally to the chemerim blood levels.

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

We are grateful to the Department of Radiology, University Hospital Osijek for their logistical support.

This study was approved by the Ethical Committee of the Faculty of Medicine Osijek: 25-1:5484-4/2013, 27.5.2013.

Assessment of endothelial dysfunction in patients with generalized periodontitis

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Key Words: endothelium dysfunction, endothelin-1, generalized periodontitis.

Introduction: The study of pathogenesis of the generalized periodontitis (GP) that develops into the research of the endothelium dysfunction of the microvasculature of periodontal tissues proved to be an extremely important achievement. Endothelin-1 (ET-1) – powerful endogenic vasoconstrictor plays a significant role in the induction of vascular disorders. The diagnosis of the initial disorders of endothelial function of microvasculature of the periodontium contributes to the determination of therapeutic measures for its correction.

Methods: The clinical, paraclinical and immunological studies were carried out in 96 patients from 25 to 50 years old, including 18 patients with the intact periodontium (comparison group). Level of endothelium dysfunction marker – ET-1 was defined in oral liquid by means of the ELISA method and a broad set of reagents Endothelin-1 ("Biomedica" Austria). The electron microscopic studies of marginal periodontium were made.

Results and discussion: We made the analysis of ET-1 concentration in patients with clinically intact periodontium and those with GP depending on the severity and course of their disease. We have found that the ET-1 concentration in oral liquid in patients with the I stage of the chronic GP was equal to 0,59±0,04 fmol/ml and it exceeded the indices in the comparison group in 1,4 times ($p<0,01$), in patients within the period of chronic GP the exacerbation it exceeded the indices in the comparison group in 1,9 times ($p<0,01$) correspondingly. There was a direct correlation between the stage of GP and levels of ET-1 in oral liquid ($r = +0,63$). In the microvasculature of gums in GP patients well expressed and polymorphic changes were found.

Conclusions: In patients with GP disorders of endothelium-dependent relaxation of blood vessels was found. The increase of ET-1 concentration in the oral liquid promotes the development of vasospasm, thrombosis and leads to the progression of pathological process in the periodontal tissues.

This study was approved by the local Ethical Committee 22.04.2013 protocol №4

Alteration of ObR expression in the Sprague-Dawley rat brain as a consequence of acute and chronic stress

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Key words: leptin receptor, stress, brain, rat

Introduction. Acute and chronic stressors challenge homeostasis which is maintained by sympathomedullary pathway and hypothalamus-pituitary-adrenal axis. Obesity is a disorder of homeostatic mechanisms which control energy balance through leptin and leptin receptor (ObR). Males are losing weight upon chronic stress while females gain weight. However, the complete mechanism of body weight maintenance during chronic stress and gender differences are still unclear. The aim of this study was to evaluate whether the acute and chronic stress affect ObR expression in the brain of male, female (NON-OVX) and ovariectomized (OVX) Sprague-Dawley rats.

Methods. Study included 72 four-months-old Sprague-Dawley rats divided in males, NON-OVX and OVX group. These groups were further subdivided in acute, chronic and control group. Acute stress was provoked by cold restraint while chronic stress by combination of different stressors within the 10-day stress session. Stress sessions were repeated 3 times with 2 week pause in-between. Sham groups were exposed to the same environment as stress groups, but with stressor excluded. When the rats were 28 weeks old brains were collected and free-floating immunohistochemical staining was performed using ObR antibody.

Results. ObR positive neurons were analyzed in the barrel field (S1BF) and periventricular (Pe), arcuate (Arc) and lateral hypothalamic nuclei (LH). While there was no alteration of ObR expression in the Pe, the differences in Arc, LH and S1BH are observed. Acute stress lowers expression of ObR in all animals and these regions with exception of LH in OVX where ObR expression is increased. Contrary, chronic stress brings expression of ObR to control levels or even higher.

Discussion and conclusion. Exposure to acute stress causes down-regulation of ObR receptor in barrel field and critical regions of hypothalamus. Homeostatic mechanisms are able to stabilize levels of ObR during chronic stress, at least in young animals.

Source(s) of research support: Internal research grant from University of Osijek, Osijek, Croatia

Acknowledgement: This study is the part of Women's Health and Cardiovascular Diseases Research Network of Regional Cooperation for Health, Science and Technology (RECOOP HST) Consortium formed by Cedars–Sinai Medical Center (CSMC), Los Angeles, CA, USA.

This study was approved by the Ethical Committee of the Faculty of Medicine Osijek; number of approval: 2158-61-07-11-51.

Abstracts

April 17, 2015

Poster Session #1

Cardiovascular Diseases

Age- and titin isoform-dependence of oxidative insults on passive force in perinatal rat left ventricular

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Key words: passive force, titin, cardiomyocytes, oxidation

Introduction: The physiological and pathological adaptation processes to the extrauterine life involves posttranslational modifications of the differentially expressed titin isoforms relevant to the passive force ($F_{passive}$) of the cardiomyocytes and hence the diastolic function of the left ventricle (LV). In this study we aimed to reveal how the titin isoform composition and oxidative insults influence the passive tension of the LV cardiomyocytes in newborn rats.

Methods: Experiments were performed at different stages of postnatal development (0, 7 and 21 days) of control Wistar rats. $F_{passive}$ was measured in single, permeabilized LV cardiomyocytes. The effects of protein oxidation on $F_{passive}$ was evaluated following *in vitro* exposures to an oxidative agent, 2,2'-dithiodipyridine (DTDP, 10 mM) in cardiomyocytes followed by the application of the antioxidant dithiotreitol (DTT, 10 mM). Titin isoform composition was analyzed by SDS-gel electrophoresis.

Results: $F_{passive}$ was significantly increased with age in a range of sarcomere lengths (SL, 1.9-2.5 μm) in rats. $F_{passive}$ was significantly lower in the 0- and 7-day-old groups than in the 21-day-old animals (0.11±0.01 kN/m^2 *vs.* 0.26±0.02 kN/m^2 *vs.* 0.56±0.02 kN/m^2,SL: 2.3 μm, $P<0.05$, n=10-13). The N2BA/N2B ratio of titin isoforms showed age-dependent characteristics (80±1%/20±1%, 41±1%/59±1%, 10±1%/90±1% in 0, 7, 21 day-old rats, n=5-7). DTDP significantly increased $F_{passive}$ in 0- and 7-day-old rats, but to a lesser extent in 21-day-old animals ($\Delta F_{passive}$ normalized to the baseline values in the three age groups: 99±26%, 65±23%, 39±21%, $P<0.05$ 0-day-old *vs*. 21-day-old group, SL: 2.3 μm, n=7-8). The effects of DTDP were completely reverted by the application of DTT in 0-, 7-, 21-day-old animals (0.10±0.01 kN/m^2, 0.21±0.02 kN/m^2, 0.51±0.08 kN/m^2, SL: 2.3 μm, n=7-8).

Conclusion: Cardiomyocyte maturation following birth is accompanied by a gradual decline in the oxidation evoked increases in $F_{passive}$, presumably because the compliant N2BA titin isoform is more sensitive to oxidative insults than the N2B titin.

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The animal research study was approved by the Ethical Committee of the University of Debrecen (Ethical Stateme

Arginase-NO-synthase system in patients with ischemic heart disease of different age groups

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Key words: ischemic heart disease, nitric oxide, NO-synthase, arginase, *L*-arginine

Introduction. Although a considerable amount of research in the biological role of NO has been done, the exact regulatory mechanisms underlying NO-homeostasis remains unclear. Research of arginase-NO-synthase system in patients with ischemic heart disease (IHD) in different age groups and clarification of the regulatory mechanisms maintaining NO-homeostasis has great importance.

Methods. Fifty patients with IHD (32 men and 18 women) aged 45-75 years (average age 56.8 ± 1.7 years) were enrolled in this research. All patients gave written informed consent to participate in research. The patients were divided into two groups with respect to their ages: group A - middle-aged patients (45-60 years), group B - elderly patients (61-75 years) (WHO, 1963). The patients of both groups matched for sex, disease duration, number of pain attacks. Patients with IHD who have not received treatment by nitro medication, but occasionally used nitroglycerin for angina pectoris were included in the research.

Results and discussion. It has been found that disturbance of endothelial function in patients with IHD is characterized by increased activity of total NO-synthase, which leads to hypersynthesis of "harmful" NO. Total NOS activity in middle-aged patient with IHD is increased in 2.2 times, in elderly patient - in 2.3 times ($P < 0.01$). It has been shown that disturbance of endothelial function in patients with IHD is characterized by reduced endothelial NO synthesis by eNOS and increased systemic NO synthesis due to increased iNOS activity.

It has been shown that increase in arginase activity is more expressed in elderly patients with IHD and is the compensatory mechanism to limit the L-arginine bioavailability.

Conclusion. These data indicate a disturbance of NO-homeostasis, lack of endothelial NO and hyperproduction of "harmful" NO by iNOS in patients with IHD, which is more expressed in elderly patients.

Acknowledgement: This study was approved by the DHLNMU Ethical Committee. Approval No 8 from October 22, 2012.

Acute TLR2 activation after stroke is modulated by transplanted mesenchymal stem cells

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Key words: in vivo imaging, stroke, inflammation

Introduction: A transgenic mouse model made by combining a Toll-like receptor 2 promoter with a luciferin cassette is used for visualization of in vivo TLR2 expression through bioluminescent signaling. TLR2 is activated by damage associated molecular patterns (DAMPs) and has been linked with microglial activation after neuronal damage in stroke.

Methods: Stroke was induced using transient middle cerebral artery occlusion (MCAO) on 3-4 month old transgenic TLR2-GFP-luc mice. The baseline and 1 day after ischemia bioluminescent signal were recorded using Xenogen IVIS imaging system. Afterwards, group of mice were transplanted with bone marrow derived mesenchymal stem cells in 0,5 µl of PBS via the stereotaxic intracerebral injection, while the control mice were injected with 0,5 µl of PBS only. TLR2 signal was followed by in vivo imaging durong the acute phase of stroke. Microglial response was evaluated using immunohistochemistry for Iba1 in transplanted and control group. For statistical analysis the regions of interest were fixed to compare individual mice and groups.

Results: TLR2 showed marked induction after MCAO with a peak at the third day after stroke, while transplanted mice showed altered intensity of inflammation. Existing variability of baseline TLR2 signal was normalized for each mouse, enabling us to define a dynamic of microglial activation through the acute phase of stroke for the transplanted and control groups. Immunohistochemistry confirmed the results acquired using in vivo imaging.

Conclusions: TLR2-GFP-luc transgenic mice represent a useful and ethically acceptable model for evaluation of overall intensity of stroke, and microglial activation in particular. It was possible to visualize in vivo the alteration of the intensity of neuroinflammation after MSC application.

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Acknowledgement: Animal Care and Use Committee Approval 380-59-10106-14-55/230 from October 23, 2014

Platelets as regulators of plasminogen activation system

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Key words: platelet, plasminogen activation, plasminogen activator inhibitor-1 (PAI-1), PAI-1 activity determination.

Introduction. Platelets bind plasminogen, its activators and contain PAI-1. Platelet membrane can be considered as a catalytic surface for plasminogen activation. The aim of this work is to investigate kinetic of plasminogen activation by tissue plasminogen activator (t-PA) in the presence of platelets and define inhibitory activity of platelet PAI-1.

Methods. Human platelets were isolated from blood of healthy volunteers. Human Glu-plasminogen was purified by lysine-sepharose chromatography. The rate of plasminogen activation by tPA was estimated by activity of new formed plasmin. Activity of platelet PAI-1 was determined by the method developed in our lab. It is based on the inhibition of plasminogen activation by tPA using bovine desAB-fibrin as stimulator.

Results. In the presence of resting platelets the catalytic efficiency of Glu-plasminogen activation increased in 8 times mainly thanks of Km decrease (0.184±0.066 μM compared with 1.117±0.086 μM in cell free system), catalytic constant had no significant change. Thrombin treatment of platelets accompanied with further increase of catalytic efficiency of plasminogen activation more than in 30 times. Stimulating effect of platelets was not related with the presence of endogenous plasminogen or its activators. PAI-1 activity of lysates of resting platelets and releasates of thrombin- and collagen-stimulated platelets was 2.04±0.70, 1.26±0.60 and 0.75±0.36 IU/10^8 cells, respectively.

Conclusion. Platelets stimulate plasminogen activation at physiological concentration of tPA. Platelets could be considered as sites of plasminogen activation and local plasmin generation. Platelets contain and release the active form of PAI-1 during activation. Quantitative assessment of its activity suggests that platelet PAI-1 makes a significant contribution to limiting of plasminogen activation and further fibrinolysis. The proposed method for quantifying PAI-1 activity in plasma and platelets can be used for evaluation of fibrinolytic potential in patients with cardiovascular diseases.

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Resrach protocols were approved by the Ethical Committee of Palladin Institute of Biochemistry of NASU (from the 4th of June 2014, protocol №2).

Hyperbaric oxygenation affects the mechanisms of acetylcholine-induced relaxation in healthy and diabetic rats

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Key words: hyperbaric oxygenation, CYP450-4A isoforms, acetylcholine, EETs, diabetes mellitus

Introduction: Our previous studies demonstrated that hyperbaric oxygenation (HBO_2) can modulate the mechanisms of vascular responses to angiotensin (1-7) in healthy and diabetic male rats, which included the role for epoxyeicosatrienoic acids (EETs) (Diab Vasc Dis Res. 2015;12(1):33-45; Undersea Hyperb Med. 2012;39(6):1053-66). The aim of the present study was to assess the possible role of arachidonic acid metabolites in acetylcholine –induced responses in HBO_2 treated healthy and diabetic rats.
Methods: The effects of hyperbaric oxygenation (HBO_2) on acetylcholine-induced vasorelaxation (AChIR) were evaluated in fifty-five male Sprague-Dawley rats randomized into 4 groups: healthy controls (Ctr), diabetic rats (DM), and control and diabetic rats that underwent hyperbaric oxygenation (Ctr+HBO_2 and DM+HBO_2). AChIR was measured in aortic rings with/without indomethacin, L-NAME or clotrimazole. CYP450-4A1,2,3 protein isoforms expression was determined by Western blot.
Results: Plasma antioxidative capacity and systemic oxidative stress were determined with FRAP and TBARS assays, respectively. Data were analyzed with Two-way ANOVA RM and Bonferroni post test.$p<0.05$ was considered significant. AChIR was preserved in all groups of rats, but mediated with different mechanisms. In the presence of L-NAME, AChIR in Ctr+HBO_2 and DM+HBO_2 rats was partially preserved in contrast to control and DM groups. Clotrimazole partially blocked vasorelaxation in all groups, but more in both DM groups.The oxidative stress was significantly higher in both DM and DM+HBO_2 groups compared to respective controls. Both HBO_2 groups had higher protein expression of all CYP450-4A isoforms compared to control and both DM groups.
Discussion: Present study demonstrated significant effects of HBO_2 on enzymes that metabolise arachidonic acid and are involved in vascular reactivity to ACh.
Conclusion: In conclusion, functional studies and expression of CYP450 in both HBO_2 groups suggest improved endothelial function by HBO_2, mediated by CYP4504A metabolites of arachidonic acid.

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Abstracts

April 17, 2015
Plenary Session #1
Neurodegenerative
and
Metabolic Diseases

A proposal to study brain insulin resistance driven by the insulin resistance syndrome as a causal factor in neurodegenerative diseases (NDGDs)

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Introduction: The insulin resistance syndrome is a condition defined largely by a set of four symptoms: systemic insulin resistance, dyslipidemia, endothelial dysfunction, and inflammation. This syndrome is a feature of many systemic disorders (e.g., obesity, type 2 diabetes [T2D], cardiovascular disease, essential hypertension and non-alcoholic fatty liver disease). It may also be a pathogenic factor in NDGDs, the prevalence of which is becoming epidemic, given evidence that the insulin resistance syndrome is present in Alzheimer's disease (AD), Parkinson's disease (PD), and vascular dementia (VD). Indeed, T2D is an established risk factor for all three of these dementia types.

Hypothesis: The insulin resistance syndrome in T2D and other systemic disorders may increase neurodegenerative dementia risk by fostering development of brain insulin resistance, which we have found to be a common and profound phenomenon in AD (Talbot et al., JCI 122: 1316, 2012). This hypothesis is consistent with reports showing (a) that diet-induced systemic insulin resistance elevates biomarkers of brain insulin resistance, (b) that diets lowering systemic insulin resistance reduce AD risk, and (c) that a class of antidiabetics (incretin receptor agonists such as Victoza) markedly reduces brain pathology, vascular damage, and cognitive deficits in animal models of AD and PD. Brain insulin resistance should greatly accelerate pathogenesis of neurodegenerative dementias, because impairments of brain insulin signaling are known to promote loss of cerebrovascular function, cell death, synaptic dysfunction, and cognitive impairment.

Approach: The present hypothesis requires testing whether or not measures of the insulin resistance syndrome (systemic insulin resistance, dyslipidemia, vasodilation and inflammation) are significantly and positively related to measures of brain insulin resistance, brain pathology, and cognitive status. This is achievable by experimental studies on rodents on normal diets vs. high fat diets inducing insulin resistance and by correlational studies on normal rodents vs. rodent models of AD, PD, and VD. Validation studies can be run on human AD, PD, and VD cases, blood samples from which allow measurement not only of systemic variables, but of neuronal proteins found in exosomes derived from the brain. This approach builds upon ongoing efforts of RECOOP scientists to understand the relationship between systemic levels of leptin, estrogen, and progesterone receptors and the Homeostasis Model Assessment of insulin resistance (HOMA-IR). Finally, as a therapeutic component, the project will test if the ability of incretin receptor agonists to reduce the insulin resistance syndrome is proportional to their ability to reduce brain insulin resistance, brain pathology, and cognitive impairment in the animal models of AD, PD, and VD as suggested, but not proven, in previous studies.

Conclusions: The insulin resistance syndrome may promote development of several neurodegenerative dementias (AD, PD, and VD) by fostering development of brain insulin resistance. This can be tested in blood samples from such cases, as well as in blood and brain samples from rodent models of the insulin resistance syndrome, AD, PD, and VD. By reducing systemic and brain insulin resistance, incretin receptor agonists may prove to be effective therapeutics for NDGDs in general.

Changes of VEGF and NPY expression in rat trigeminal ganglion induced by diabetes

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Key words: trigeminal neuropathy, diabetic neuropathy, VEGF, NPY

Introduction: Peripheral diabetic neuropathy (DPN) is common early complication of diabetes mellitus (DM), characterized by structural alterations in the peripheral nervous system. It influences the trigeminal nerve function by changing the pain response and transduction of the orofacial sensory pathways. NPY is a neuropeptide that modulates inflammation and pain during nerve injury. Increased expression of NPY was observed in sensory ganglia in different neuropathic models. Although known as an angiogenic factor, VEGF also plays a crucial role in the neurogenesis, axonal outgrowth and restoration of nerve function in animal models of ischemic and DPN. The aim of the study was to investigate expression of NPY and VEGF in neurons of trigeminal ganglion (TG) in experimental models of type I (DM1) and II of DM (DM2).

Methods: DM1 was induced with intraperitoneal (i.p.) application of 55 mg/kg of the streptozotocin (STZ) in citrate buffer (pH 4.5). DM2 rats were fed with the high fat diet and received i.p. 35 mg/kg of STZ after the beginning of the treatment. Rats were sacrificed two weeks or two months after DM induction. TG ganglia were harvested and embedded in paraffin. Five μm sections were double stained for VEGF-IB4 or NPY-IB4 and percentage of different neuronal populations were quantified.

Results: Significant increase of percentage of NPY-immunoreactive neurons was observed in DM1 and DM2 rats, two weeks, as well as two months after induction. Expression of VEGF in TG increased after two weeks and then decreased until two months after induction of DM1 or DM2. Observed changes were not specific for particular neuronal subpopulations.

Conclusion: The obtained results provide better insight in pathophysiology of diabetic TG neuropathy. Modification of VEGF and NPY could be toll for prevention and treatment of diabetic trigeminal neuropathy.

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Acknowledgements: The study was approved by the Ethical Committee of the University of Split, School of Medicine and was performed according to the European Union Directive (2010/63/EU)-approval for the HRZZ; grant no. 02.05./28.

The quantitative assessment of corneal nerve structure in an animal model of type 2 diabetes mellitus

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Key words: diabetes mellitus, diabetic neuropathy, cornea, image processing

Purpose: The cornea is one of the most densely innervated tissues of the body; therefore, it may enable the non-invasive assessment and follow-up of systemic neurodegenerative diseases. Our purpose was to visualize nerves below the level of resolution of confocal scanning microscopy (0,5 µm) in an animal model of type 2 dibetes mellitus.

Methods: Type-2 diabetic 'Zucker Diabetic Fatty' (ZDF, n=4) and non-diabetic ZDF Lean control (n=4) rats were examined and euthanized at the age of 32 weeks. After harvesting the corneas, the specimens were fixed in 4% paraformaldehyde solution. Whole-mount preparations were immunohystochemically stained with anti PGP 9.5 and photographed using confocal microscopy (Zeiss LSM 780). 10 central, 4 periferal and 4 intermediate subbasal and 12 stromal stacks were taken of each cornea. The subbasal and stromal nerves were segmented using open source software (Image J and Neuron J). For the comparison of the control (C) and diabetic (DM) groups unpaired t-test was used.

Results: Altogether 2698 images were compared in 156 stacks. Integrated density and raw integrated density showed singnificant difference in the central and indermediate zones of the subbasal plexus between the two groups (central mean±SEM: C: 6,02±0,18 vs DM: 16,66±1,21 and C: 28,43±0,85 vs. DM: 41,16±1,08; intermediate: C: 9,23±0,36 vs. DM: 14,59±1,8, C: 43,61±1,7 vs. DM: 38,47±1,7). No significant difference was found between the examined parameters (total length, integrated density, raw integrated density) in the stroma.

Conclusion: Corneal nerve structures can be visualized and quantitatively analyzed by our methodology. The examination of the subbasal nerves in the central and intermediate zones could be used in our further trials, however, the increase of the subbasal nerual density in diabetic rats needs to be explained. After validating on a larger sample, our methodology might provide an alternative to investigate surrogate markers of diabetic neuropathy in laboratory research.

Source(s) of research support: OTKA PD100245

Acknowledgements: The animal research study was approved by the local Ethical Committee Number: 22.1/1162/3/2010

A New Model of Stroke in Type-1 Diabetic Rats

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Key words: Model, Cerebral ischemia, Transient, stroke, middle cerebral artery, diabetes, rats, female

Introduction: Aim of this study was to propose a new model and determine what duration of t-MCAO is optimal for stroke research in female diabetic SD rats and therefore offer unifying research protocols and easier comparability of results.

Materials and Methods: Type-1 diabetic Sprague–Dawley female rats (N=25), 12 weeks old, with high chronic hyperglycemia, were divided in 5 groups (N=5 in each group) and subjected to different duration of t-MCAO (20 min., 30 min., 45 min., 60 min. and 90 min.) and reperfusion, under LDF monitoring. 24 hours after reperfusion infarction volumes were evaluated by TTC staining and infarct volume analysis.

Results: Intra-ischemic reductions of CBF, were similar in all groups, ranging between 25 % and 32% of baseline values. Reperfusion was significantly impaired in 90 minute group compared to other groups (56-62% of baseline vs. 80 to 125 % of baseline in other groups).20 minutes t-MCAO did not induce brain infarction. 30 minutes ischemia produced significantly larger infarct (46.44±6.99%) that affected half of striatum and half of cortex area compared to 20min.45 and 60 minute ischemia resulted in significant spreading of infarction area to almost whole striatum (16.83±1.84% and 18.53±3.36% respectively), and significant portion of cortex (34.90±9.99% and 40.35±5.24%of one hemisphere). Ischemia of 90 minutes led to massive hemispheric stroke of 88.96±5.57% of hemisphere and whole striatum (22.21±3.22 %) and almost whole MCA irrigated cortex was infarcted (66.75±5.77%).

Discussion and Conclusion: Present study suggests that diabetic rat stroke model has to be different from non-diabetic. Our results demonstrated that female type-1 diabetic SD rats are highly sensitive to brain ischemia and it is necessary to significantly shorten duration of brain ischemia compared to non-diabetic rats. Optimal duration of t-MCAO seems to be 30 minutes, producing significant stroke useful in therapy options studies, but probably not definite, too large or fatal.

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Prenatal dietary load of Maillard reaction products combined with postnatal Coca-cola drinking affects metabolic status of female Wistar rats

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Key words: MRPs – Maillard reaction products, bread crusts, Coca-cola, offspring, female Wistar rats

Introduction: Diet rich in Maillard reaction products (MRPs) is presumed to impose increased risk of development of cardiometabolic afflictions, such as obesity or insulin. A present epidemics of such diseases has also been linked to the rising consumption of saccharose/fructose sweetened soft drinks.
Aim: To assess the impact of prenatal exposure to MRPs-rich diet on metabolic status of female offspring, and its further modification by Coca-cola consumption.
Methods: At the first day of pregnancy, female Wistar rats were randomized into 2 groups, pair-fed either by standard rat chow (MRP-), or MRPs-rich diet (MRP+). Offspring from each group of mothers were divided into 2 groups, and provided either water (cola-) or Coca-cola (cola+) for drinking *ad libitum* for 18 days. Oral glucose tolerance test was performed, circulating markers of inflammation, oxidative stress, glucose and lipid metabolism were assessed.
Results: Higher weight gain was observed in MRP+ groups, significant only in rats drinking water. Both prenatal and postnatal intervention increased carboxymethyllysine levels and semicarbazide-sensitive amine oxidase activity, significance reached between MRP-/cola- and MRP+/cola+. Total antioxidant capacity was lower in MRP groups, the effect significant in cola+ groups. Rats drinking Coca-cola had higher insulin, HOMA-IR, heart rate, advanced oxidation of protein products, triacylglycerols and oxidative stress markers measured as TBARS, with no visible effect of MRPs-rich diet.
Conclusion: Metabolic status of offspring was affected both by prenatal and postnatal dietary intervention. Our results suggest that combined effect of prenatal and postnatal dietary pattern may play a role in development of metabolic disorders in later life.

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Experiments were approved by the Institutional Animal Care and Use Committee (IACUC). The institutional ethical committee approval number**:** 025/2013/UPF, 6.6.2013

Let's check this one more time.. decision change frequency during recognition as a predictor of working memory capacity

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Key words: recognition memory, working memory capacity (WMC), eye movements

Aims: Working memory capacity (WMC) is an important cognitive trait predicting higher order abilities, decision making or fluid intelligence, among others, as well as a cognitive decline related to aging or psychiatric diseases (e.g. schizophrenia). It is usually assessed with long, computerized battery of tests (i. e. different so called "span tasks"). Here we present a simple, noninvasive and non-verbal way of measuring ones WMC efficiency.

Methods: Forty participants (20 females) took part in the experiment (20-29 year old). They performed the gaze-contingency new/old recognition memory paradigm . Each participant's short-term and working memory was also examined by the Operational span (OSPAN) task and a modified Wechsler's Digit span tests (forward and backward).
High working memory scores correlated with the performance of new/old recognition paradigm but, what was of special interest here, we found that WMC was also, and much stronger, related to a number of decision changes (operationalized as a number of saccades between options "new" and "old" before final answer) performed during recognition phase. The higher WMC the lower the number of decision changes (r=0.36*).

Results: The results clearly show that people with different scores in WM tests are characterized by different pattern of eye movements during recognition. Our results suggest that there is a possibility of assessing someone's WMC by analyzing pattern of eye movements during performing a simple memory task.

Discussion – Conclusion: It could offer a new way of cognitive functioning assessment which can be of special interest when the measurement errors associated with both explicit (e.g. participant's intention to cheat) or implicit (one's perceptual speed or muscle strength) factors has to be limited.

Acknowledgement: University of Social Sciences and Humanities Ethical Committee has reviewed and accepted experimental protocol: 01.26.2015 (2/2015).

Nociceptin and nocistatin in the myometrium: relaxing effect of two neuropeptides

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Key words: nociceptin, nocistatin, pregnant myometrium, rat, human, preterm, calcitonin-gene related peptide, calcium-dependent potassium channels, cyclic adenosine monophosphate

Introduction: The effects of neuropeptides nociceptin (N/OFQ) and nocistatin (NST) in the central nervous system have been described, but less data are available on their peripheral functions including uterine smooth muscle.

Methods: The myometrial contractility was measured by isolated organ bath. The mRNA and protein expressions were detected by RT-PCR and Western blot technique, respectively. The myometrial cAMP accumulation was measured by enzyme immunoassay kit. The plasma levels prepronociceptin and NST were determined by radioimmunoassay.

Results: The level of their precursor prepronociceptin is elevated in the preterm human myometrium as compared with full-term samples, whereas it gradually increases toward term in the pregnant rat uterus. Both N/OFQ and NST inhibit myometrial contractions, an effect which can be enhanced by naloxone and blocked by Ca^{2+}-dependent K^+ channel (BK_{Ca}) inhibitors. Both compounds increase the myometrial cAMP level which may be responsible for the activation of this channel and subsequent intracellular hyperpolarization. NST releases calcitonin gene-related peptide from the sensory nerve ends, which explains its cAMP-elevating effect.

Discussion and Conclusion: In contrast with the nervous system, where they behave as antagonists, N/OFQ and NST are able to potentiate the uterine-relaxing effect of each other in both rat and human tissues. Further studies are required to clarify the roles of N/OFQ and NST in the regulation of the myometrial contractions and the perception of pain during delivery.

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All experiments were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: IV/198/2013). The collection of human samples was approved by The Ethical Committee of Albert Szent-Györgyi Clinical Center, University of Szeged ((registration number: 114/2009).

$GABA_B$ receptor positive allosteric modulator rac-BHFF: Influence of on the extracellular level, uptake/release of [^{3}H]GABA, Em and synaptic vesicle acidification in cortical and hyppocampal presynaptic nerve terminals

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Keywords: $GABA_B$ receptors, GABA transporters, exocytosis, nerve terminals, rat brain cortex, hippocampus, thalamus

Introduction: Positive allosteric modulators of $GABA_B$ receptors have great therapeutic potential for medications of anxiety, depression, etc.
Methods: preparative biochemistry, radiolabel assay. 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 # 1 from 19/09-2012). 40 animals were used in the study.
Results and discussion: The effects of recently discovered positive allosteric modulator of $GABA_B$ receptors rac-BHFF on the key characteristics of GABAergic neurotransmission were investigated in cortical and hypocampal presynaptic nerve terminals (synaptosomes). The ambient level of [^{3}H]GABA that is a balance between release and uptake of the neurotransmitter increased in the presence of rac-BHFF (at concentrations more than 10 μM). The initial velocity of synaptosomal [^{3}H]GABA uptake was suppressed by the modulator. In the presence of GABA transporter blocker NO-711, it was shown that rac-BHFF increased tonic release of [^{3}H]GABA from synaptosomes. Rac-BHFF within the concentration range of 0.3 – 10 μM did not enhance inhibiting effect of baclofen on depolarization-induced exocytotic release of [^{3}H]GABA. Above effects were not related to acting of rac-BHFF on presynaptic $GABA_B$ receptors or specific inhibition of GABA transporters but associated with ability of rac-BHFF (0.3-30 μM) to cause dose-dependent depolarization of the plasma membrane and dissipation of the proton gradient of synaptic vesicles in synaptosomes that was shown in the presence of $GABA_B$ receptor antagonist saclofen using fluorescent dyes rhodamine 6G and acridine orange, respectively.
Conclusion: Therefore, drug development strategy of positive allosteric modulation of $GABA_B$ receptors is to eliminate these side effects of rac-BHFF in presynapse, and *vice versa*, these new properties of rac-BHFF may be exploited appropriately in other way.

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Abstracts

April 17, 2015

Plenary Session #2

Cardiovascular Diseases

Shared decision making in life style and nutrition for intervention in women with risk factors in cardiovascular health

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Key words: cardiovascular health, risk factors, life style, nutrition, women.

Introduction: Cardiovascular diseases (CVD) are the leading cause of death for women in industrialized countries. The aim of our study was to determine the impact of physicians' interventions on women's decisional conflict surrounding lifestyle changes necessary to minimize CVD risks.

Methods: This prospective, interventional study included 102 women of different reproductive status (3) and one or more of cardiovascular risk factors: obesity, high cholesterol levels, tobacco exposure, and high blood pressure. Our educational intervention included a lecture on life styles and diets associated with CVD, as well as individually tailored decisional aids and calculated 10-year CVD risk (4). Immediately before and after the educational intervention, we measured decisional conflict (DC) (5), state hope (IHS), eating habits (EH) (6) and participants knowledge of CVD. After a three-month period, we re-examined their cardiovascular risk factors, measured DC, HIS, EH and calculated their 10-year CVD risk.

Results: Immediately following the intervention, decisional conflict about lifestyle changes was considerably reduced (Md=33, 95% CI 30-36 vs. Md=25, 95% CI 20-25) and remained so even three months following the intervention (Md=27, 95% CI 25-30, $P<0.001$). 65 (64%) women reduced their CVD risk (median reduction Md= 0.14, 95% CI 0.1-0.5) and weight (median loss 1 kg, 95% CI 0-1). Women were extremely satisfied with the support they received from their family physician (M=8.9 95% CI 8.5-9.3).
Discussion: Detection, treatment and life style interventions are meant to reduce major risk factors and instances of cardiovascular diseases. Our individually-tailored intervention lowered the decisional conflict of women on their necessary lifestyle changes as well as their CVD risks.

Conclusion: Our study demonstrates that a single educational intervention conducted by family physician can lead to significant life changes, primarily through weight reduction and decrease in the conflict which women face when attempting to change their life style and habits.

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We also thank Dr. Ian Pitha, MD, PhD, Head of Laboratory for Atherosclerosis Research, Centre for Experimental Research, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic for the critical review of our research plan.

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Estimation of central obesity in Slovak adults: comparison of waist circumference vs. waist-to-height ratio

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Key words: central obesity, waist circumference, height-to-weight ratio, index of central obesity, prevalence

Introduction: Visceral adipose tissue accumulation is an independent risk factor for the development of obesity-associated comorbidities. Professional associations recommend estimating central obesity (CO) measuring waist circumference (WC) employing gender and ethnicity-specific cut-points. Recent attitudes advocate correction of WC for height, as WC might underestimate CO in short, and overestimate in tall subjects. We studied the degree of concordance/discrepancy of CO estimation using WC vs. waist-to-height ratio (index of central obesity, ICO) in Slovak adults.

Methods: Data from 18-63 years-old males (n=2137) and females (n=2531) were analyzed. CO was classified either as WC≥94cm (males) and ≥80cm (females), or as ICO>0.50 (both genders). Discrepancy of the results was analyzed according to SD of height, or decades of age.

Results: 34% of males presented CO according to WC; 46% according to ICO. Males' height averaged 179±7cm (mean ± SD). In short subjects CO was underrated by WC in comparison with ICO (height ≤-2SD in 35%; -1SD and average height up to 19%); in tall subjects WC overrated CO (≥2SD up to 12%). Females: Employing WC 34%, according to ICO 28% were classified as CO. Height averaged 166±6cm. WC underrated CO in short females (≤-2 SD) up to 16%, and overrated CO in all other SD subgroups by 4%-to-26%. WC and ICO correlated significantly with age in both genders. Prevalence of discrepancy between WC and ICO classification did not differ significantly in age-decades groups in either gender.

Discussion: WC classifies similar proportion of Slovak males and females as centrally obese, while employing ICO less females than males present CO. Interestingly, in Slovaks discrepancy between WC and ICO in estimation of CO does not follow the Gaussian distribution of height, differing profoundly among males and females.

Conclusion: Further studies in different countries are definitely needed to clarify the degree of discordance between the mentioned methods.

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Gender differences in morphological and functional characterization of athlete's heart in a rat model

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Key words: cardiac hypertrophy, gender differences, pressure-volume analysis

Introduction: Long-term exercise training is associated with characteristic morphological and functional myocardial changes, termed athlete's heart. Referring to the latest studies, sex hormones may be involved in the regulation of exercise-induced left ventricular (LV) hypertrophy. We aimed at understanding the gender-specific changes in the heart and the underlying signaling pathways following a training period in a rat model.

Methods: The animal male and female rat's groups were divided into control and swimmer groups. Athlete's heart was induced by swim training. Swimmer groups swam 200 min/day for 12 weeks, while control rats swam 5 min/day. Following the training period we assessed LV hypertrophy with echocardiography. LV pressure-volume (P-V) analysis was performed to investigate in vivo cardiac function. Finally, molecular biological studies (qRT-PCR, Western blot) were performed.

Results: Echocardiography showed LV hypertrophy which was confirmed by LV wall thickness and mass values, nevertheless it was more pronounced in females. Heart weight/tibial length ratio also verified gender differences in LV hypertrophy. The induction of Akt signaling was more significant in females that could led to the more pronounced cardiac hypertrophy. There is also a characteristic difference in the mitogen-activated protein kinase (MAPK) pathway. The α-myosin heavy chain (MHC)/β-MHC ratio did not differ in males, but increased significantly in females.

Discussion: Despite the more significant hypertrophy in females, characteristic functional parameters of athletes' heart did not show notable differences between the genders. P-V analysis showed improved contractility, mechanoenergetics and unaltered LV stiffness in both males and females.

Conclusion: The results confirm that there is a greater exercise-induced hypertrophy in females which has no functional consequence compared to the males. The gender-specific response of the LV to exercise is modulated by characteristic molecular pathways.

Acknowledgement: All procedures and handling of the animals during the study were reviewed and approved by the Ethical Committee of Hungary for Animal Experimentation, approval # 22.1/1162/3/2010, 06.22.2010.

Macrophage phenotype in the adipose tissue of postmenopausal women

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Key words: atherosclerosis – adipose tissue – inflammation – macrophages

Introduction: Atherosclerosis and its clinical complications are still the most common cause of death in developed countries. In addition to hypercholesterolemia, atherosclerosis is driven by inflammatory cells, mainly by macrophages. In our project we studied phenotypes of macrophages connected with proinflammatory status in adipose tissue of healthy living kidney donors.
Methods: Anthropometric characteristics were collected from all subjects. Samples of 2,5 grams of subcutaneous, perirenal and perivascular adipose tissue of kidney donors were exposed to collagenase and repeatedly filtered and stroma vascular fraction (SVF) was eluted from the sample. SVF was then labelled with monoclonal antibodies conjugated with fluorochromes (CD14, CD16, CD36, CD163, CD68 and calprotectin) and subsequently analyzed by flow cytometry. The same surface markers were determined in blood samples of the subjects.
Result: Blood monocytes did not express calprotectin and only a minor population expressed CD16 whereas high expression of calprotectin and CD16 in adipose tissue macrophages of living kidney donors was found. Only in the subcutaneous adipose tissue we observed a positive correlation ($p<0.05$) of number of CD14+ monocytes and CD14+calprotectin+monocytes with body mass index. Two groups of female kidney donors were distinguished: the premenopausal and postmenopausal group. The postmenopausal women had a higher number of CD14+CD16+ macrophages than the premenopausal group in perirenal ($p<0.04$) and perivascular ($p<0.05$) adipose tissue. Similarly the postmenopausal women had higher number of CD14+CD16+ macrophages than men in perirenal ($p<0.05$) and perivascular ($p<0.04$) adipose tissue.
Conclusion: We demonstrated a positive correlation of BMI with number of CD14+ monocytes and CD14+calprotectin+ monocytes in subcutaneous adipose tissue of kidney donors. We observed significant differences in numbers of CD14+CD16+ macrophages in premenopausal and postmenopausal women and men. The higher number of CD14+CD16+ macrophages may be related to proatherogenic role of this subpopulation of macrophages in postmenopausal women.

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Ethical Committee Approval: date: 27.6.2012, number: 1041/12 (G 12-06-11)

Characterization of right ventricular function using 3D and speckle tracking echocardiography in patients after heart transplantation

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Keywords: heart transplantation, right ventricle, echocardiography, speckle tracking, strain

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Introduction: Reduced right ventricular (RV) function is a common finding in patients underwent heart transplantation (HTX). However, certain limitations apply regarding conventional echocardiographic measures of RV function. We were aimed at investigate RV function using three-dimensional (3D) and speckle tracking echocardiography (STE) and correlate them with standard parameters in patients after HTX.

Methods: Thirty patients after HTX were enrolled (mean age 54±14 years, 15 patients within one year, 15 over one year after HTX). Beyond the measurement of tricuspid annular plane systolic excursion (TAPSE), we acquired 3D datasets from apical view using multi-beat reconstruction from 4 or 6 cardiac cycles (Philips EPIQ 7G, X5-1 transducer). Using a dedicated software for RV quantification (TomTec 4D RV-Function 2), RV end-diastolic (EDV), end-systolic volumes, ejection fraction (EF) were measured and furthermore, free wall longitudinal strain were quantified using STE.

Results: EDV remained in normal range in our cohort (87±22 mL). Average value of TAPSE was 15±4 mm, which indicated significantly depressed RV longitudinal function. However, EF was found maintained or just mildly decreased (45±7%). There was no correlation between TAPSE and 3D derived EF. Free wall longitudinal strain correlated with EF ($r=0.39$, $p<0.05$). Patients over one year after HTX had better TAPSE (17±4 vs. 14±4 mm in patients within one year, $p<0.05$). EF did not differ between the two groups (43±6 vs. 46±7%). TAPSE correlated with the time elapsed after HTX ($r=0.62$, $p<0.01$).

Discussion and Conclusion: The widely used TAPSE is not a reliable measure of RV systolic function in patients underwent heart transplantation. Free wall strain describing longitudinal shortening provides a better estimate. However, the maintained EF suggests a relevant radial component in RV function. In time, longitudinal function can recover. 3D and STE have a remarkable value in the assessment of RV function in patients underwent HTX.

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Semmelweis University, Institutional Committee for Science and Research TUKEB 175/2012

Sex-specific chronic stress response at the level of adrenal gland modified sexual hormones and leptin receptors

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Key words*:* adrenal gland, chronic stress, sexual hormones receptors, leptin receptor

Aim To compare cardiometabolic risk-related biochemical markers, sexual hormone and leptin receptors in the adrenal gland of rat males, non-ovariectomized females (NON-OVX), and ovariectomized females (OVX) under chronic stress.
Methods Forty six 16-week-old Sprague-Dawley rats were divided into male, NON-OVX, and OVX group and exposed to chronic stress or kept as controls. Weight, glucose tolerance test (GTT), plasma concentration of glucose, and cholesterol were measured. Adrenal glands were collected at the age of 28 weeks and immunohistochemical staining against estrogen beta (ERβ), progesterone (PR), testosterone (AR), and leptin (Ob-R) receptors was performed.
Results Body weight, GTT, serum cholesterol and glucose changed in response to stress as expected and validated the applied stress protocol. Males had significantly higher number of ERβ receptors in comparison to control group (P=0.028). NON-OVX group had significantly decreased AR in comparison to control group (P=0.007). The levels of PR did not change in any consistent pattern. The levels of Ob-R increased upon stress in all groups, but the significant difference was reached only in the case of OVX group compared to control OVX group (P=0.033).
Conclusion Chronic stress response was sex specific. OVX females had similar biochemical parameters as males. Changes upon chronic stress in adrenal gland were related to an increase in testosteron receptor in females and decrease in estrogen receptor in males.

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Ethical approval was received from local ethics committee of Faculty of Medicine Osijek, J. J. Strossmayer University of Osijek and Croatian Ministry of Agriculture. September 4th 2013. Enlisted Under: 602-04/11-08/09.

Effect of resveratrol on caspase 3 activation in primary mouse fibroblasts

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Introduction: Resveratrol is a flavonoid compound isolated from the skin of red grape. Its effect on the apoptosis is contradictory in the literature, as both pro- and antiapoptotic effect were described. The cell type used can be one of the major differences between the various models and the compound might differentially affect tumorigenic and non-transformed normal cells.

Aims: We aimed to study the effect of resveratrol on survival and caspase 3 activation in non-transformed cells after serum deprivation.
Methods: In this study primary mouse embryonic fibroblasts were used. Apoptosis was induced by serum deprivation. Caspase 3 activation, lactate dehydrogenase release as cell viability measure were assayed by fluorescent methods. The involvement of various signaling pathways was also examined.

Results: Serum deprivation of primary fibroblasts induced significant activation of caspase 3 within 3 hours and reduced cell viability after 24 hours. Resveratrol dose dependently prevented caspase activation and improved cell viability with IC50 value of about 50-100 μM. It was also capable of reducing the already upregulated caspase 3 activity suggesting its rescue effect. Among the major signaling pathways p38 kinase was found to be critical in the protective effect of resveratrol.

Conclusion: Our results show that resveratrol exerts dose-dependent protective effect on non-transformed primary fibroblasts. It prevents caspase 3 activation *via* p38 kinase dependent pathway suggesting the role of mild stress in its effect. Furthermore, due to its rescue effect it may be capable of not only preventing, but treating of aging-related degenerative diseases.

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All animal procedures were approved by the ethical committee of the Semmelweis University (22.1/606/001/2010, February 5, 2010) and were in accordance with the EU Council directives on laboratory animals (86/609/EEC).

Relationship of semicarbazide sensitive amine oxidase/vascular adhesion protein-1 and insulin resistance in lean vs centrally obese male students

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Key words: semicarbazide sensitive amine oxidase, soluble vascular adhesion protein-1, insulin resistance, central obesity

Introduction: Semicarbazide sensitive amine oxidase (SSAO) is heterogeneous enzyme, which breaks down amines, resulting in the production of H_2O_2 and (among others) methylglyoxal. Enhanced oxidative stress alters the effects of insulin and glucose transport. Methylglyoxal might induce insulin resistance (IR) as well. SSAO is functionally identical with vascular adhesion protein-1 (VAP-1). Soluble VAP-1 levels are elevated in hyperglycemia, diabetes, and are linked to cardiovascular complications, and glucose homeostasis.
We asked whether alteration in SSAO/sVAP-1 system occurs already in early stages of IR, with regard to presence/absence of central obesity (CO).
Methods: In a cross-sectional study apparently healthy Caucasian non-diabetic males (n=127) aged 17-to-20-years were classified into 4 groups according to CO and IR as follows: lean insulin sensitive (LIS) n=33, lean IR (LIR) n=31, CO insulin sensitive (OIS) n=32, CO-IR (OIR) n=31. CO was defined as waist-to-height ratio>0.5, IR by QUICKI <0.319. The radiometric method was used to determine enzyme activity of SSAO. Levels of sVAP-1 were determined using the commercial ELISA.
Results: We did not ascertain differences either in the activity of plasma SSAO (LIS=61±18, LIR=59±21, OIS=61±16, OIR=60±14 pmol/mg/h), or the levels of sVAP-1 between the mentioned groups. Plasma enzyme activity of SSAO directly and significantly correlated with the levels of circulating sVAP-1 ($p<0.001$, $R^2=0.33$).
Discussion: CO, IR and their interaction are not associated with changes in enzyme activity of SSAO or levels of sVAP-1 in adolescent males. Increased SSAO/sVAP-1 probably occurs under hyperglycemia, or in the presence of diabetes. Evidence of positive association between SSAO and sVAP-1 is important especially from methodological point of view, as it implies that methods of determination SSAO/sVAP-1 could substitute one another.
Conclusion: In non-diabetic adolescent males presence of cardiovascular risk factors such as CO, IR or their concurrent manifestation is not associated with altered activity of SSAO or circulating sVAP-1 levels.

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Acknowledgement: The study was supported by Cedars Sinai Medical Center's International Research and Innovation Management 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).

Abstracts

April 17, 2015

Poster Session #2

Nanomedicine and Medical Imaging

Assessment of Immunotoxic effects of TiO_2 Nanoparticles

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Key words: immunotoxicity, nanoparticles, titanium dioxide

Introduction: Development in nanotechnology provides promising source of beneficial materials for common use and application in many industries including nanomedicine. Therefore, the hazard assessment to ensure safety of newly engineered nanoparticles (NPs) is important. Significant part of toxicity testing is assessment of the effect of NPs on the immune response. Titanium dioxide (TiO_2) is one of the most used NP for example in foods, medicines, cosmetics, paints, plastics and papers.
Methods: Human peripheral blood cultures (n=8-10) were treated with TiO_2 NPs (of nominal size 21nm, size in dispersion RPMI medium, 10% FCS: 102±15/285±67nm) in three different concentrations: 0.12, 3 and 75μg/cm^2 and four time intervals: 72h, 48h, 24h and 4h. Lymphocyte transformation assay was used to assess the effect of NPs on lymphocyte function. Phagocytic activity and respiratory burst of leukocytes and natural killer cell activity were measured using flow cytometry. Electron microscopy imaging of thin-sections of peripheral blood mononuclear cells was used to examine possible cell damage caused by NPs.
Results: No cytotoxic effect of selected doses of TiO_2 NPs was observed when measured as basal proliferative response of peripheral blood cells. Significant stimulatory effect of high dose of TiO_2 NPs was mostly pronounced in cell cultures exposed to NPs for last 4h of 72h cultivation period. Four hours exposure to high dose TiO_2 NPs significantly stimulated T-cell and T-dependent B-cell response in presence of phytohaemmagglutinin, concanavalin A and pokeweed mitogen. On the other hand, proliferative response of T-lymphocytes through the T-cell receptor displayed suppression without clear dose-dependence. Phagocytic activity and respiratory burst of granulocytes were significantly stimulated when cells exposed 24h to NPs. Natural killer cell activity was enhanced also in cultures stimulated with TiO_2 NPs for 24h.
Discussion and conclusions: Our findings indicate different response of B- and T-lymphocytes, phagocytic cells and natural killer cells to TiO_2 NPs.

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We thank Helena Nagyova and Edita Mrvikova for their excellent technical help and Dagmar Bilanicova, Giulio Pojana, Antonio Marcomini (Venice University, Italy) for NP characterization.

Cytotoxicity of maghemite core/polyaniline shell nanoparticles

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Keywords: Maghemite, nanoparticles, core–shell, polyaniline, cytotoxicity

Introduction: Superparamagnetic iron oxide nanoparticles (NPs) found a wide range of applications in medicine and biotechnology due to their magnetic properties. Polyaniline is an important conducting polymer. Purpose of this research was to synthesize new hybrid polyaniline-coated magnetic NPs and to investigate their electrical and magnetic properties and cytotoxicity on the human neuroblastoma cell line SH-SY5Y.

Methods: Maghemite (γ-Fe_2O_3) NPs were prepared by the coprecipitation of Fe^{2+} and Fe^{3+} salts with ammonium hydroxide which was followed by controlled oxidation with NaOCl. The polyaniline (PANI) shell was obtained by polymerization of aniline hydrochloride with ammonium peroxydisulfate in aqueous solution of poly(*N*-vinylpyrrolidone) in the presence of γ-Fe_2O_3 NPs. The resulting γ-Fe_2O_3@PANI NPs were characterized by scanning and transmission electron microscopy, dynamic light scattering, FTIR spectroscopy and elemental analysis. Cytotoxicity was determined by MTT assay.

Results and Discussion: The number-average diameter of the starting γ-Fe_2O_3 NPs was 9 nm. After the modification with PANI, the size increased to 200 nm; particle size distribution was moderately broad. The NPs formed a stable aqueous colloid. Electrical conductivity of prepared composite films was $\sim 10^{-4}$–10^{-5} S cm^{-1}. In the biological experiments, toxicity was determined using the SH-SY5Y cells incubated in DMEM medium with the γ-Fe_2O_3@PANI NPs (up to 12.5 μg/ml) at 37 ºC for 24 and 72 h. The results of thorough characterization of the hybrid particles will be presented on the conference.

Conclusions: New conducting and magnetic γ-Fe_2O_3@PANI core/shell nanoparticles have been successfully synthesized and proved to be non-toxic under investigated conditions. Their biomedical applications, in particular, in biosensors are in progress.

Acknowledgement: Support of the Ministry of Education, Youth and Sports of the Czech Republic (LH14318) and RECOOP HST Association and Cedars Sinai Medical Center is acknowledged.

Improvement of biocompatibility in laboratory rats of synthetic 4-thiazolidinone derivatives by complexing these drugs with polymeric nanocarrier

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Key words: 4-thiazolidinone derivatives, polymeric nanocarrier, toxicity, biocompatibility

Introduction. Enzymatic markers and the content of other indicators of cardio-, hepato- and nephrotoxicity were measured in blood serum of rats treated with novel 4-thiazolidinone derivatives – 3882, 3288 and 3833, and doxorubicin used as positive control. The effects of free forms of these drugs and of their complexes with new PEG-containing polymeric comb-like nanocarrier (VEP-GMA-PEG) were compared.
Methods and Materials. The substances were injected daily intraperitoneally. The experiments lasted for 10 days for rats treated with doxorubicin, and 20 days for rats treated with synthetic substances. The following biochemical indicators of general toxicity were studied: aspartate aminotransferase, alanine aminotransferase, α-amylase, gamma-glutamyltransferase, alkaline phosphatase, creatine kinase, and lactate dehydrogenase activity in rat's blood serum. Besides, concentration of total protein, urea, creatinine, glucose, cholesterol, triglycerides, calcium, sodium, chlorides, and iron ions was measured.
Results and Discussion. Normalization of activity of the enzymatic markers was revealed at application of complexes of doxorubicin and 4-thiazolidinone derivatives with novel nanocarrier, compared with the elevated activity of studied enzymes at the action of free forms of drugs. This suggests a decrease in cardio-, hepato- and nephrotoxicity of drugs immobilized on the nanocarrier. Other studied indicators were less informative.
Conclusion. The obtained data, as well as results on enhancement of antineoplastic action of 3882, 3288 and 3833 compounds and doxorubicin after their immobilization on the nanocarrier and data on their biocompatibility suggest perspectives of nanocarrier application for drug delivery.

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

BioEthics Committee Approval. All animal experiments were conducted keeping the European Convention on Protection of Vertebrate Animals (Strasbourg, 1986) and corresponding Law of Ukraine (N944, 14.12.2009). Structure of this study and experimental procedures were approved by Ethical Committee of Lviv National Medical University (N2, 17.02.2010).

Potentials of C60 Fullerene as a biocompatible platform for drug delivery: in vitro and in vivo studies

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Keywords: C_{60} Fullerene, Doxorubicin, Drug Complexes, Apoptosis, Treatment *in vitro* and *in vivo.*

Introduction: Development of novel nanocarriers for effective drug delivery to the molecular targets in tumor cells is an actual problem in modern pharmaceutical chemistry. The main goal of this study was to investigate if pristine C_{60} fullerenes can an effective drug delivery platform for well-known anticancer drug doxorubicin (Dox) for its further use in clinical practice.

Materials and methods: DCFDA/DHE assays for measurement of specific ROS production, JC-1 and Rhodamine-123 assays for study of mitochondrial depolarization, annexin V/PI and DAPI staining for analysis of apoptosis under action of C60-Dox complexes on various tumor cell lines were applied. Animal study was carried out on 28 male mice of C57Bl/6J line bearing Lewis lung carcinoma, divided into 4 groups. The injections of Dox, C_{60} fullerene or C_{60}+Dox complex were started on a 2nd day after tumor transplantation. The growth kinetics of Lewis lung carcinoma in mice was characterized by changing its size from the 10th till the 22nd day. An average life span of animals was estimated in all experimental groups.

Results: Dox conjugation with C_{60} fullerene led to 1.5-2-fold increase in Dox toxicity towards various human tumor cell lines, compared with such effect of free drug. Cytomorphological studies demonstrated that C_{60}+Dox complexes killed tumor cells by apoptosis induction. The effect of such complex towards tumor-bearing mice was even more pronounced than that in the *in vitro* experiment, leading to 2,5-fold decrease of tumor volume and 63% increase of average life span of treated animals compared with control.

Conclusion: Novel drug delivery systems based on C60 fullerene were synthesized and demonstrated high activity and specificity of action both in vitro and in vivo, suggesting great promise of their application for chemotherapy of malignant tumors. It is also planned to immobilize 4-thiazolidinone derivatives synthesized at the RECOOP member (DHLNMU)

on the C60 fullerene and thus use it as a platform for delivery of these novel drugs to tumor and immune cells.

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Acknowledgement: Experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Institute of Cell Biology NASU: 01.09.2014, №1

Ligand attraction with PEG-monooleate as efficient method of inorganic nanocrystals transfer to water

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Keywords: nanocrystals, ligand attraction, PEG-monooleate, cytotoxicity

Introduction: In the past two decades quantum dots (QDs) have gained tremendous attention due to their potential for use in variety of applications, ranging from cellular imaging to electronic devices. High-quality QDs are desirable for biological detection because of their unique optical features. Nevertheless, the utility of QDs is restricted by their potential cytotoxicity and by the necessity of ultraviolet and visible light excitation, which usually causes the autofluorescence of the biological sample and signal interference. To address the aforementioned issues, a new class of lanthanide-doped nanomaterials has emerged. Termed as upconversion nanoparticles they have unique advantages such as the lack of autofluorescence, low cytotoxicity, long emission decay and deeper light penetration in the so-called therapeutic window. However, the utility of nanoparticles in biological applications requires that they are fully dispersible in aqueous solutions. Therefore, we conducted a surface modification of $NaGdF_4$:Yb^{3+},Er^{3+} and CdSe/CdS QDs by ligand attraction method.

Methods: Transfer to water was obtained by ligand attraction in which amphiphilic ligand (PEG-monooleate) uses the Van der Waals interactions to create the bilayer formation with original ligand on the surface of nanocrystals. The structural properties were characterized by transmission electron microscopy (TEM). The luminescent properties were investigated by photoluminescence and photoluminescence decay measurements.

Results and Discussion: The procedure of nanocrystals transfer to water was successful. Unlike for other methods, ligand attraction allows to conserve optical properties of nanocrystals. Functionalized upconversion nanoparticles were stable in water longer than QDs. However, the upconversion nanoparticles showed instability once exposed to cells, which resulted in significant cytotoxicity. It may be caused by the dissociation of the amphiphilic ligands of the formed bilayer.

Conclusion: Despite the successful transfer to water the as-prepared nanocrystals cannot be used at this stage in bioapplications due to their cytotoxicity. Improved stability may be achieved by cross-linking of amphiphilic ligands.

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Application of novel radiopaque ZrO_2-Gd_2O_3 nanocomposite functionalized with hyaluronic acid for repair of bone defects in experimental animals

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Keywords: regeneration of bone defects, rats, novel osteoplastic material, polymer-mineral nanocomposite, ZrO_2–Gd_2O_3 core, functionalization with hyaluronic acid.

Introduction: Creation of new materials for replacement of bone defects of various aetiology, the ways of improvement of their biocompatibility and osteogenous potential, as well as providing of osteoinduction, osteoconduction and radiopacity, are important tasks in surgical practice.
Aim: of this work was to use novel polymer-mineral composites based on nanoparticles of ZrO_2-Gd_2O_3 and functionalized with the hyaluronic acid for regeneration of bone defects in experiment and conducting radiological and histological study of the reparative osteogenesis.
Materials and methods: Animal study was carried out on 24 female rats aged 8-9 months with 300-350 g body weight. Artificial bone defects were formed in rat's caudal vertebra. The animals were divided into 4 groups each composing of 6 rats whose bone defects were treated with: 1) novel radiopaque ZrO_2-Gd_2O_3 nanocomposite with hyaluronic acid in its coating; 2) commercial synthetic material «Easy-Graft™» (DS Dental, Switzerland) prepared on the basis of beta-tri-calcium phosphate; 3) bone regeneration material «Stimulus-Oss» based on animal collagen with addition of 2% chlorohexidine bigluconate and hydroxyapatite; 4) control in which bone defect was sutured under the blood clot. Bone (vertebrae with the regenerate) material was collected for study in 30 days after surgical intervention. Vertebra macropreparations were investigated morphologically, roentgenologically, histologically, and histochemically.
Results: Application of new experimental model (regeneration of bone defects artificially formed in rat's caudal vertebra) proposed by the authors has demonstrated its advantages in the reparative osteogenesis. Among 3 different osteoplastic materials used in this study, novel radiopaque ZrO_2-Gd_2O_3 nanocomposite with hyaluronic acid in its coating showed the highest effectiveness in regeneration of the osseous tissue. This was confirmed by the morphological, as well as by histological and histochemical study of wound repair. New material has demonstrated bio-tolerance and high integration with the osseous tissue. Due to its radiopaque core, the developed ZrO_2–Gd_2O_3 nanocomposite can be used for monitoring of treatment course, since it can be easily detected by X-ray method. The obtained results are the basis for new studies with perspectives of their implementation in clinical periodontal practice, maxillofacial surgery, and implantology.
Conclusion: Novel polymer-mineral nanocomposite with ZrO_2–Gd_2O_3 core functionalized with hyaluronic acid is effective in regeneration of bone defects, demonstrates high integration in osseous tissue and bio-tolerance. Its application provides a possibility for monitoring the process of treatment of bone damages since novel nanocomposite can be easily detected at X-ray study.
Ethical Committee Approval was received under No.7/2012-09-24 from BioEthics Committee of Danylo Halytskyi Lviv National Medical University (Lviv, Ukraine).

Abstracts

April 17, 2015
Breakaway Sessions

Fetal Sex Disparities in Vitamin D levels of Smokers and Non Smokers

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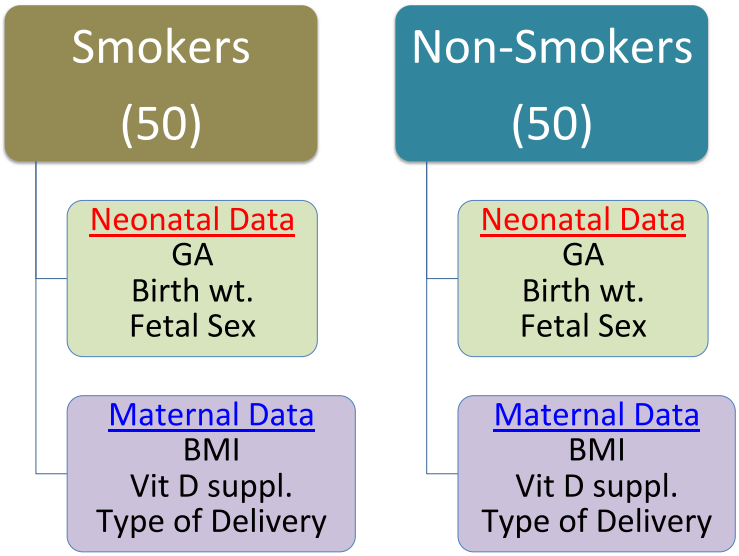
Objective: The study aims at following two objectives:
1. To assess the influence of smoking on vitamin D levels in pregnant women at the time of delivery.
2. To evaluate fetal sex disparities in vitamin D levels of smokers and non-smokers pregnant women.

Background: Smoking is embedded in Central and Eastern European lifestyle. Smokers are lean and have reduced levels of circulating estrogens due to an increased hepatic turnover. Maternal vitamin D status early in pregnancy was associated with risk of low birth weight and small-for-gestational age infants in one study, whereas another study found this relation only among white women. Polymorphisms in the vitamin D receptor gene may contribute to vitamin D-related disparities in fetal growth. Evidence from recent studies suggests an early prenatal influence of maternal vitamin D status on fetal skeletal development, with lasting postnatal effects. During pregnancy, the requirements based on fetal sex disparities are not well known.

Method: Vitamin D (1,25 $(OH)_2$ D)levels would be assayed using three blood spots at the time of delivery from each of 50 smokers and 50 non-smokers. In addition, maternal and neonatal data would be collected at the time of birth. This study would explore potential association of smoking with vit D levels at the time of delivery and fetal sex disparity in Central and Eastern Europe (CEE). Partners of Mother and Child Health Research (M&CH) Network of the Regional Cooperation in Health, Sciences and Technology (RECOOP HST) Association, would like to identify this association from any or all of the five sites in CEE; Czech Republic, Hungary, Romania, Slovakia and Ukraine.



Abstracts

April 18, 2015

Poster Session #3

Infection and Immunology

Cervical microbiota in women with preterm prelabor rupture of membranes

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Objective: To analyze cervical microbiota in women with preterm prelabor rupture of membranes (PPROM) by pyrosequencing and to document associations between cervical microbiota, cervical inflammatory response, microbial invasion of the amniotic cavity (MIAC), histological chorioamnionitis (HCA), and intraamniotic infection (IAI).

Study design: Sixty-one women with singleton pregnancies complicated by PPROM were included in the study. Cervical and amniotic fluid was collected at the time of admission. Cervical microbiota was assessed by16S rRNA gene sequencing by pyrosequencing. Interleukin (IL)-6 in the cervical fluid and amniotic fluid was assayed by ELISA and lateral flow immunoassay, respectively.

Results: Four bacterial community state types [CST I (*Lactobacillus crispatus* dominated), CST III (*Lactobacillus iners* dominated), CST IV-A (non-*Lactobacillus* bacteria dominated), and CST IV-B (*Gardnerella vaginalis* and *Sneathia sanguinegens* dominated)] were observed in cervical microbiota of women with pPROM. Cervical fluid IL-6 concentrations differed between CSTs (CST I 145 pg/mL, CST III 166 pg/mL, CST IV-A 420 pg/mL, and CST IV-B 322 pg/mL; $p = 0.004$). There were also differences in the rates of MIAC, of both MIAC and HCA, and of IAI among CSTs. No difference in the rate of HCA was found among CSTs.

Conclusion: Cervical microbiota in PPROM women can be characterized by four CSTs. The presence of non-*Lactobacillus* CSTs is associated with strong cervical inflammatory response and a higher rate of MIAC, both MIAC and HCA, and IAI, representing a PPROM subtype with pronounced inflammation. CST I represents the most dominant type of PPROM with a low rate of MIAC, both MIAC and HCA, and IAI.

Source(s) of research support: This research work was supported by a grant from the Ministry of Health of the Czech Republic (NS 13461-4/2012), Charles University in Prague, the Faculty of Medicine in Hradec Kralove, Czech Republic, project "PRVOUK" P37/10, and the Faculty Hospital in Hradec Kralove (a long-term organization development plan).

Acknowledgements: the study was approved by the Institutional Review Board committee (August 10, 2010; No. 201012 S15P).

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Effect of DSS on Bacterial Growth in Gastrointestinal Tract

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Key words: IBD, colitis, bacteria growth, Salmonella SL7207, E. coli Nissle 1917

Introduction: Inflammatory bowel disease (IBD) includes ulcerative colitis and Crohn's disease. Probiotics such as Escherichia coli Nissle 1917 (Eco) and Salmonella enterica Typhimurium SL7207 (SL7207) are known to help with different gastrointestinal problems. The aim of our study was to find suitable bacterial vector for gene therapy of colitis.

Methods: C57BL/6 male mice were divided into 2 groups – control (n=6) and 2% dextran sodium sulfate (DSS) treated (n=5). At day 10 mice were sacrificed and samples collected of contents and homogenates from stomach, proximal and distal small intestine (SIP and SID), caecum, proximal and distal colon. Samples were homogenized and plated onto micro titration plates. SL7207 and Eco (OD_{600}=0.4) were added to samples or to PBS as control. Bacterial growth was measured each hour and growth curve was calculated based on optical density.

Results: Growth of Eco in all samples except contents of SIP and SID from DSS-treated animals was inhibited compared with its growth in PBS. Growth of SL7207 in all samples was enhanced or unaffected in comparison with PBS, but did not reach growth rates of Eco. The bacterial growth was inhibited in gastrointestinal contents from DSS-treated animals compared to healthy controls. In homogenates of SID and proximal colon DSS stimulated growth of both bacterial strains.

Discussion: Treatment with DSS inhibits the growth of Eco more effectively than SL7207 in all samples. DSS has stimulating effect on growth of both strains in homogenates of SID and proximal colon. Gastrointestinal tract, both contents and tissues, does not inhibit growth of SL7207 compared with negative control (PBS) and provides more suitable environment for growth of SL7207 compared with Eco.

Conclusion: Salmonella enterica Typhimurium is more suitable as potential bacterial vector for targeted intestinal cell therapy since it survives better in tissue homogenates of DSS treated mice.

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Acknowledgement**:** The animal experiments were approved by the institutional review board and Ethics Committee of Comenius University Faculty of Medicine (Bratislava, Slovakia) at 16.6.14 under number 00201435.

Ureaplasma species and *Mycoplasma* hominis in cervical fluid of pregnancies complicated by preterm prelabor rupture of membranes

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Objective: To evaluate Ureaplasma species and Mycoplasma hominis DNA in the cervical fluid and their association with microbial invasion of the amniotic cavity (MIAC) and/or histological chorioamnionitis (HCA) in pregnancies complicated by preterm prelabor rupture of membranes (PPROM).
Study design: A prospective study of 68 women with singleton pregnancies complicated by PPROM between $24^{0/7}$ and $36^{6/7}$ weeks was conducted. Cervical fluid and amniotic fluid were collected from all women at the time of admission. The Ureaplasma species and Mycoplasma hominis DNA in the cervical fluid was identified using specific real-time PCR.
Results: Ureaplasma species and Mycoplasma hominis DNA was identified in 59% (40/69) of the cervical fluid samples. Women with the presence of Ureaplasma species DNA with and without Mycoplasma hominis DNA in the cervical fluid had a higher rate of MIAC alone [35% (14/40) vs. 11% (3/28); p = 0.02] and a higher rate of the presence of both MIAC and HCA [30% (12/40) vs. 4% (1/28); p = 0.01] than women without Ureaplasma species and Mycoplasma hominis DNA in the cervical fluid.
Conclusions: The presence of Ureaplasma species DNA with and without Mycoplasma hominis DNA in the cervical fluid is associated with a higher risk of MIAC or MIAC and HCA together in pregnancies complicated by PPROM.

Source(s) of research support: This work was supported by a grant from the Ministry of Health of the Czech Republic (NT14104-5/2013) and by Charles University in Prague, Faculty of Medicine in Hradec Kralove, Czech Republic, project “PRVOUK” P37/10.

Acknowledgements: the study was approved by the Institutional Review Board committee (August 10, 2010; No. 201012 S15P).

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Interaction of Recombinant Diphtheria Toxin Molecule Fragments with Resistant to Toxin Cells

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Key words: CRM197, diphtheria, endocytosis, L929 cells, proHB-EGF.

Introduction. Some mammalian species show considerable resistance to diphtheria toxin (DT). But the clear view on possible mechanisms of resistance implementation in cells does not exist. The possibility of DT binding and internalization by the resistant cells is not certain demonstrated.
Methods. The methods of protein expression in *E. coli* and their purification, eukaryotic cell cultures technique, confocal microscopy and flow cytometry were used during this work.
Results. It was showed that resistant to toxin L929 cells derived from mouse (*Mus musculus L*) connective tissue efficiently bind and internalize recombinant fluorescent derivatives of DT, such as B-subunit and R-domain. Besides, in L929 cells the changes in the number and size of the endosomes containing B-subunit is different compared to sensitive Vero cells originated from the kidney of green monkey (*Cercopithecus aethiops L*). It was also found that Vero cells have absorbed more B-subunit than R-domain during incubation of cells simultaneously in presence of B-subunit and R-domain in the media. However, L929 cells absorbed more molecules of R-domain than of B-subunit compared to Vero cells. In addition, L929 cells were characterized by rapid and almost complete colocalization of R-domain and B-subunit in the early stages of incubation, whereas in Vero cells colocalization occurred slowly and gradually at each time point of incubation in presence of these molecules.
Discussion. This data indicates that the cells interact with B-subunit similarly to entire toxin and that resistant to DT cells can bind and internalize toxin molecules in the same manner as sensitive cells. It is possible that differences in the intracellular transport of the toxin-receptor complex may reflect the presence of certain differences in the functions of proHB-EGF in L929 and Vero cells.
Conclusion. Our results may indicate some differences in intracellular traffic and different biological role of DT receptor proHB-EGF in toxin-sensitive and toxin-resistant cells. Deeper understanding of the molecular mechanisms of cell interactions with DT and realization of DT resistance in unsusceptible cells can open the way for the development of pharmacological therapies aimed to prevent toxic action of DT in cells. Also, this knowledge will help in the development of recombinant means designed for drug delivery into cells using DT moieties.

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Examination of antibacterial effect of neutrophilic granulocytes derived microvesicles

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Keywords: extracellular vesicles, microvesicles, antibacterial effect, neutrophilic granulocytes, bacteria

Introduction: Almost every cell type can form extracellular vesicles spontaneously or upon stimuli. The literature refers to the extracellular vesicles which shed from the plasma membrane and have a size between 100-1000 nm as microvesicles. Our group has previously described that the human neutrophilic granulocytes can produce microvesicles with antibacterial properties as an answer to complex biological activation (Tímár et al. 2013). Now, we examined the dose-dependency of the previously described antibacterial effect in different bacterial strains.

Methods: We used neutophilic granulocytes isolated from healthy human blood and stimulated with yeast opsonised with mixed human serum. We obtained the microvesicles by two-step centrifugation and filtration, and then we prepared dilutions of the microvesicles. As an initial sample we used microvesicles derived from 10^7 neutrophils. Compared to this, we prepared samples containing 10-fold, 2-fold less and 5-fold more quantity of vesicles. We applied heat inactivated microvesicles as negative control and neutrophilic granulocytes as positive control. We examined the antibacterial effect with bacterial survival test (Rada et al. 2004) on *Staphylococcus aureus* and *Escherichia coli* strains. We also carried out this test with our new method with flow cytometer.

Results: In the case of *S. aureus* strains, the results showed lower bacterial number in comparison with the heat inactivated samples. The antibacterial effect increased with the increasing doses of the microvesicles. This dose-dependency was also detectable with the flow cytometric survival test. However, in the case of the Gram-negative *E. coli* the antibacterial effect was below that we found in the Gram-positive *S. aureus* strains.

Conclusion: Neutrophilic granulocyte derived microvesicles inhibit dose-dependently the growth of *S. aureus*. Nevertheless, the microvesicles' antibacterial capacity was lower in *E. coli* strains. The flow cytometric results strongly corralated to this and the speed of this method offers advantages for the following examinations.

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Venus blood was drawn from healthy adult volunteers according to procedures approved by the Institutional Review Board of the Semmeweis Universtiy, permission number: BPR/021/12661-2/2014.

Abstracts

April 18, 2015

Plenary Session #3

Molecular Biology

Infectious Diseases

Immunology

Translation of Stem Cell Therapies for Skeletal Disorders

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Key words: Osteoporosis, Vertebral Compression Fracture, Mesenchymal Stem Cells (MSC), Parathyroid Hormone (PTH), Stem Cell Imaging

Introduction: Osteoporosis-related vertebral compression fractures (OVCFs) occur at a rate of 750,000 per year – twice the rate of hip fractures. Once OVCFs occur, there are limited treatment options. We hypothesized that an intravenous injection of MSCs combined with PTH administration would induce stem cell homing to vertebral defects followed by osteogenesis and defect repair. In this study, we investigated the strategy to regenerate vertebral defects in both, a rodent (rat) and pig models.
Methods: We induced osteopenia in nude rats by ovariectomy and four months of low calcium diet (LCD). Human bone marrow-derived (BM)-MSCs were labeled with Luciferase reporter gene using lentiviral vectors. Multiple vertebral defects were created in lumbar vertebrae of nude rats and lumbar vertebrae of minipigs. Treatment included multiple i.v. injections of labeled cells and daily SQ injections of PTH (in different concentrations) or saline for 4 weeks (for both animals). For pigs, porcine MSCs were administrated i.v. once a week. Cell survival and homing to the defect site were monitored using bioluminescent imaging (BLI) in rats. Bone regeneration was monitored using μCT in vivo for rats and clinical X-ray scanner and μCT ex vivo in pig studies after harvesting. Histological analysis and immunofluorescent staining were done on the treated vertebrae.
Discussion: BLI detected MSC homing to the lumbar region of the animals few days after the intravenous delivery. Vertebral defects in osteopenic rats treated with the combined stem cell-and-PTH therapy resulted in 2-fold increase in bone volume density two months after treatment when compared to defects treated with PTH only. The vertebrae in the untreated rats did not heal after 8 weeks. In the porcine model of multiple vertebral defects, remarkable healing of the defect was observed as early as 5 weeks after the surgery. Notably the combined stem cell-and-PTH therapy succeeded to regenerate the defect in a much more efficient way than each treatment alone.
Results: The results show that vertebral defects in osteopenic rats and pigs were efficiently repaired when treated with human MSCs and PTH, compared to the controls. This study provided evidence for future therapies that could revolutionize the treatment of vertebral and other complex fractures especially in osteoporotic patients.

Funding: CIRM Early Translational II Awards, TR2-01780; NIH R01 DE019902
The study was approved by Cedars-Sinai Institutional Animal Care and Use Committee
Rat Study: IACUC003609
Pig Study: IACUC004928

Study of immunoglobulin IgG glycosylation alteration in systemic and organ-specific autoimmune disorders

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Key words: autoimmune disorders, antibodies, glycosylation, lectins, ELISA.

Introduction. The N-glycosylation on human immunoglobulins, especially on IgGs, plays a critical role in the bioactivity of this group of important proteins, e.g. in rheumatoid arthritis (RA) patients a decrease in the terminal galactose content of the N-linked glycans at the conserved Fc region (Asn297) glycosylation site of IgG occurs. However, it is still not clear whether altered glycosylation is attributable to only systemic (or rheumatic, like SLE, RA) disorders, or it also can be observed in autoimmune conditions involving specific organ (like diabetes mellitus type 1 (DM1), autoimmune thyroiditis, etc).

Methods. In this work we studied glycosylation of IgG molecules of RA patients at different stages of disease progression, and of patients with DM1, using aged-matched groups of NHD as a control. To analyze glycan exposure in IgG molecule we used previously developed and described lectin-based ELISA method (Sjöwall C., Lupus, 2014), which provides information about accessibility of glycans on IgG molecules, besides representative samples of each groups were also analysed with capillary electrophoresis with laser induced fluorescent detection (CE-LIF) to detect the complete IgG glycoforms for specific patients.

Results. Obtained data showed altered fucose availability, alteration is sialylation and level in patients with RA. However exposure of glycan residues was not altered significantly in the organ-specific autoimmune disorder like DM1.

Discussion. Population of RA and DM1 patients were studied and compared. Obtain data allow us to correlate the glycosylation profile of IgG in different populations with the type of autoimmune disorder based on the involvement of specific organs or systemic disorder.

Conclusion. Altered glycosylation was clearly associated with rheumatic conditions, but not with organ specific autoimmune disease.

Funding: The work was supported by the WUBMRC and IVF Scholarship awarded to A. Karmash; DAAD and Fritz Thyssen Foundation scholarships awarded to I. Magorivska.

Ethical Committee approval was received from Ethics Committees of DH LNMU No.3/2010-03-22, UHO (25-1:11861-3/2012), FM UJJSO (2158/61-07-13-31).

Acknowledgement: The study was supported by Cedars Sinai Medical Center's International Research and Innovation Management 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).

Importance of dosage and immunization schedule on the adjuvancity of poly(lactide-co-glycolide) particles as antigen carriers for immunization *per os*

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Key words: oral vaccination, immunity, nanoparticles, PLGA, diphtheria toxin

Introduction: Diphtheria toxin (DT) is the main pathogenic factor of Corynebacterium diphtheria produced by bacteria cells at their colonization sites in respiratory tract mucous membranes. Strong induction of antitoxic immunity in mucosal tissues can protect the body against the infection. The aim of this work was to evaluate the dosage-dependent immunogenic properties of poly(lactide-co-glycolide) (PLGA) adjuvant particles coated with cellobiose as antigen carriers for oral immunization.

Methods: Two types of PLGA-cellobiose particles (PLGA-cellobiose-1 ~ 0,8 μm and PLGA-cellobiose-2 ~ 1,2 μm) containing non-toxic recombinant fragment B of diphtheria toxin fused with enhanced GFP (EGFP-SbB) were characterized *in vitro* for their size, shape, antigen loading and ability to induce phagocytosis. BALB/c female mice of the same age and weight were treated *per os* with different doses of antigen immobilized on the particles (2,5 μg, 25 μg, 250 μg and 2500 μg per 1 kg of body weight) 3 times with intervals of 2 weeks. Sera were collected one week after each immunization. The antigen-specific IgG and IgA antibodies were estimated in serum of immunized mice by ELISA.

Results: Increase in concentration of blood antitoxic antibodies was detected in mice predominantly after the first immunization. Antigen dosage 250 μg per 1 kg body weight was the most immunogenic for IgG antibodies induction for both types of PLGA-cellobiose particles. At the other hand, antigen dosages 25 and 2,5 μg per 1 kg body weight were the most immunogenic for IgA antibodies induction by PLGA-cellobiose 1 and 2 particles, respectively. Second and third treatment had no significant effect on the immune response or even reduced it, what could be explained by immune tolerance induction by antigens delivered *per os*.

Conclusion: Obtained results suggested that correct dosage of PLGA-cellobiose particles loaded with antigen could significantly increase the humoral immune response against introduced antigen after first immunization, but high doses of delivered antigen and continuous immunizations could lead to immune tolerance. Thus, only one immunization in appropriate dosage of PLGA particles needed for successful stimulation of protective immune response. That is why PLGA particles can be considered as a potent component for oral vaccines.

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Acknowledgement: 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 №1 approved on 04/02/2013).

Thiazolidinone-based design of new antitrypanosomal agents

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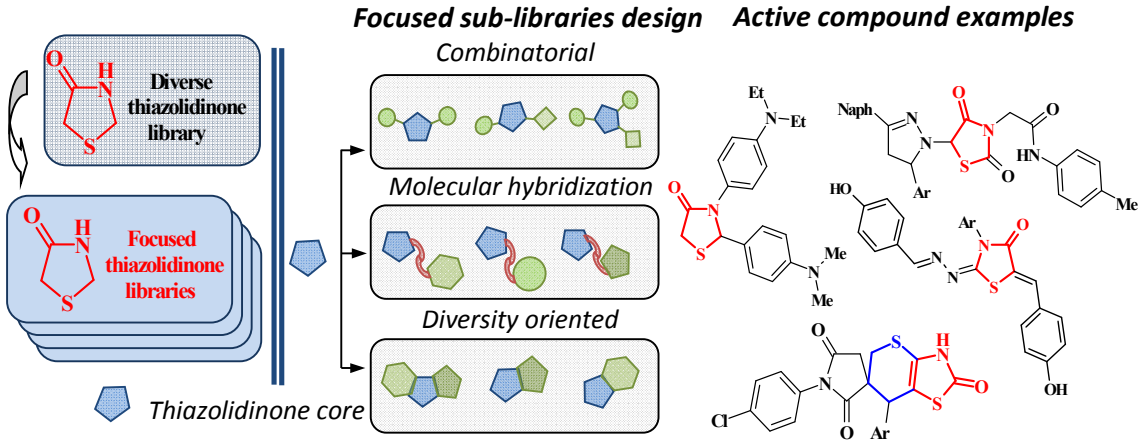
Introduction. Human African Trypanosomiasis (sleeping sickness) and American Trypanosomiasis (Chagas disease) are among the most serious regional neglected tropical diseases caused by Trypanosoma (*T.*) which have now spread to other continents. Despite the increasing interest in the search for new antitrypanosomals, and the impressive advances in understanding the biology of *T.* the treatment of trypanosomiasis urgently requires new effective and non-toxic drugs (since eflornithine was approved in the 90[s] no new drugs were marketed). Following the current trends in the new antitrypanosomanl agents design thiazolidinones are of special interest as *i*) mimics of thioureas/thiosemicarbazones *ii*) attractive scaffolds for structure-based design. Limitations of highly active ligands (to validated targets) application are due to low effectiveness in vivo or toxicity. The present project is an extension of our ongoing efforts towards search new thiazolidinone-based antiparasitic agents.

Methods. organic synthesis, structure-based design, antitrypanosomal activity assay, (Q)SARs.

Results and discussion. The project realization involves the several stages: *1*) primary screening of antitrypanosomal activity (*T. btucei, T. cruzi*) of the diversity thiazolidinones library; *2*) SAR analysis, design and synthesis of focused sub-libraries within combinatorial and privileged-substructure-based diversity oriented synthesis strategies and molecular hybridisation; *3*) sub-libraries screening, hits and leads identification; *4*) (Q)SAR(P) analysis and formation of the direction for structure optimization; 5) *in depths* study of mode of action, toxicity evaluation. The set of thiazolidinone-based anticancers was involved into the study following the new findings about the simultaneous anticancer and antitrypanosomal activities.



Based on preliminary results the focused sub-libraries of active thiazolidinones were selected, set of hit- and lead-compounds were detected (among screened compounds the most efficient trypanocidals (*in vitro*) were found to be active against *T.b.b.* and *T.b.g.* with IC_{50} 0.01– 0.10 μg/mL) as well as some modifications items were discovered.

Conclusion. The set of active thiazolidinone-based antitrypanosomal agents was identified and the routes for new active compounds have been designed and confirmed.

Characterization of the effect of D- and L-limonene on pregnant rat myometrium *in vitro*

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Keywords: limonene, pregnancy, nifedipine, paxilline, antioxidant

Introduction: Generation of reactive oxygen species can produce abnormal contractility in the pregnant uterus therefore there is a growing interest of dietary antioxidants such as vitamin C, tocopherols, carotenoids and terpenoids in protection of the foetus against oxidative stress and for prophylactic treatment of premature labor. The antioxidant limonene is a major compound of citrus essential oils. Its applications in food, cosmetics and household products have multiplied significantly, but its effects on the pregnant myometrium have not been investigated.

Methods: Our aim was to study the effects of limonene on pregnant Sprague-Dowley rat myometrium on the last day of pregnancy *in vitro*, and to explore its mechanism of action. Uteri were removed from rats on day 22 of pregnancy. Muscle rings were sliced from the uterine horns and mounted vertically in an organ bath.

Results: D- and L-limonene (10^{-13}-10^{-8} M) caused myometrial contraction in a dose-dependent manner. Pretreatment with nifedipine (10^{-8} M), tetraethylammonium (10^{-3} M) and theophylline (10^{-5} M) attenuated the contracting effects of D- and L-limonene, while in the presence of paxilline (10^{-5} M) D- and L-limonene were ineffective. The two enantiomers decreased the myometrial cAMP level, but after paxilline pretreatment the cAMP level was not altered as compared with the control value. Additionally, L-limonene (10^{-6} M) proved to diminish the contractile consequences of the oxidative damage caused by methylglyoxal ($3x10^{-2}$ M), whereas the D enantiomer was ineffective.

Discussion: Our findings suggest that, besides the antioxidant action of L-limonene, D-and L-limonene cause myometrial smooth muscle contraction through activation of the A_{2A} receptor and opening of the voltage-gated Ca^{2+} channel.

Conclusion: Our results raise the possibility of the pregnant uterine contraction-increasing effects of limonene-containing products during pregnancy. Although limonene is an antioxidant agent, its use should be avoided during pregnancy, with the perspective of improving successful family planning.

Acknowledgement: Experiments were approved by the Hungarian Ethical Committee for Animal Research: IV./198/2013.

Experimental oral and intraperitoneal infection of Swiss albino mice with two coxsackievirus strains

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Key words: Coxsackie B viruses, route of infection, Swiss albino mice, viral RNA, pancreas, heart.

Introduction: Coxsackie B viruses (CVB) have been associated with chronic diseases such as type 1 insulin-dependent diabetes mellitus (T1D). Serological and epidemiological studies relate CVB4 infection to prediabetic and diabetic individuals. Aim of our study was to investigate the pathogenic potentials of two different strains (diabetogenic: CVB4-E2, cardiotropic: CVB3-Nancy), using outbred mouse model, oral and intraperitoneal (i.p.) routes of infection, main focus on the heart and pancreas.
Methods: Different groups (6 mice/group) of 3-4 weeks old, 15-17g, male Swiss albino mice were infected with CVB3-Nancy and CVB4-E2 orally and intraperitoneally (dose of $2x10^6$ $TCID_{50}$), mock infected controls were included. Glucose tolerance test (GTT), viral titers, presence of viral RNA, and histopathology of the pancreases and hearts of infected mice were studied.
Results: Mortality was observed only after the oral infection with CVB4-E2 at days 5 and 7 post infection (p.i.). Hyperglycemia was absent in all infected mice. After infection with CVB4-E2, virus was detected by PCR at day 49 in the pancreases, but not at day 105, whereas CVB3-Nancy was detected by PCR at days 49 and 105 p.i., irrespective of the infection route. Viral RNA was detected at days 49 and 105 p.i. in the hearts of 6/6 (oral) and 5/6 (intraperitoneal) mice infected with CVB4-E2, and 3/6 (oral) and 2/6 (intraperitoneal) mice infected with CVB3-Nancy, Histopathology showed inflammation in the exocrine pancreas only in the i.p. infected mice by both the viruses during the acute phase of infection. Mild necrotic foci were observed at day 10 p.i. in the hearts of all infected mice, and a reparative necrosis was observed in all CVB3-Nancy infected mice.
Conclusion: Affliction of the pancreas of CVB infected mice depends on the virus strain, host and the route of infection.

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The study was approved by the Ethical Committee of the Slovak Medical University Date: 5th November 2006 and the State Veterinary and Food Control Authority of the Slovak Republic. Date: 22nd March 2007. Number: C.k Ro 3035/07-221/3.

Abstracts

April 18, 2015

Plenary Session #4

Nanomedicine

Medical Imaging

Oncology

NanoBioTech activity at Institute of Cell Biology, NAS of Ukraine: Achievements in 2014, existing problems, and perspectives within RECOOP-HST Network.

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Key words: nanomaterials, treatment, diagnostics, anticancer drugs, tumor cells, apoptosis.

In 2014, several NanoBioTech-related projects were realized in the Department at the ICB.

Project 1: **Imaging of formalin-fixed, paraffin-embedded melanoma tissues for *in vivo* labeled mouse melanoma tissue**. Thermodestruction of melanoma tissue was achieved for a specific lectin-functionalized nanocrystal (NC) developed at Wroclaw Technical University (Poland). The existing problems are the lack of access to the hardware for whole animal imaging and poor compatibility with used type of the NC. The future studies are focused on detection of functionalized NC using X-ray excitation for X-ray compatible detection systems. Collaboration has started with NanoBioTech-interested laboratories at Cedars-Sinai Medical Center (Los Angeles, USA).

Project 2: **Biomedical applications of novel polymeric particles of the nanoscale with a reactive shell suitable for bio-functionalization**. Enhanced anticancer activity and circumvention of drug resistance mechanisms was achieved by applying novel polymeric/phospholipidic nanocarriers synthesized at Lviv National Polytechnic University (Ukraine). Novel oligoelectrolyte-based non-viral gene delivery systems for genetic transformation of the eukaryotic (yeast, plant, mammalian) cells were also created. It should be noted that there is no big interest to these drug and gene delivery systems within the RECOOP-HST Network. However, active collaboration is developing with Lviv National Medical University and outside RECOOP-HST Association.

Project 3: **Biomedical and biotechnological application of novel superparamagnetic nano- and microparticles** developed at the Institute of Macromolecular Chemistry (Prague, Czech Republic) also collaborating with the Institute of Biochemistry (Kyiv, Ukraine). Although several joint articles were already published by collaborating RECOOP-HST teams, the magnetic properties of created new materials have not been adequately exploited in the research *in vitro* and *in vivo*.

Acknowledgement: We thank for Cedars Sinai Medical Center's International Research and Innovation Management 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).

Bioluminescent imaging of the mouse brain molecular response after stroke

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Key words: in vivo imaging, stroke, inflammation, axonogenesis, apoptosis

Introduction. TLR2 (Toll-like receptor 2), GAP43 (Growth-associated protein-43), and CASP3 (Caspase 3) represent major aspects of events after brain damage: inflammation, axonogenesis, and apoptosis respectively.
Methods. To visualise the molecular events related to the activity of TLR2, GAP43, and CASP3 in the brain of the living mouse bioluminescent imaging of luciferase reporters mirroring their activity was applied. Two transgenic mouse model were generated C57Bl/6-Tg(Tlr2-luc/gfp)10Kri and C57Bl/6-Tg(Gap43-luc/gfp)Kri carrying TLR2-luc and GAP43-luc cassettes respectively. Ischemic brain lesion corresponding to the ischemic stroke in humans was induced using transient middle cerebral artery occlusion (MCAO) on 3-4 months old transgenic mice. Together with luciferin, an alternative luciferase substrate DEVD-aminoluciferin (VivoGlo™ Caspase 3/7 Substrate; Promega, US) was used. This substrate was cleaved in the brain by CASP3 (Caspase 3) and subsequently luciferin was available for luminescence reaction . Imaging was performed by IVIS Spectrum Pre-clinical In Vivo Imaging System (Perkin Elmer, US) in living animals by recording the emitted light from the brain.
Results. TLR2, GAP43, and CASP3 were upregulated after stroke. To analyze the apoptosis in subset of GAP43 cells the imaging with DEVD-aminoluciferin was performed. The in vivo bioluminescence imaging was validated and quantified by imunostaining. The findings suggested that CASP3 activity, not necessarily associated with neuronal apoptosis, increased, and CASP3 and GAP43 might be part of a common molecular pathway involved in early stress response after stroke.
Conclusion. Neuronal stress in addition to inflammation, repair, and apoptosis can be assessed by the bioluminescent imaging as important processes during the brain response to stroke.

Source of research support: The study was supported by EU FP7 grant GlowBrain (REGPOT-2012-CT2012-316120).

The study was approved by Animal Care and Use Committee 380-59-10106-14-55/230 from October 23, 2014

Response Evaluation after Primary Systemic Therapy of Her2 Positive Breast Cancer – an Observational Cross-Sectional Study

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Keywords: *breast cancer, primary systemic therapy, trastuzumab, molecular imaging, FDG PET/CT*

Introduction: There are no uniformly accepted methods to predict which HER2 positive breast cancer patients are more likely to achieve pathological complete remission (pCR) after primary systemic therapy (PST). Our aims were to evaluate (I) the benefit of trastuzumab containing PST in human epidermal growth factor receptor 2 (HER2) overexpressing breast carcinomas;(II) to explore the differences between responder and non-responder patients and (III) to analyze the predictive power of hybrid clinical-imaging modalities in the monitoring of tumor response.

Methods: Altogether 188 cases who received PST between 2008 and 2014 were reviewed. Finally 43 HER2 positive (HER2+) breast cancer patients [subtypes: 28 Luminal-B-HER2+ and 15 HER2+] were enrolled in the present study. 26 patients received mostly taxane-based PST without trastuzumab (Gr1) and 17 patients received trastuzumab containing PST (Gr2). pCR rate was evaluated according to EWGBSP recommendations. Regarding pCR we compared the predictive value of (1) breast-ultrasound (US); (2) FDG-PET/CT evaluated by changes in FDG-uptake (metabolical response) and (3) PET/CT evaluated by combined (metabolical and morphological remission) criteria. Sensitivity (sens), specificity (spec), positive (PPV) and negative predictive values (NPV) were calculated.

Results: Ten patients (38.5%) achieved pCR in Gr1, while 8 (47%) in Gr2. According to pCR rate significant differences were detected between the two subtypes: pCR was more frequent in the HER2+ compared to Luminal-B-HER2+ tumors in both treatment groups (Gr1 p=0.04, Gr2 p=0.03). PET/CT evaluated by combined criteria (3) separated the cases presenting pCR vs. non-pCR more accurately in both patient groups (in Gr1 sens=77.8% spec=100% PPV=100% and NPV=71.4%; in Gr2: sens=87.5%, spec=62.5%, PPV=70% and NPV=83.3%). Considering metabolism based PET/CT evaluation alone (2) PPV was higher, but sensitivity decreased markedly. With US (1) the results were the following: in Gr1 sens=83.3% spec=25% PPV=62.5% NPV=50%; in Gr2: sens=100% spec=12.5% PPV=41.6% NPV=100%), respectively.

Discussion and Conclusion: Regarding pCR rate, the benefit of trastuzumab-containing PST was defined in HER2 overexpressing breast cancer. Luminal-B-HER2+ subtype need further analysis to identify patients who would surely benefit from PST. Combined evaluation of tumor metabolism and morphology gave better results in separating pCR/non-pCR patients than only viability- or morphology based criteria.

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Funding: None was involved.
Ethical Committee Approval: Ethical approval for the study was given by the Semmelweis University Institutional Review Board. Written informed consent was waived. Date and number of the ethical approval: 12-June-2013; TUKEB No.120/2013.

Pyrazoline-thiazolidinone hybrids in the design of new anticancer agents

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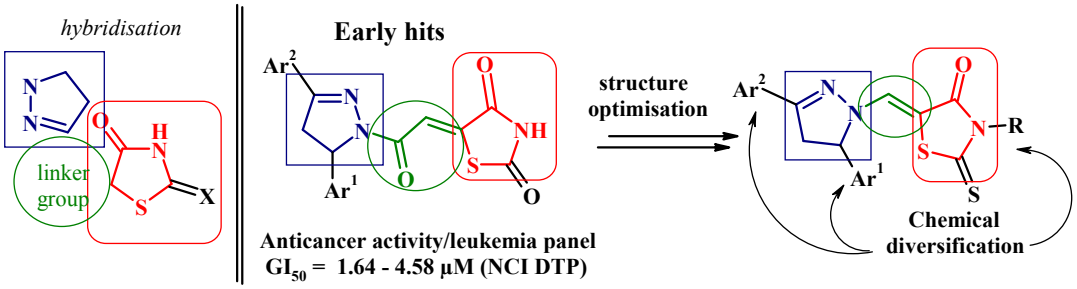
Introduction. Creation of the hybride molecules is one of the most employed approaches in designing new anticancer drugs. Moreover, the combination of privileged scaffolds within such approach has been regarded as a benefit. Thiazolidinones and pyrazolines as the examples of such scaffolds have been reported to possess promising chemotherapeutic properties including antitumor activity. Anticancer effect of 4-thiazolidinones is related with affinity to JNK-stimulating phosphatase-1, TNFα, anti-apoptotic Bcl-X_L-BH3 complex, integrin $\alpha_v\beta_3$ receptors and others. Pyrazole and pyrazoline derivatives have been identified as inhibitors of cyclin-dependent kinases, heat shock proteins, vascular endothelium growth factor, P-glycoprotein, others. The present work is an extension of our ongoing efforts towards searching new thiazolidinone-based anticancer agents.
Methods. Structure optimization, organic synthesis, structure analysis, anticancer activity (*in vitro*).
Results and discussion. Based on the (Q)SAR data and previously hits structures, the focused sub-library of pyrazoline-thiazolidinone hybrids have been designed and synthesized within the hybrid-pharmacophore approach. The molecular structures of these compounds were confirmed (NMR, MS, X-ray).



The results of screening anticancer activity in *Jurkat T, HL-60, HL-60/ADR, MCF-7, HCT-116* cell lines confirmed the expected high cytotoxic effect of new 4-thiazolidinone derivatives. Among tested compounds ***Les-4368***, ***Les-4956***, ***Les-4370*** and ***Les-5579*** showed the highest level of activity that exceeds doxorubicin's one. Leukemia panel was identified as most sensitive cell lines. Based on the in-depths study of anticancer effect apoptosis-related mitochondria-depended mechanism was suggested for thiazolidinones' action that involves prooxidant phase with increasing ROS generation.

Conclusion. New thiazolidinones were designed within hybride-pharmacophore approach and were shown to use the apoptosis-related mechanism of action towards leukemia cells.

Acknowledgement: The authors thank Cedars Sinai Medical Center's International Research and Innovation Management Program and the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support.

The surface modified magnetic iron oxide nanoparticles; interactions of nanoparticles with cells *in vitro*

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Key words: magnetite nanoparticles, uptake, toxicity, oxidative stress, cytoskeleton dynamics

Introduction. Magnetite nanoparticles (MNPs) are one of the most promising types of nanoparticles for diagnostic and therapeutic purposes; therefore the mechanisms of MNPs interactions at cellular and molecular levels as well as their bio-safety are of great concern.
Methods. The surface-modified MNPs were characterized in-depth by different physico-chemical methods. MNPs behavior in culture media was determined by DLS, the MNPs uptake was visualized by TEM and quantified by AAS/ICP-MS. The toxicity of MNPs was measured by MTT and the oxidative DNA damage was evaluated by SCGE. The ROS levels and cytoskeleton dynamics were determined by the fluorescence, the antioxidant enzyme activities and the total antioxidant capacity (TAC) were analyzed by spectrophotometry. The glutathione level was determined by flow cytometry.
Results. The cytotoxicity of MNPs increased proportionally with the particle size and correlated with the internalized amount of particles. The internalized MNPs were detected as vesicle-bound aggregates localized in the cytoplasm. All MNPs induced certain levels of ROS in A549 cells, but none of them produced any significant increase in oxidative damage to DNA. Indeed, no changes in the TAC, iGHS and GPx, and only discreet changes in SOD activity were observed in MNPs-treated cells. In contrast, all MNPs caused visible changes in cell morphology and disruption of cytoskeleton structure.
Discussion and Conclusion. Our data indicate that oxidative stress plays, at most, only a marginal role in the biological activity of surface-modified MNPs in A549 cells. We suppose that rather severe disruption of cytoskeleton structure might underlie the toxicity of the surface-modified MNPs. Consistent with our results, great cytoskeleton changes, disorganization of actin fiber and tubulin networks were observed in human endothelial cells treated with coated MNPs (Wu et al., Int. J. Nanomedicine. 5:385-399, 2010). Further studies are required to thoroughly investigate the MNPs' interactions with cytoskeleton structure in human cells.

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Abstracts

April 18, 2015

Poster Session #4

Molecular & Cell Biology

Inhibitory κBα is a Direct Bone Morphogenetic Proteins Target Gene and an Essential Mediator of Anti-catabolic and Joint Regenerating Effects of BMPs

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Introduction: Loss of articular cartilage in the joints is a common problems in rheumatoid arthritis patients. Increasing evidence supports a role for BMP7 in preventing joint damage and in its regeneration in part by rescuing the catabolic effects of TNFα and IL-1β. The precise molecular mechanism mediating the preventive and regenerative effects of BMP7 is unknown. Our transcriptional profiling studies revealed that the activation of BMP signaling leads to increased expression of *Inhibitory κBα* (IκBα), that is a key negative regulator of a major proinflammatory NF-κB pathway. We therefore explored whether anti-catabolic effects of BMPs in cartilage and bone are mediated by BMP-induced IκBα expression.

Methods: cDNA microarrays-based gene expression profiling was used to discover novel BMP target genes. Positive hits were confirmed using Northern and Western blotting and Real-Time PCR. Luciferase reporter assays and ChIP assay were used to characterize the BMP-responsive region in IκBα promoter. Anti-catabolic effects of BMPs were validated *in vitro* using preosteoblastic cell lines and primary human mesenchymal stem cells (hMSC) osteoblast differentiation, EMSA and lentiviral shRNA approach. To validate *in vivo* the importance of our findings we used a model of experimentally induced arthritis in DBA/1 mice. All experiments were approved by the Ethics Committee for Animal Research (KU Leuven, Belgium; P198/2012).

Results: Real-Time PCR showed that activation of IκBα mRNA by BMPs does not require *de novo* protein synthesis, thus suggesting IκBα is a direct BMP target gene. Using ChIP assays we demonstrated that Smad1/5 and Smad4 bound to the highly conserved proximal region of IκBα promoter. A proximal fragment of IκBα promoter was found to be activated by BMP2 and BMP7. EMSA assay showed that BMP7-induced IκBα expression blocks formation of TNFα-induced NF-κB transcriptional complex. Furthermore, BMP treatment was found to inhibit TNFα and LPS-induced NF-κB transcriptional response in mouse preosteoblasts and hMSC and rescued the differentiation of MSC from proinflammatory inhibition. shRNA-mediated knockdown of IκBα expression confirmed an essential role of IκBα in mediating of anti-catabolic effects of BMPs. Anti-catabolic effects of BMPs *in vivo* are currently under investigation.

Conclusion: BMP-induced IκBα expression is a key mechanism mediating preventive and regenerative effects of BMP7 on degrading cartilage and bone. These results may lead to the development of novel strategies for cartilage and bone regeneration, using the combination of BMP7 with pharmacological agents.

Plasma membrane Ca^{2+}-pump new inhibitor and supressor of myometrium spontaneus relaxation

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Key words: myometrium, calix[4]arene, contractility, cell calcium, plasma membrane calcium pump (PMCA)

Introduction: Plasma membrane Mg^{2+},ATP-dependent Ca^{2+}-pump (PMCA) is involved in diseases connected with smooth muscle contraction. Nonetheless, prior to our research there were no low-molecular and selective inhibitors for it. We show that calix[4]arene C-90 (5,11,17,23-tetra(trifluoro)methyl(phenyl-sulfonylimino)-methylamino-25,26,27,28-tetrapropoxycalix[4]arene), which is easy to obtain, inexpensive in synthesize, low-molecular, lipophilic compound, efficiently and selectively inhibits the ATPase activity of PMCA in the myometrium.
Methods: Experiments were carried out in accordance with the European Guidelines and International Laws and Policies. The protocols were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol # 4 from 21/10/2012). Calix[4]arene C-90 was characterized with infrared spectroscopy and nuclear magnetic resonance methods. PMCA activity was determined on swine myometrium by measurements of P_i. Cell calcium ($[Ca^{2+}]$) was measured with fluorescent microscopy (LSM 510 META) with fluo-4. Laser correlation spectroscopy ("ZetaSizer-3") was used to determine effective hydrodynamic diameter (EHD) of smooth muscle cells (SMC).
Result: The inhibition coefficient $I_{0,5}$ was equal to 20.2±0.5, and calix[4]arene C-90 (100 μM) decreased PMCA activity velocity maximum by 75%, selectively comparing to other ATPases located in plasma membrane. The inhibitory effect of C-90 on PMCA activity associated with cooperative action of four sulfonylamidine groups. Calix[4]arene C-90 increased $[Ca^{2+}]$ in myometrium quiescent SMC by 45±9% comparing to control, however, during 1.5-2 minutes $[Ca^{2+}]$ was returning to initial level. Additionaly, C-90 (50 μM) caused SMC EHD decrease by 26% like as oxytocin (100 nM), and in concentration 10 μM decreased uterine smooth muscle relaxation velocity maximum by 20% (measured in spontaneous muscle activity).
Discussion and Conclusion: Since calix[4]arene C-90 effectively and selectively suppresses PMCA activity, increased $[Ca^{2+}]$ in SMC is connected with low PMCA activity, i.e. PMCA takes control of basal $[Ca^{2+}]$ in SMC. Returning to initial level means that other calcium-control systems become involved in Ca^{2+} extrusion, if PMCA is inhibited. Due to results of EHD and relaxation velocity decrease, considering availability of C-90 synthesis calix[4]arene C-90 may be applied as contraction agent in smooth muscles.

Acknowledgements: We appreciate S.O.Kosterin and V.I.Kalchenko for cooperation.

Histopathological Analysis of Subchronic Toxicity on Genetically Modified Maize Pioneer and Monsanto Mon810

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Key words: GMO risk assessment, subchronic toxicity, maize MON810

Introduction: A primary objective of GRACE project is to improve the availability and presentation of the scientific information about GMO risk assessment. We present the results of histopathological analysis of 90-day subchronic toxicity of GM plant: Monsanto and Pioneer maize MON810.

Methods: The study was conducted in agreement with the OECD method No. 408 and in accordance with GLP. Wistar Rcc Han rats were used and two varieties of GM maize from two different companies were tested. Dietary treatments represent groups of 11% GMO, 33% GMO and control group. The test substance was applied through feed every day over a period of 90 days. The GM feed was prepared by a certificated producer of laboratory animals feed. A complete necropsy was performed in all 120 animals at the end of the feeding study on day 91. The wet-weight of selected organs was recorded. Histological evaluation of tissue specimens was done in 10 animals per group. Collected tissues were preserved in the fixative medium (neutral buffered 10% formalin) and processed for histopathological examination.

Results: Lympho-epitheloid granuloma was observed in small intestine (6 animals, both genders) in experimental groups as well as in control group. Inflammatory prostatitis (6 animals) observed in 33% GMO group and in control group is supposed to be nonbacterial origin. Follicular cysts in ovary (2 females) were found in 33% GMO group. Other findings as cysts on the margins of the atrioventricular valves, myocardial necrosis and lesions of the acinar pancreas observed in 4 males are relatively common in aged rats.

Conclusion: No specific pathological lesions were found in examined organs that could be associated with the administration of the test item and related to subchronic toxicity in our experiment.

The research study supported by the European Commission 7th Framework Programme for the project *GRACE 311957.

Acknowledgement: The study was approved by the Ethical Committee of the Slovak Medical University on November 11, 2011.

Effect of perinatal hypoxia on GABA transporter functioning in cortical, hippocampal and thalamic nerve terminals of the developing rat brain

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Keywords: perinatal hypoxia, plasma membrane GABA transporters, nerve terminals, rat brain cortex, hippocampus, thalamus

Introduction Perinatal hypoxia lead to life-long cognitive disability, behavioral abnormalities and even epilepsy that are suggested to be due to neurodevelopmental disorders in network construction and maturation. Considering a special role of GABA for an immature brain, a model of hypoxia-induced seizures in rat pups was used for studying on transporter-mediated uptake of [^{3}H]GABA.

Methods Wistar rat pups underwent hypoxia and seizures in an airtight chamber infused by atmosphere composed of 4% O_2 and 96% N_2 at the age of 10-12 postnatal days (pd 10-12) (Jensen F.E., et al, J Neurophysiol., 1998) The experiments with rat brain cortical, hippocampal and thalamic nerve terminals (synaptosomes) were performed at pd 17-19, pd 24-26, pd 38-40 and pd 66-73 in the control (12 animals) and after hypoxia (12 animals). Difference between two groups was compared by two-tailed Student's *t*-test. The differences were considered significant, when $P \leq 0.05$.

Results The initial velocity of [^{3}H]GABA uptake was higher in the young rats (pd 17-19) of both groups. The rate of uptake decreased with different intensity in all studied brain regions with age: in the cortex and thalamus it decreased abruptly and more than twofold for the period from pd17-19 to pd38-40. In the hippocampus, a decrease in the rate during the same period was equal to 20%. Exposure to hypoxia had no effect on the intensity of GABA uptake in the cortex and thalamus, but caused a significant age-dependent attenuation of the uptake intensity in the hippocampus.

Discussion and Conclusion Such a response of the hippocampus to hypoxia indicates a larger vulnerability of the hippocampus compared to the cortex and thalamus to the action of hypoxia. These results are in agreement with our previous findings on the alterations in the ratio of active GAT1/GAT3 expressed in the plasma membrane of nerve terminals after perinatal hypoxia (Pozdnyakova N. et al, Croat Med J., 2014). Therefore, modulation of GABA transporter activity, and so GABAergic neurotransmission in the brain, is one of the effective approaches in the treatment of neurological disorders.

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Acknowledgements: Thank you for Cedars Sinai Medical Center's International Research and Innovation Management 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). 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 №1 approved on 19/09/2012).

Analysis of NMDA modulators with CE-LIF in different biological samples

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Key words: capillary electrophoresis, D-amino acids, NMDA receptor, chiral separation

Introduction: L-amino acids are the building blocks of proteins and only their formation and presence in biological systems were believed till a few decades ago. However, recent results suggest that several D-amino acids like D-aspartate and D-serine occur in many living organisms, including the human body. These amino acids possess neuromodulator function in the Central Nervous System (CNS) as their main target is the N-methyl-D-aspartate receptor, which plays important role in neuroplasticity, memory formation, learning processes and some pathological conditions.

Aims: Our aim was to develop a chiral capillary electrophoresis method to quantitate D-aspartate and D-serine in biological samples.

Method: Capillary electrophoresis method for enantioseparation of aspartate and serine has been developed and applied for their determination in various brain areas of newborn and adult mice after the animals were sacrificed. All animal procedures were approved by the ethical committee of the Semmelweis University (22.1/606/001/2010, February 5, 2010) and were in accordance with the EU Council directives on laboratory animals (86/609/EEC).

Results: For the sensitive laser-induced fluorescence (LIF) detection of amino acids their derivatization with NBD-F was used. An amino-modified ß-cyclodextrin, HPA-β-CD was found suitable for chiral analysis of various amino acids. Using 50 mM pH 7 HEPES buffer containing 6 mM concentration of this chiral selector provided baseline separation of aspartate and serine enantiomers. All determinations were accomplished in a polyacrylamide coated capillary using reverse polarity for the analysis of the negatively charged analytes. The developed method has been validated. The applicability of the method was tested by determination of D-amino acid neuromodulators in brain samples from newborn and adult mice. The brains from newborn mice contain D-aspartate in higher concentration compared to the adult brain samples, while the opposite was found in case of D-serine. Among analysed adult brain samples, amygdala, hippocampus and prefrontal cortex were rich in both D-amino acids.

Discussion: The higher level of D-aspartate found in brain samples of newborn mice is in line with previous results suggesting its role in neurogenesis. Higher level of NMDA receptor modulators could be detected in brain regions playing important role of memory formation.

Conclusion: The developed capillary electrophoresis method is suitable for quantitation of D-aspartate and D-serine in brain samples from laboratory animals of various disease models.

Acknowledgement: All animal procedures were approved by the ethical committee of the Semmelweis University (22.1/606/001/2010, February 5, 2010) and were in accordance with the EU Council directives on laboratory animals (86/609/EEC).

Abstracts

April 18, 2015

Poster Session #4

Translational Life Sciences

Alterations of peritubular capillaries in experimental renal fibrosis

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Key words: angiogenesis, endothelium, ultrastructure

Introduction: Renal fibrosis is associated with rarefaction of peritubular capillaries (PTCs). However, functional and ultrastructural alterations of the renal microvasculature in renal fibrosis are not well described.

Methods: We studied three murine models of fibrosis with distinct mechanisms of injury, i.e. unilateral ureteral obstruction (UUO, day 1, 3 and 5), unilateral ischemia-reperfusion injury (IR, day 14 and 21) and Col4A3 deficient (Alport) mice. In all models we quantified PTC to tubule ratios using immunohistochemistry, analyzed vascular leakage using Evans Blue dye and fibrinogen extravasation and ultrastructure by electron microscopy.

Results: Compared to healthy kidneys, we found significantly lower numbers of PTC in UUO day 3 (-11%) and day 5 (-16%), but not day 1. Capillary rarefaction was also observed after ischemia/reperfusion injury on both days 14 (-12%) and 21 (-17%). Compared to healthy kidneys, we observed significantly higher extravasation of Evans blue in UUO day 3 (+250%) and day 5 (+167%), but not day 1, ischemia/reperfusion injury day 14 (+460%) and day 21 (+157%) and in Alport mice (+100%). Compared to healthy kidneys, we found significantly more interstitial deposition of fibrinogen in the fibrotic kidneys in all three models (+109 to +459%). In fibrotic kidneys, ultrastructural studies revealed loss of fenestrations, increased thickness of endothelial cell soma and lamina densa of the PTC basal membrane and PTC widening.

Discussion and Conclusions: Independent of the underlying mechanism, all fibrosis models were characterized by progressive loss of renal microvasculature, a significant increase in vascular leakage and substantial alterations of the endothelial ultrastructure. These data show that renal fibrosis not only involves loss of PTC but also significant functional alterations of remaining capillaries.

Acknowledgement: Institutional Animal Care and Use Committee Approval: Approval Nr. 84-02.04.2011.A213 issued on 30.01.2012

Stress monitoring on gastrointestinal smooth muscle by electromyography

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Key words: stress, SEMG, smooth muscle, electric activity

Introduction: Smooth muscle electromyography (SEMG) is a method to observe the gastrointestinal and myometrial activity. Our research team has developed a new method and equipment to detect and analyze the electric activity of the smooth muscles in vivo. Our aim was to differentiate and characterize the signals from the different sequences of gastrointestinal (GI) tract and to detect changes in SEMG signal caused by stress.

Methods: For the SEMG signal characterization we used male SPRD rats. The stomach or small intestine or large intestine stayed intact; the rest of the GI tract was removed. We used two types of electrodes: filament electrodes were inserted into the target organ, while subcutaneous electrodes were placed above the organ.
In stress experiments we used awake, gastrointestinally intact rats with subcutaneous electrodes. These rats were recorded without restriction in their movements, and then immobilized, under stressful condition. At the beginning and at the end of these experiments we took plasma samples to measure the cortisol level.

Results: Characteristic signals of the stomach, ileum and coecum can be found at 3-4, 20-25 and 0-3 cycle per minute (CPM), respectively. Immobilization increased the electric activity of the GI tract. The intensities of electric signals were increased by 90%, 100% and 140% in the stomach, ileum and coecum respectively. These results and the effectiveness of the stress model have been confirmed by the changes in plasma cortisol level.

Discussion and Conclusion: We proved that the SEMG signals from GI tract can be characterized and separated from each other. We also revealed changes during in the GI tract, by experimentally-induced stress. SEMG seems to be useful method both for invasive and non-invasive detection of smooth muscle function in vivo.

Sources of funding: This work was supported by project PIAC_13, Ministry of National Development, Hungarian Government

Acknowledgement: Authors thank for Cedars Sinai Medical Center's International Research and Innovation Management 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).

All experiments were carried out with the approval of the Hungarian Ethical Committee for Animal Research (registration number: IV/198/2013).

Risk Assessment of the Genetically Modified Maize Pioneer Mon810

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Key words: GMO, subchronic toxicity studies, clinical biochemistry

Introduction: A key objective of GRACE project is to conduct 90-day animal feeding trials, animal studies with an extended time frame on genetically modified (GM) maize in order to comparatively evaluate their use in GM plant risk assessment. We present results of 90-day feeding study with GM maize Pioneer MON810 varieties, their near-isogenic non- GM varieties and two additional conventional maize varieties.

Methods: The rat feeding study on Pioneer MON810 was performed by taking into account the EFSA Guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed (EFSA Scientific Committee, 2011) and the OECD TG 408. Three dietary treatments represent the groups "control", "11% GMO" and "33% GMO". Two additional groups consisting of two conventional maize varieties with the same sample size per gender and group were included. Consequently, the factor "group" has five levels, namely "control", "11% GMO", "33% GMO", "conventional 1" and "conventional 2".

Results and Discussion: No statistically significant differences in feed consumption and in body weights of animals were observed in male as well as female rats in all five experimental groups. No signs of morbidity and mortality were observed throughout the 90-day feeding period. Histological changes were sporadically observed in the control and 33% GMO groups. In 11% GMO diet and 33% GMO diet groups the concentration levels of Ca and urea levels statistically significantly increased when compared to the control group. The Na concentration levels significantly decreased in all GMO groups against to the control group. Haematological changes were observed in all test groups.

Conclusion: Preliminary results show that the Pioneer MON810 maize at two various levels of diet did not cause significant adverse effects on health of male and female Wistar Han RCC rats after subchronic exposure. The study requires further experiments.

The research study supported by the European Commission 7th Framework Programme for the project GRACE 311957.

Acknowledgement: The study was approved by the Ethical Committee of the Slovak Medical University on November 11, 2011.

Animal model for the better understanding of bronchopulmonary dysplasia

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Key words: Animal model; bronchopulmonary dysplasia; pathomechanism; lipopolysaccharide; intrauterine infections

Introduction: The chronic lung disease of preterm infants is called bronchopulmonary dysplasia (BPD). In the Hungarian NICUs more than 50% of preterms born with less than 1000 grams are affected by BPD. The pathophysiology of the disease is complex, the exact mechanism and the mediators are still not known, so there is no proper therapy as well. There is an urgent need for an adequate animal model to reveal the underlying pathomechanisms.
The aim of our study was to create a relevant rat model with good reproducibility to investigate the formation and course of BPD.

Methods: Our study is based on the known clinical fact, that the intrauterine infections, inflammations have an important role in the disease. So pregnant Wistar rats were treated by intra-amnional endotoxin (lipopolysaccharide: LPS) injection to induce inflammation on the 20th gestational day. Control animals were injected with saline. The respiratory function and the carbachol - induced (11 and 22 mmol) bronchial resistance of the pups (n=6/group) were measured by whole-body pletysmography on the 2nd and 4th postnatal weeks. The structural alterations of the lung were analyzed by micro computertomoghraphy (CT) and routine histology methods.

Results: The offspring of LPS-treated mothers showed signs of inflammatory hyperreactivity on the 4th postnatal week. The bronchial resistance induced by muscarine-receptor agonist carbachol was significantly higher than that of the control group. The CT examination revealed decreased air content and increased inflammation of the airways on the 2nd week and these findings further increased by the 4th week. The routine histologic examination showed elevated number of granulocytes and lymphocytes. The lung structure of control pups was normal.

Discussion and Conclusion: We created a reproducible animal model with the characteristic functional and morphological changes of BPD. This model could be a useful to identify the mediators, target molecules and even to test new therapeutic methods.

Acknowledgement: Experiments were approved by the Institutional Animal Care and Use Committee (IACUC) Approval BA02/2000-5/2011

Changes in superior cervical ganglion of adult rats induced by gonadectomy

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Key words: superior cervical ganglion, sympathetic activity, postpubertal development, gonadectomy

Introduction: The superior cervical ganglion (SCG) is a paravertebral chain ganglion that supplies sympathetic innervations to the head and neck. It is a good model for studies of sympathetic nervous system. Neurons in the SCG could be distinguished in subpopulations and it was proved that neurochemical coding is specific to neurons that supply different tissues. Recently, a significant increase in the number of neurons in peripheral ganglia was observed between the third and eight month of age, due to a protracted maturation process. It was known that gonadal hormones have a significant influence on the number of neurons and the density of synapses in the SCG during the early postnatal period. Nevertheless, most of the studies included gonadectomy of neonatal rats, assuming that only in the early postnatal development substantial changes in the SCG could take place.
The aim of studies were to investigate changes in neurochemical subpopulations of SCG-neurons and dynamic of expression of nestin, a marker of neural progenitor cells caused by gonadectomy of sexually mature rats.

Methods: Male and female Sprague-Dawley rats were gonadectomized at the age of two months. After 30 days, they were sacrificed. Additionaly, male rats were sacrificed at 2, 3 and 6 months of age. SCGs were harvested and processed immunohistochemically.

Results: Significant decrease of nestin expression was observed between 2nd and 3rd month of age. The nestin-expression was significantly higher in SCG of female in comparison to male rats. Ovariectomy resulted in a decrease, while orchidectomy caused an increase of nestin expression. Gender and gonadectomy significantly influenced on mean diameter, density and neurochemical specificity of neurons in SCG.

Conclusion: Gonadal activity strongly influences the postpubertal development of SCG. That could be an explanation of gender differences in sympathetic activity, as well as the related diseases.

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Inhibitory effect of original synthetized isoquinoline derivatives for the rat uterus contraction

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Key words: isoquinoline, inhibition,Rho-kinases, rat uterus

Introduction: The Rho-kinases, members of Rho-family, have a pivotal role in regulation of smooth muscle contractility. ROCK I and ROCK II phosphorilate the myosin-phosphatase and the myosin light chain, that induces contraction in myometrium.
Earlier in studies have investigated the affinity of isoquinoline alkaloids (e.g.HA-1077, H1152P) to the Rho-kinases and these compounds notably inhibited the Ca^{2+} -independent process resulting smooth muscle relaxation.

Aim: In the present study we examined the influence of the newly synthetized isoquinoline derivatives for the Rho-kinases on the rat uterus.

Methods: Quantitative real-time PCR and Western blot analysis were used to determine the mRNA and protein expression of ROCK I and ROCK II in the non-pregnant, pregnant (day 5, 9, 13, 15, 18, 20, 22), during parturition and postpartum rat uterus (1,3,5,7 day after labour). The effects of isoquinoline derivatives on the uterine rings (non-pregnant,20 day of pregnancy and during parturition) have been investigated in an in vitro organ bath system in the concentration range of 10^{-10} – 10^{-5} M in cumulative manner.

Results: The mRNA and protein expression of Rho-kinases significantly decreased in the early stage of gestation and were constant to the 22 day of pregnancy. The level of ROCK I and ROCK II increased remarkable during parturition. In the course of in vitro contractility study we found some isoquinoline derivatives with uterus-relaxant effect during parturition and in non-pregnant rat uterus.

Discussion: Our results clearly demonstrated that, (1) the RhoA/ROCK system may plays an important role for regulation of uterine contraction during parturition, (2) the inhibitory effect of some isoquinoline derivatives significantly higher, when the expression of Rho-kinases are increased.

Conclusion: It may further be concluded that the new isoquinolines may be of therapeutic relevance as tocolytic agents in the future.

Acknowledgement: Thank you for Cedars Sinai Medical Center's International Research and Innovation Management 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).

All experiments involving animal subjects were carried out with the approval of the Hungarien Ethics Committee for Animal Research (registration number: IV/198/2013).

Abstracts

April 18, 2015

Poster Session #4

Clinical Research

Single-neuron novelty responses in the human substantia nigra during recognition memory

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Key words: substantia nigra, recognition memory, single neuron, DBS

Aims: The ability to distinguish novel from familiar stimuli is an essential part of learning. It was theorized that the substantia nigra acts as a novelty detector and thus supports the process of forming new memories.

Methods: We investigated this hypothesis by recording neuronal activity with microelectrodes during surgery for implantation of a deep brain stimulation device in the subthalamic nucleus. We presented awake patients with a sequence of images and asked them to classify each as novel or familiar. Simultaneously, we recorded single unit neuronal activity in the substantia nigra from five participants.

Results: Ten units (29 %) responded significantly different to novel compared to familiar pictures, with the majority (70 %) exhibiting higher firing rates to novel stimuli.

Discussion- Conclusion: This is the first evidence provided by human intracranial recordings that neurons in substantia nigra are indeed involved in detection of novel visual stimuli. This indicates that in addition to its role in motor learning, the substantia nigra is involved in the learning of visual information.

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Research was approved by Cedars-Sinai Medical Center's Institutional Review Board protocol nr Pro00030163

Evaluation of patient's opinion about herbal medicines and phytotherapy in Ukraine

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Key words: herbal medicines, rational phytotherapy, compliance, pharmaceutical care

Introduction: According to the WHO about 65-80% of the world's population that is living in developing countries depends essentially on herbal medicine (HM) for primary health care.

Methods: Survey of patients carried out with structured *questionnaire (*Ethical Committee Approval: 2014-05-26; No 5), elaborated at the department. We conducted the questionnaire polls of respondents at the pharmacies on one's own. The group of respondents was formed by random sampling. Statistical analysis of results was performed using computer software package STATISTICA v6.0.

Results: Questionnaire polls of 538 respondents on the application of HM and level of awareness of rational phytotherapy in Ukraine have been conducted. Patients, who consider HM effective (87.7%) use them in greater amount (4.20±2.87, $p<0.01$), and respondents who think that HM are ineffective, use only (1.94±2.15, $p<0.01$) these drugs. 68.4% of patients requires more information on phytotherapy and rational use of HM. Respondents, who need information on HM, use in average (4.07±2.86, $p<0.01$) drugs. Patients, who would like that HM for pharmacotherapy will be prescribed, use the greater amount of these medicines (4.22±2.94, $p<0.01$) as opposed those who do not want prescribing HM (2.33±1.82, $p<0.01$).

Discussion: The results of the evaluation of patients opinion regarding the use of HM and phytotherapy revealed a number of problems associated with the use of HM: an increased risk of adverse events (45.9% of patients believe that HM is absolutely safe); risk of adverse drugs interactions (40.8% of patients use HM simultaneously with synthetic drugs); polypharmacy (64% of patients use 3 or more HM simultaneously), that makes it necessary to analyze, monitor and resolve these problems, in particular, through system of pharmaceutical care.

Conclusion: We discovered the insufficient compliance between patient and physician concerning HM application and confirmed that the efficiency, safety and quality of phytotherapy depend on the completeness, accuracy and content of information received by patients.

Disturbance of regulatory mechanisms of spermatozoa in patients with infertility

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Key words: male infertility, spermatozoa, regulatory mechanisms, calcium, nitrix oxide.

Introduction. Infertility is a widespread complex problem affecting approximately 15 % of couples. Studies indicate that more than 40 % of infertility is related to male factor. Study of functioning of male germ cells, which play a crucial role in reproductive function, is crucially important. It is known that calcium ions and NO are second messengers regulating many *cellular functions*.

Methods. Spermatozoa of patients with male infertility and healthy donors were used. All patients and donors gave written informed consent to participate in research. The activities of ion-transporting systems were determined on the saponin-permeabilized spermatozoa. ATP-hydrolase activity was determined spectrophotometrically, registering process of ATP hydrolysis by the accumulation of inorganic phosphate. *Determination* of NO_2^- level was carried out using the *Griess reaction*.

Results and discussion. It has been shown that values of Ca^{2+}-ATPase activity of endoplasmic reticulus increased significantly in patients with infertility. Contrary to this, the activity of Ca^{2+}-independent ATPase systems (Na^+, K^+-ATPase, "basal" Mg^{2+}-ATPase and H^+-ATPase) decreases. Changes in ATPases activities indicate disturbance of ionic homeostasis in spermatozoa. It has been found that with increasing degree of oligozoospermia the calcium level increases in semen, spermatozoa and spermal plasma.
Numerous functions of spermatozoa are mediated by NO and its stable metabolites, acting as modulators of spermatozoa mobility, resistance to changes in environmental conditions, etc. In patients with oligozoospermia the NO_2^- level is not changed in comparison with control group (healthy donors). However, in patient with asthenospermia the NO_2^- level is significantly increased.

Conclusion. It is assumed that development of pathospermia correlates with impaired functioning of membrane-bond enzymes, including ion-transporting systems, disturbance of ionic homeostasis and NO-homeostasis of spermatozoa in patients with infertility

Acknowledgement: This study was approved by DHLNMU Ethical Committee No 8 from October 22, 2012).

Computer modeling assistance in planning of endoscopical osteosynthesis of the mandibular condyle

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Key words: mandibular condyle fractures, endoscopy, osteosynthesis.

Introduction: The frequency of mandibular condylar fractures varies from 8 to 76% of all the lower jaw fractures. Today, promising are endoscopic or endoscopically assisted osteosynthesis of mandibular condyle using intraoral access or transbuccal devices. Computer technologies and mathematical calculations are essential in planning and forecasting such operating procedures.

Materials and methods: To study mandibular condyle osteosynthesis three approaches were used: 1) plate with intraosseous rod; 2) one straight plate; 3) two straight plates. To study the fixative properties of the plates simulation models of computer (PCM) was used and the finite element method, implemented in today's complex software (*COSMOSWorks, Comsol Multiphysic*) to calculate the stress-strain state of the "condyle-device".

Results: A comparison of the reliability mandibular condyle osteosynthesis using plate with intraosseous rod, straight plate or two straight plates under conditions of masticatory forces was processed. In case of two plates osteosynthesis the largest tensions arise in plate, which is fixed with two screws. In case of osteosynthesis using plate with intraosseous rod maximum stress occurs in the region of the transition from direct plate to intraosseous rod. In case of one straight plate osteosynthesis tension occurs (249.9 MPa), significantly higher than the corresponding strain in the osteosynthesis of two plates (197.3 MPa) and a plate of intraosseous rod (87.1MPa).

Conclusions:

1. From the biomechanical point of view all the plates, can be successfully used for mandibular condyle osteosynthesis.
2. Using two straight plates for mandibular condyle osteosynthesis, in terms of strength, is much better than one straight plate, but intraorally it's technically difficult to install 2 plates, but it is possible in combination with transbuccal access.
3. Application of intraosseous rod plate for mandibular condyle osteosynthesis by intraoral access is reliable as under chewing and under the combined action of the lateral pterygoid and chewing muscles.

Acknowledgements: Authors thank to the Head of Department of Applied Mathematics of Ivan Franko Lviv National University, Doctor of Science, Professor Ya.G. Savulya for collaboration in theoretic-experimental part.

Experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of the Lviv National medical University, №4 from 10.05.2014.

April 19, 2015 (Sunday)

Departure

Goodbye

See you CSMC - RECOOP RESEARCHER at 6th TriNet Meeting in Prague on October 16 -18, 2015

RECOOP Visegrad Scholarship Program

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

The top ten young scientists selected during the Bridges in Life Sciences Annual Conferences have the opportunity to 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.

RECOOP HST Association in 2014 won two Visegrad Scholarships:

Post-Master's Scholarship:

Ivana Koborová
Institute of Molecular Biomedicine
Medical Faculty, Comenius University, Bratislava, Slovakia

Research project at the Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary from September 2014 to January 2015:

"Relationship of SSAO/VAP-1 and insulin resistance in adolescents"

In-Coming Scholarship:

Alexander Karmash
Intern at the Department of Regulation of Cell Proliferation and Apoptosis
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Research project at the Horváth Laboratory of Bioseparation Sciences at the Research Centre for Molecular Medicine, University of Debrecen, Hungary, September 2014 – January 2015:

"Role of disease-related changes in immunoglobulin IgG glycosylation"

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 Scholarships 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 maximum 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:
IKEM - Institute for Clinical and Experimental Medicine, Prague, Czech Republic
Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic
University of Debrecen, Hungary
University of Pecs, Hungary
University of Szeged, Hungary
Slovak Medical University, Bratislava, Slovakia

RECOOP HST Association Member Organizations allegeable for the In-Coming scheme
Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Kyiv, Ukraine
Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

RECOOP HST Association's Cedars – RECOOP Research Center (CRRC) could participate
Semmelweis University, Budapest, Hungary
Comenius University in Bratislava, Slovakia
Institute of Physics, Wroclaw University of Technology, Wroclaw, Poland
University Hospital in Hradec Kralove, Czech Republic

2015 Summer School of Scientific Communication: Publishing Research for Multidisciplinary Audiences
June 30 – July 3 2015
http://wp.ffzg.unizg.hr/sssc15/

2015 Summer School of Scientific Communication: Publishing Research for Multidisciplinary Audiences on June 30 – July 3 2015. CSMC – RECOOP will pay their travel and accommodation expenses.

Organized by: University of Split, Split, Croatia, and University of Zagreb, Faculty of Humanities and Social Sciences, Zagreb, Croatia

Place: University of Split
Number of participants: 16-20
Language of the Summer School: English
ECTS points for students: 4

Chairs of the School:
Prof. Ana Marušić, MD, PhD, University of Split School of Medicine, Split, Croatia
Prof. Ida Raffaelli, PhD, University of Zagreb Faculty of Humanities and Social Sciences, Zagreb, Croatia

Lecturers/Scientific Committee:
Prof. Elizabeth Wager, PhD, Sideview, UK
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The Summer School of Scientific Communication is organized by a group of journal editors with experience in teaching in scientific writing. The Summer School is the continuation of international writing workshops organized by the editors of the Croatian Medical Journal and the editors of other medical journals such as The Lancet, BMJ, JAMA and Annals of Internal Medicine.

The Summer School is targeted to doctoral students and young postdoctoral fellows from all scientific fields who are preparing their first manuscript or would like to improve their skills in writing and publishing in scientific journals. Participants are required to bring a draft of their manuscripts for the Paper Clinic of the Summer School.

Summer School in Split is unique in its multidisciplinary approach, both in the composition of its teaching team, selection of the participants and the topics of their manuscripts. Previous Summer Schools attracted young researchers from all over Europe and from different research fields, ranging from medicine and biotechnology to law, archaeology, linguistics and art history. Another unique feature of the Summer School is the focus on practical work with participants' manuscripts, in which formal lectures are arranged around Paper Clinic, where lecturers work through the manuscript with a small group of participants.

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