General Information

Arrival on October 10 (Thursday evening)

4th RECOOP TriNet Meeting

On October 11 (Friday), 2013 from 8:30 to 13:30 in the conference room
On October 12 (Saturday), 2013 from 9:00 to 18:00 in the conference room

Departure on October 13 (Sunday), 2013

Location of the meeting, accommodation and meals:

HOTEL DUJAM *** & YOUTH HOSTEL, Velebitska 27, 21000 Split – Croatia, Tel: + 385 (0) 21/273-080, fax: + 385 (0) 21/273-081, e-mail: info@hoteldujam.com, web: www.hoteldujam.com.

Arrival on October 10, 2013

You have to take care of your airport transfer and local transportation!

20:00 - 22:00 TriNet Kick off Dinner
Agenda

On October 11 (Friday), 2013 - 4th RECOOP TriNet Meeting – Day 1

Conference room

8:30 – 9:00 Welcome and RECOOP Review
Sandor G. Vari
Director, International Research and Innovation Management Program, Cedars-Sinai Medical Center & President of the RECOOP HST Association

9:00 – 9:15 Influence of body weight on bone mineral density in rats in experimental model of osteoporosis
Martin Gajdoš and Patrícia Kramárová
Faculty of Medicine, Slovak Medical University in Bratislava; Slovakia

9:15 – 9:40 Impact of Obesity and Stress on Cardiovascular Function
Marta Balog
Laboratory of neurobiology, Department of Medical Biology, School of Medicine Osijek, Croatia

9:40 -10:05 Effects of high fat diet, ovariectomy and exercise on obesity (OB) receptor expression in rat perirenal fat tissue and brain
Senka Blažetić
Department of Biology, University of Osijek, Osijek, Croatia

10:05 -10:20 SSAO/VAP-1 activity in the aorta and adipose tissues – the role of gender, obesity and stress
Tamas Tábi
Department of Pharmacodynamics, Semmelweis University, Budapest, Hungary

10:20 -10:35 Impact of ovariectomy, high fat diet, physical activity and antioxidant rich cookies on oxidative/antioxidative status in rat liver
Rosemary Vuković
Department of Biology, University J. J. Strossmayer of Osijek, Osijek, Croatia

10:35 – 10:50 Investigation of the possible role of stress and female hormones in the development of cardiomyocyte dysfunction
Attila Borbely
Institute of Cardiology, Division of Clinical Physiology, Medical and Health Science Center, University of Debrecen, Hungary

10:50 – 11:15 Coffee Break
11:15 – 11:30 Smooth muscle electromyography: an old-new method for myometrial and gastrointestinal investigations in vivo
Robert Gaspar
Department of Pharmacodynamics and Biopharmacy, University of Szeged, Hungary

11:30 – 11:45 The effects of prenatal stress on the early neurobehavioral development of rat pups
Tímea Kvárik
Department of Obstetrics and Gynaecology, Medical School, University of Pécs, Pécs, Hungary

11:45 – 12:00 The effects of intraganglionic injection of calcium/calmodulin-dependent protein kinase II inhibitors on pain-related behavior in diabetic neuropathy
Antonia Jelicic Kadic
Laboratory for Pain Research, University of Split School of Medicine, Split, Croatia

12:00 – 12:15 Intrathecal inhibition of calcium/calmodulin-dependent protein kinase II in diabetic neuropathy adversely affects pain-related behavior
Matija Boric
Laboratory for Pain Research, University of Split School of Medicine

12:15 – 12:30 Central regulation of food intake in stress and obesity
Katarina Sebekova
Institute of Molecular BioMedicine, Medical Faculty, Comenius University, Bratislava, Slovakia

12:30 – 12:40 FlexiForm 2.0 Electronic Data Entry Form
Gyula Markovics
Praxinfo, Miskolc, Hungary

12:40 – 13:00 Stress, Obesity, Bone Density, Metabolic and Cardiovascular Diseases Panel discussion
Sandor G. Vari

13:00 – 14:00 Lunch

14:00 – 19:00 Bland into Split and mingle with RECOOP Scientists

14:00 – 18:00 Optional sightseeing with bus – free of charge – participants should give three swab samples from their throat.
No pain just gain of knowledge about the city and about the enteroviruses we share in the same room.

19:00 – 21:00 Dinner
On October 12 (Saturday), 2013 - 4th RECOOP TriNet Meeting – Day 2

Conference room

9:00 – 9:15 Review the Progress
Sandor G. Vari

9:15 – 9:30 Collaboration in 2013 of the Institute of Cell Biology (Ukraine) with other institutions of the RECOOP-HST Association
Rostyslav S. Stoika
Institute of Cell Biology (ICB) Lviv, National academy of Sciences of Ukraine

9:30 – 9:45 Combining Stem Cells and Biomaterials for Brain Repair – Unlocking the Potential of the Existing Brain Research through Innovative In Vivo Molecular Imaging
Marija Lovrić
Senior Scientist on GlowBrain project, University of Zagreb, School of Medicine, Croatia

9:45 – 10:00 Recent progress in light emitting inorganic nanocrystals growth, their functionalization and potential use in biomedicine
Artur Podhorodecki,
Institute of Physics, Wroclaw University of Technology, Wroclaw, Poland

10:00 – 10:15 Native and synthesized magnetic nanoparticles and their excitotoxic potential
Tatiana Borisova
Department of Neurochemistry, Palladin Institute of Biochemistry (Kiev) National Academy of Sciences of Ukraine

10:15 – 10:30 In vivo monitoring of transport of poly(L-lysine)-modified iron oxide nanoparticle-labeled macrophages in a rat
Daniel Horák
Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic

10:30 – 10:45 Potential capacities of MRI and Bioluminescent Imager in GlowBrain for in vivo detection of nanoparticles
Marija Lovrić
Senior Scientist on GlowBrain project, University of Zagreb, School of Medicine, Croatia

10:45 – 11:00 Detection of nanoparticles in Blood – Brain and Human Placenta Barrier
Sandor G. Vari
Director, International Research and Innovation Management Program, Cedars-Sinai Medical Center & President of the RECOOP HST Association
11:00 – 11:15 Coffee Break

11:15 -11:45 Nanomedicine Panel Discussion
Sandor G. Vari

11:45 – 12:00 Dry throat swab sampling for enterovirus PCR diagnosis
Shubhada Bopegamage
Enterovirus Laboratory, Slovak Medical University, Bratislava, Slovak Republic

12:00 – 12:15 The importance of estimating the correct gestational age and monitoring the pregnancy in preterm birth
Cristian Poalelungi
“Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania

12:15 – 12:30 Murine cytomegalovirus defective in myeloid-cell dissemination functions exhibit decrease CNS invasiveness in newborn animals.
William Britt
Division of Infectious Diseases, Department of Pediatrics, University of Alabama at Birmingham, Birmingham, Alabama, USA

12:30 – 12:45 Cervical fluid IL-6 - a possible predictor of early onset sepsis in pregnancies complicated by late PPROM.
Marian Kacerovsky
Department of Obstetrics and Gynecology, University Hospital in Hradec Králové, Czech Republic

12:45 – 13:00 Mother and Child Health Panel Discussion
Sandor G. Vari

13:00 -14:00 Lunch Break

14:00 – 14:15 Testing the Timing Hypothesis of Atherosclerosis Prevention in Women
Jan Pitha
Laboratory for Atherosclerosis Research, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic

14:15 – 14:30 Impact of adipose tissue and related inflammatory mechanisms on the atherosclerosis
Králová Anna
Laboratory for Atherosclerosis Research, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic

14:30 – 14:45 Risk factors and gender differences at all sites in the RECOOP WH and CVD Retro Study
Dražen Mlinarević
Emergency Cardiology, Clinical Hospital Osijek and Department of Medical Biology, School of Medicine Osijek,

14:45 – 15:15 Women’s Health and CVD Panel Discussion: Intervention of Cardiovascular Risk Factors in Women Exposed to Different Hormonal and Environmental Impacts
Moderator: Jan Pitha

15:15 – 16:00 RECOOP Project Review
Sandor G. Vari

CEE Public Health Awareness Program
CEE NIH Visiting Fellow program
Capacity building for science communication and grant writing

16:00 – 16:30 Coffee Break
Consultation - FlexiForm 2.0 Electronic Data Entry Form
Gyula Markovics

16:30 – 17:30 All About Health Care Data
Linn Defensor
Office of Research Compliance and Quality Improvement, Cedars-Sinai Medical Center, Los Angeles, CA, USA and RECOOP HST Consortium CTSMN Project Leader

17:30 – 18:30 Team and Project Building in the RECOOP Research Networks

Mother and Child Health
Moderator: Chander P. Arora, Research Project Adviser, International Research and Innovation Management Program, Cedars-Sinai Medical Center, Los Angeles, CA, USA

RETRO Manuscript
Relativity of risk factors
Prospective Studies:
   Cytomegalovirus Screening in Mothers and Newborns
   Coxsackievirus B (CVB) Study in Mothers and Newborns
   IL-6 in cervical fluid in late PPROM pregnancies

Women’s Health & CVD
Moderator: Zoltan Papp, Institute of Cardiology, Clinical Physiology Department
University of Debrecen, Hungary

RETRO Manuscript
Prospective Study: Lifestyle Intervention in Women in Different Reproductive Stage with Different Risk Factors
Shared decision making in CVD lifestyle intervention with primary care physicians and patients
Translational Research:
- Obesity
- Stress
- Central regulation of food intake in stress and obesity
- Mitochondrial Apoptosis and dysfunction in obesity, diabetes and CVD

**NanoBioTechnology**
Moderator: Rostyslav Stoika

- Investigating use of nanocrystals in biomedicine (melanoma, brain and breast tumor)
- Modified iron oxide nanoparticle in MRI imaging
- Femtonics two-photon (2P) microscope for test
- MRI and Bioluminescent Imager for in vivo detection of nanoparticles in Blood – Brain and Human Placenta Barrier
- Drug delivery via nanoparticles

**October 13 (Sunday), 2013 - Departure**
CEE Public Health Awareness Program

IVF Standard Grant Application ID 21320228 “Cartoonists and Scientists Improve the Knowledge on Brain Disorders in V4 and Neighboring Countries”

RECOOP’s research activities could be reviewed in the Biopolymers and Cell Journal (www.biopolymers.org.ua); 2010-13 Vol. 26., 27., 28., 29., N2 supplementary. RECOOP and the Brain Sneezing Group – BSG (http://cartooneast.com/category/index/item_id/2) built a creative platform for cartoonists and medical professionals could bring some fresh ideas into the way of thinking of scientists, physicians and cartoonists. In 2013 implemented the CEE Public Health Awareness Program and will organize the “Brain Sneezing Day” (BSD) as part of the Dana Foundation’s worldwide Brain Awareness Week - BAW (bawinfo@dana.org). Following that every year RECOOP and BSD will organize Public Health Awareness Days for prevention of cardiovascular diseases (Heart Beat Together), obesity (Beat the Drum and Loose the Fat), diabetes (Eat Less to Prevent Diabetes), preterm birth (Healthy Mother - Happy Child) and cancer (Together we Prevent to be a Ghost). We would like to involve the Clinton Foundation.

CEE NIH Visiting Fellow program

IVF Standard Grant Application ID 21320181 “Continuity and Sustainability of Cross Atlantic Life Science Collaborations in the V4 Countries”

RECOOP HST Association would like to build a joint venture with the Clinton Foundation to establish a Central and Eastern European (CEE) NIH Visiting Fellow program for young physicians and post docs. The NIH training program has two phases. Phase I., is postdoctoral research training has to be completed at NIH Institutes. The pre-selection will be made by RECOOP will guaranty young scientists have secured research projects at home (Croatia, Czech, Hungary, Romania, Slovakia &Ukraine). NIH already partners with several countries/regions are partially funding Phase I. https://www.training.nih.gov/international_career_transition_awards. The annual cost of one fellow is 70K. RECOOP shall secure 50% partially fund of annual cost of the CEE NIH Visiting Fellows. RECOOP is creating a CEE NIH Visiting Fellow Fund to provide support for 10 young scientists from CEE (35K/young scientists/year total 350K/year). In Phase II seventeen Cedars–RECOOP Research Centers will host and provide re-entry grants with home country co-funding.
Capacity building for science communication and grant writing

Visegrad 4 and Eastern Partnership Flagship Project ID 31350027 “Capacity-Competitiveness Building in V4 - EaP countries for science communication and grant writing”.

RECOOP HST Association and NIH/NCI would like to increase the number of young scientists capable of writing good quality peer-review articles and competitive grants. In May 2014 Split, Croatia a two days workshop, in August 2014 (Prague, Czech Republic) and in July 2015 Lviv, Ukraine five days Summer Schools are proposed by the RECOOP HST Association with the Center for Global Health of the U.S. National Cancer Institute (NCI) of the National Institutes of Health to discuss the most important aspects of writing a research article, specifically the different sections and the language that can be used to accomplish one’s goals in those sections of an article. A considerable amount of time will be spent on how authors can analyze texts to understand language choices and see how writing is much more than having the right general organization and achieving technical accuracy. The same method will be used for grant writing. The participating young scientists with the help and supervision of the tutors will convert their Young Scientist Research Award Phase I application prior sent to the tutors into Phase II.
Abstracts

Faculty of Medicine, Slovak Medical University in Bratislava; *Faculty of Public Health, Slovak Medical University in Bratislava, ** Faculty of Medicine, Comenius University in Bratislava

**Background:** In general, overweight and obesity are considered to be the significant risk factors of development and progression of several chronic diseases, whereas the influence of body weight and obesity on bone mineral density is traditionally regarded as beneficial. Currently, this paradox is not clearly explained.

**Aim:** Aim of the study was to investigate the influence of standard diet and high fat diet on body weight and bone mineral density in ovariectomized and non-ovariectomized rats.

**Materials and methods**

**Animals and study design:** Study was approved by the Ethical committee for animal experimentation of Slovak Medical University and by the State Veterinary and Food Administration of the Slovak Republic. Thirty-two female Wistar rats (4 weeks old, body weight 130-150 g) were housed in an air-conditioned room (relative humidity 55±5%, 20-24°C) under a 12-h light/dark cycle and given free access to food and tap water. After 7 days quarantine acclimated rats were randomly divided into two dietary groups, either a standard diet group (SD; SP1, Top Dovo, Czech Republic) or high fat diet (HFD; D12451 (I) mod. 45 kJ% fat, ssniff Spetzialdiätten GmbH, Soest, Germany). During the all experiment, food intake and body weights were measured one times every week. Following the eight-week dietary intervention, the rats from both dietary groups were subjected to either ovariectomy (OVx) or sham (SH) surgery. After 2 weeks of convalescence, all rats continued in standard or high fat diet next 8 weeks. Total body composition and bone mineral density were measured a week before the surgery and at the end of experiment.

**Ovariectomy:** In 16 rats (8 from SD group, 8 from HFD group), bilateral OVx was performed using the dorsal approach. The next 16 rats from both dietary groups were subjected to sham surgery.

**Total body composition and bone mineral density:** After anesthesia total BMD (bone mineral density), total BMC (bone mineral content), total tissue, total fat and total lean were analyzed by using a Lunar Prodigy Advance with Encore 2011 software version 13.60 (Ge Medical Systems, Madison, WI).

**Results**

Values of body weight, total bone mineral content gain (ΔtBMC) and total bone mineral density gain (ΔtBMD) after 10 days of SD/HFD administration in all groups (OVx/SH) are shown in Table 1.

*p<0.05, **p<0.01, ***p<0.0001
We observed the increase of body weight and ΔtBMC in SD-OVx group and HFD-SH group if compared to SD-SH group, ovariectomy had the lowest effect. The combination of both of these factors (HFD-OVx) had the most significant effect. The influence of these factors on ΔtBMD was opposite, the administration of high fat diet to animals with ovariectomy had the lowest effect.

**Discussion**

The positive relationship between body weight and tBMC and clearly confirmed inverse relationship between body weight and tBMD show that the quality of bone tissue decreases in rats with increased body weight. Because both the total body weight and the total bone mass content increase, we can assume that the increased tBMC is not adequate for keeping the sufficient quality of bone tissue. These findings do not confirm the assumption of preventive effect of increased body weight on bone quality.

"This article was created by the realization of the project "Center of excellence of environmental health", ITMS No.24240120033, based on the supporting operational Research and development program financed from the European Regional Development Fund."

**Acknowledgement:**

The presented research work is part of a multisite study “Obesity, Bone Density and Cardiovascular Diseases” supported by 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).
Impact of Obesity and Stress on Cardiovascular Function
Senka Blažetić¹, Barbara Viljetić², Marta Balog³, Sandor Varí⁴, Martin Gajdoš⁵, Irena Labak¹, Marija Heffer³

¹ Department of Biology, University of Osijek, Osijek, Croatia;
² Department of Chemistry and Biochemistry, School of Medicine, University of Osijek, Osijek, Croatia;
³ Department of Medical Biology, School of Medicine, University of Osijek, Osijek, Croatia;
⁴ International Research and Innovation Management Program, Cedars - Sinai Medical Center, Los Angeles, CA, USA; President of the RECOOP HST Association
⁵ Department of Clinical and Experimental Pharmacotherapy, Medical Faculty, Slovak Medical University

Abstract

Aim of the whole study is identification of differences in development of cardiovascular pathology (heart, autonomous ganglia, blood vessels and fat tissue) upon acute and chronic stress. We analyzed plasma levels of glucose, C-reactive protein (CRP), uric acid and cholesterol as cardiovascular diseases markers in male, female and ovariectomized rats under acute and chronic stress. Chronic stress was performed during 10 days and rats were sacrificed at the age of 3 months. The results of this study demonstrate a significant difference between tested groups in plasma glucose, uric acid and cholesterol levels upon acute stress. One part of the results was written as a paper "Plasma Content of Glucose, C-reactive Protein, Uric Acid and Cholesterol in Male, Female and Ovariectomized Rats Upon Acute and Chronic Stress – a Path for Development of Cardiovascular Diseases" which was published in Collegium antropologicum journal.

We improved the initial pilot study on stress and obesity, so new study is in progress. Chronic stress protocol was repeated 3 times during 10 days, while rats were sacrificed at the age of 6 and a half months. At the moment only female rats (ovariectomized and non-ovariectomized) were included in the study, but male group will also be included at the end of October 2013. Longer and repeated chronic stress protocol showed differences in glucose tolerance test results. Anthropological data showed that ovariectomy has an impact on medistinum and heart weight. Samples are being analysed at this moment.

In April 2013, collaboration with prof. Gajdoš group from Bratislava Medical School was established. Main goal was to see the impact of ovariectomy and exercise among female rats under standard or high fat diet. Anthropological data show distinct pattern of changes. Samples we collected (brain, fat tissues, ganglia, heart) are being analysed at this moment.

This study is part of Women’s Health and Cardiovascular Diseases Research Network of RECOOP HST Consortium formed by CSMC, Los Angeles, CA, USA.
Effects of high fat diet, ovariectomy and exercise on OB receptor expression in rat perirenal fat tissue and brain

Senka Blažetić¹, Barbara Viljetić², Marta Balog³, Sandor Vari⁴, Martin Gajdoš⁵, Irena Labak¹, Marija Heffer³

¹ Department of Biology, University of Osijek, Osijek, Croatia;
² Department of Chemistry and Biochemistry, School of Medicine, University of Osijek, Osijek, Croatia;
³ Department of Medical Biology, School of Medicine, University of Osijek, Osijek, Croatia;
⁴ International Research and Innovation Management Program, Cedars - Sinai Medical Center, Los Angeles, CA, USA; President of the RECOOP HST Association
⁵ Department of Clinical and Experimental Pharmacotherapy, Medical Faculty, Slovak Medical University

Abstract

Obesity is a result of an imbalanced diet and energy expenditure. Ob protein or leptin is a product of the ob gene, and it is produced by white adipose tissue.

The aim of this study was to determine effects of high fat diet (HF), ovariectomy (OV) and physical activity (PA) on OB receptor distribution in two different brain regions: barrel cortex (S1BF) and lateral hypothalamus (LH). Also, to see if there is a correlation between brain OB receptor distribution and perirenal and subcutaneous fat tissue OB receptor concentration.

The distribution of OB receptor was determined by free-floating immunohistochemistry on coronal sections of rat brain using OB receptor antibody, while fat tissue OB receptor was detected by Western blotting.

Western blot analysis of perirenal fat showed that OB receptor is expressed in HF rats with no PA. Ovariectomy didn’t cause any effect on OB receptor expression in same group of rats. Based on the brain immunohistochemistry expression of OB receptor is increased in S1BF region in HF-OV-PA group of rats. Contrary to S1BF region in LH region there is decrease in OB receptor expression.

This study shows that there is a correlation between high fat diet followed by physical activity and expression of OB receptor in brain and perirenal fat tissue of rats. 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
SSAO/VAP-1 activity in the aorta and adipose tissues – the role of gender, obesity and stress

Tábi, T., Szökő, É.

Department of Pharmacodynamics, Semmelweis University, Nagyvárad tér 4., H-1089 Budapest, Hungary

Semicarbazide Sensitive Amine Oxidase/Vascular Adhesion Protein 1 (SSAO/VAP-1) is an enzyme that functions also as an adhesion protein. It metabolizes primary amines to aldehyde, hydrogen peroxide and ammonia. Its adhesion molecule function is dependent on its enzyme activity likely by using leukocyte surface bound amine as substrate. SSAO/VAP-1 participates in the accumulation of leukocytes at the site of inflammation. In addition to the membrane bound form it is also present in the plasma in soluble form.

The SSAO/VAP-1 can contribute to cardiovascular diseases by several mechanisms.

The enzyme is highly expressed in the vascular endothelium and smooth muscle and produces toxic compounds that may induce oxidative and carbonyl stress. This process can contribute to the development and progression of endothelial dysfunction and atherosclerosis. As an adhesion protein it participates in the leukocyte adhesion, migration and infiltration to the site of inflammation, thus this way SSAO/VAP-1 may contribute to the development of low grade inflammation characteristic for the cardiovascular diseases. Pharmacological inhibition of the enzyme function may be valuable as it is able to reduce both the oxidative stress and the inflammation.

SSAO/VAP-1 is also highly abundant in the adipose tissues, where it is upregulated during adipocyte maturation. It may participate in the leukocyte invasion to the adipose tissue and consequently in the development of specific inflammation in obesity. On the other hand, via the hydrogen peroxide formed it can augment the effect of insulin on glucose uptake. In this work we have studied the SSAO activity in the aortae and adipose tissues of rats to examine the gender difference and the effect of obesity and stress. The tissue samples were provided by Marija Heffer and Radivoje Radic from Osijek, Croatia and Zora Krivosikova from Bratislava, Slovakia.

The role of the gender was found to be moderate and mainly affected the subcutaneous adipose tissue. The obesity on the other hand accompanied with increased enzyme activity in all examined tissues and the elevation was ameliorated by training. Stress again had less pronounced effect and mainly influenced the enzyme activity in the adipose tissues.

Acknowledgement:

The presented research work would not have been possible without the support of 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).
Impact of ovariectomy, high fat diet, physical activity and antioxidant rich cookies on oxidative/antioxidative status in rat liver

Rosemary Vuković, Senka Blažetić, Ivana Oršolić, Marija Heffer, Sandor Vari, Elizabeta Has-Schön

1 Department of Biology, University J. J. Strossmayer of Osijek, Osijek, Croatia; 2 Department of Medical Biology, School of Medicine, University J. J. Strossmayer of Osijek, Osijek, Croatia; 3 International Research and Innovation Management Program, Cedars - Sinai Medical Center, Los Angeles, CA, USA; President of the RECOOP HST Association

The lack of protective action of estrogens is known to cause serious metabolic disturbances, and oxidative stress is thought to be one of the suspected mechanisms. Numerous in vitro studies have indicated possible relationship between estrogens and liver oxidative damage, while oxidative stress being one of the key pathophysiological mechanism in liver disease associated with obesity, may serve as a predictor of cardiovascular diseases. The aim of this study was to estimate the effect of high-fat diet (HFD) on the oxidative/antioxidative status in liver of the ovariectomized rats, and to investigate possible ameliorating effect of lifestyle modifications (such as physical activity or antioxidant rich dietary supplements) on oxidative damage in liver. As an indicator of liver oxidative damage, LPO levels expressed in terms of thiobarbituric acid reactive substances (TBARS) were determined, while liver antioxidative status was determined by catalase (CAT), glutathione peroxidase (GPX), glutathione S-transferase (GST), glutathione reductase (GR) activities and glutathion (GSH) content. Eight groups of rats were tested: sham operated and ovariectomized rats that received standard diet (SD) or HFD, sham operated and ovariectomized rats that received HFD and were subjected to physical activity, and ovariectomized rats received HFD supplemented with anti-oxidant rich “cookies“, with and without physical activity. Results showed that HFD significantly increased TBARS content in liver relative to control groups that received SD. Furthermore, HFD decreased antioxidant defense capacities, as evaluated by the significantly decrease in the activities of CAT, GR and GST as well as the level of GSH. GPX activity remained unchanged in all group tested. Physical activity and “cookies” showed protective effect through increased CAT activity in ovariectomized rats, while had no effect on other measured parameters. These results indicate that high-fat diet affected oxidative processes as well as antioxidative defense mechanisms of liver in both ovariectomized and sham-operated groups.
Investigation of the possible role of stress and female hormones in the development of cardiomyocyte dysfunction

J. Kalász, A. Tóth, E. Pásztorné-Tóth, I. Édes, Z. Papp, *M. Heffer-Lauc, A. Borbély
University of Debrecen, Medical and Health Science Center, Institute of Cardiology, Division of Clinical Physiology, H-4032 Debrecen, Móricz Zsigmond krt. 22, Hungary,
*Department of Biology and Neuroscience, School of Medicine, Osijek, Croatia

Background: The relationship between female hormones, stress and cardiovascular function was suggested previously. Epidemiological studies indicate that chronic heart failure is more prevalent in men in comparison with premenopausal women. Stress-induced (takotsubo) cardiomyopathy, however, is more likely to occur in elderly female patients. Limited data are available on the effects of acute and chronic stress and female hormones in the development of cardiac contractile dysfunction.

Aim: To perform a comparative mechanistic and biochemical study on myocardium derived from of ovarectomized and non-ovarectomized female rats exposed to acute and chronic stress.

Materials and methods: Left ventricular myocardial samples of sham operated (control), ovarectomized and non-ovarectomized female rats subjected to acute (immobilization at +4 °C for 3 hours) and chronic stress (immobilization at +4 °C for 3 hours daily for 14 days) were used for isometric force measurements in permeabilized, isolated cardiomyocytes (determination of Ca-dependent active force (F_{active}), Ca-independent passive force (F_{passive}) and Ca-sensitivity of force production (pCa_{50})). Stress-induced myofilament protein alterations will be assessed using Pro-Q® Diamond phosphoprotein and Sypro® Ruby protein stainings.

Results: Based on our preliminary results cardiomyocytes of rats exposed to acute stress have significantly lower pCa_{50} than chronically stressed and control animals. This difference in acutely stressed animals seems to be blunted after ovarectomy. No major changes could be observed in cardiomyocyte F_{active} between the animal groups. Interestingly, F_{passive} in cardiomyocytes of ovarectomized rats subjected to chronic stress was two-fold higher compared to controls or acutely stressed animals. Biochemical assessment of the samples is under evaluation.

Conclusions: Female hormones, intensity and the duration of the stress seem to be an important contributor in the development of left ventricular contractile dysfunction. Acute stress may alter cardiomyocyte function via decreasing Ca sensitivity of force production, hence chronic stress in the lack of female hormones seems to impair Ca-independent force generation.

Acknowledgements: This research was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TÁMOP 4.2.4. A/2-11-1-2012-0024 ‘National Excellence Program’ and by the OTKA PD 108614 grant.

Keywords: cardiomyocytes, contractile dysfunction, stress, female hormones, myofilaments.
Smooth muscle electromyography: an old-new method for myometrial and gastrointestinal investigations in vivo

Robert Gaspar PharmD, PhD, Department of Pharmacodynamics and Biopharmacy, University of Szeged, Hungary

The beginning of smooth muscle electromyography (SEMG) goes back to 1920’s when the first electrogastrogram was recorded. During decades the technique was tried to use for prediction of the functional disturbances of gastrointestinal system. In contrast to the EMG of the skeletal musculature, cardiac muscle and electroencephalography, the density of information of SEMG is low and sensitive to interference due to respiration, or electrical heart activity. For this reason, smooth muscle electromyograms are difficult to record. That was the reason that the interest was reduced toward the method in 1990’s. However, the development of computing opened a new window for SEMG involving sophisticated filters and recording techniques for more precise analysis of smooth muscle electrical signals separating them from cardiac, brain and other electrical interferences.

Cooperating with Experimetria Ltd. and Adexgo Ltd., Hungary, we developed SEMG equipment which is useful even in alert (unanaesthetized) rats. Electrodes were inserted into the smooth muscles (myometrium, bowel), or placed subcutaneously. We were able to follow the changes of uterine activity in late pregnancy, even during labor. We proved that the electric signals from myometrium and gastrointestinal tract can be characterized and separated from each other by fast Fourier transformation and filtering of the primary curves. The effects of uterorelaxant and uterine stimulant agents were also detectable and correlated with the changes in mechanical contractions. We also revealed changes during pathophysiological condition of the colon, like experimentally-induced colitis.

SEMG seems to be a useful method both for invasive and non-invasive detection of smooth muscle function even in alert animals. This technique may have great importance as method of integrative pharmacology and a potential tool for human diagnostic.
The effects of prenatal stress on the early neurobehavioral development of rat pups

Mammel B\textsuperscript{1,2}, Kvárik T\textsuperscript{1,2}, Bodzai G\textsuperscript{1}, Matkovits A\textsuperscript{1}, Gyarmati J\textsuperscript{2}, Ertl T\textsuperscript{2}, Reglődi D\textsuperscript{1}, Tamás A\textsuperscript{1}, Kiss P\textsuperscript{1}, Farkas J\textsuperscript{1}

\textsuperscript{1}Department of Anatomy, PTE-MTA Lendulet Research Group, \textsuperscript{2}Department of Neonatology, Medical School, University of Pécs, Szigeti út 12., Pécs, Hungary

Introduction: Pre- and perinatal periods are the most important periods of ontogeny, during which the developing fetus may be exposed to several harmful stimuli. Evidences from epidemiological and clinical studies indicate that these stimuli have short and long-term effects on the newborns. The aim of the present study was to investigate the influence of maternal stress during different terms of pregnancy on the early physical and neurological development of the rat pups.

Methods: Pregnant Wistar rats were exposed to restrain stress for 1 hour per day in different periods of pregnancy (1, 2. and 3. term) by restricting them in moving. After delivery, the offspring were tested for somatic and neurobehavioral development daily for the first 3 weeks. Data were compared to those of the control group - made up of pups from the same age group–by using ANOVA as the statistical method.

Results: Our results showed that maternal stress during the second term of pregnancy retarded the development of several somatic signs and neurological reflexes (incisor eruption, ear unfolding, ear twitch, forelimb placing, acustic startle, air righting). We also observed a delay in the development of maturation signs of the pups whose mother had been stressed in the first term of pregnancy (ear unfolding, ear twitch, eyelid reflex, forelimb placing and grasp, auditory startle). In contrast, third-term maternal stress did not have a marked influence on the development of the newborns.

Conclusion: These data suggest that exposure to stress in the early and middle term of pregnancy may impair the somatic and neurological development of the rat pups. On the other hand, third-term maternal stress had no marked effects on the examined parameters. Our findings may help to reveal the later, adulthood effects of fetal stress.

Acknowledgements: OTKA K104984, TAMOP (4.2.1.B-10/2/KOV-2010-002, 4.2.2.B-10/1-2010-0029, 4.2.2.A-11/1/KOV-2012-0024), Arimura Foundation, PTE-MTA “Lendület” Program.
The effects of intraganglionic injection of calcium/calmodulin-dependent protein kinase II inhibitors on pain-related behavior in diabetic neuropathy

Antonia Jelicic Kadic*,#, Matija Boric*, Sandra Kostic*, Damir Sapunar*, Livia Puljak*

Introduction: Calcium/calmodulin-dependent protein kinase II (CaMKII) has been implicated in transmission of nociceptive input in diabetic neuropathy. The aim of this study was to test whether intraganglionic injection of CaMKII inhibitors may alleviate pain-related behavior in diabetic rats.

Methods: Diabetes was induced in Sprague-Dawley rats using 55 mg/kg streptozotocin intraperitoneally. Two weeks after diabetes induction, CaMKII inhibitors myristoil-AIP and KN93 were injected directly into dorsal root ganglion (DRG). Behavioral testing with mechanical and thermal stimuli was performed before induction of diabetes, the day preceding the injection, as well as 2 h and 24 h after the intraganglionic injection. The expression of total CaMKII and its alpha isoform in DRG neurons was analyzed using immunohistochemistry.

Results: CaMKII inhibitors attenuated pain-related behavior in a modality-specific fashion. Attenuation of nociceptive behavior was accompanied with corresponding decrease of CaMKII alpha expression in DRG neurons on the side of injection. Significant decrease of CaMKII alpha expression was seen in small and medium-sized neurons.

Discussion: Studies using intraganglionic delivery of pharmacological agents have been seldomly used in the basic studies of nociception. Most of the studies are using only one CaMKII inhibitor (KN-93) and one-time assessment. Pretreatment, posttreatment, as well as combination of both were studied. In this study, only posttreatment approach was used, since diabetic neuropathy occurs after the onset of the disease. We found that two CaMKII inhibitors, with different mechanism of action, may attenuate pain-related behavior in a modality-specific fashion.

*Affiliation: Laboratory for Pain Research, University of Split School of Medicine, Soltanska 2, 21000 Split, Croatia

#Corresponding author: E-mail address: antonia.jelicic@mefst.hr (A.Jelicic Kadic)
Intrathecal inhibition of calcium/calmodulin-dependent protein kinase II in diabetic neuropathy adversely affects pain-related behavior

Antonia Jelicic Kadic*, Matija Boric*,#, Lejla Ferhatovic*, Adriana Banozic*, Damir Sapunar*, Livia Puljak*

Introduction: Calcium/calmodulin-dependent protein kinase II (CaMKII) is considered an important enzyme contributing to the pathogenesis of persistent pain. The aim of this study was to test whether intrathecal injection of CaMKII inhibitors may reduce pain-related behavior in diabetic rats.

Methods: Male Sprague-Dawley rats were used. Diabetes was induced with intraperitoneal injection of 55 mg/kg streptozotocin. Two weeks after diabetes induction, CaMKII inhibitor myristoil-AIP or KN-93 was injected intrathecally. Behavioral testing with mechanical and thermal stimuli was performed before induction of diabetes, the day preceding the injection, as well as 2 h and 24 h after the intrathecal injection. The expression of total CaMKII and its alpha isoform in dorsal horn was quantified using immunohistochemistry.

Results: Intrathecal injection of mAIP and KN-93 resulted in significant decrease in expression of total CaMKII and CaMKII alpha isoform activity. Also, mAIP and KN93 injection significantly increased sensitivity to a mechanical stimulus 24 h after i.t. injection.

Discussion: A study of two CaMKII inhibitors, employing a full range of behavioral tests, has shown that reduction of CaMKII expression may contribute to increased pain-related behavior in streptozotocin-diabetic rats. Results of this study are opposing current opinion in the field. There are several potential molecular mechanisms that could explain our findings. An increase of CaMKII and its phosphorylation could be protective in short or long run for certain aspects of pain. Additionally, increase of CaMKII and its phosphorylation may be just one of the mechanisms involved in pain-related behavior. Further studies should elucidate the exact role of CaMKII in nociceptive processing in dorsal horn of diabetic models.

*Affiliation: Laboratory for Pain Research, University of Split School of Medicine, Soltanska 2, 21000 Split, Croatia

#Corresponding author: E-mail address: matija.boric.st@gmail.com (M.Boric)
**Study plan: Stress, obesity and central regulation of food intake**
Professor Katarina Sebekova, Institute of Molecular BioMedicine, Medical Faculty, Comenius University, Bratislava, Slovakia

**Professor Sebekova recommendation for studies to investigate**

- Food-related odors affect the expression of orexigenic/anorexigenic receptors, and food and satiety-related signaling
- Effects in the brain areas involved in the central regulation of food intake, control of the body energy homeostasis, and/or in the brain reward centers

**Study 1: Insulin resistant states affect the expression of insulin and leptin receptors**

Under physiological conditions anorectic peptides insulin and leptin enhance the excitability of olfactory sensory neurons in the absence of odorants, but reduce the odorant-induced transduction currents and receptor potential (Pan & Kastin, 2007). In rats, food-related odors affect the expression of orexigenic/anorexigenic receptors, and food and satiety-related signaling (c-Fos expression) in various brain regions (Šebeková et al., 2012). The highest concentrations of insulin and insulin receptors within the central nervous system (CNS) are found in the olfactory bulb, which also exhibits the highest insulin transport rate across blood-brain barrier, and the highest rate of insulin degradation (Niswender & Schwartz, 2003). While BBB transporter for leptin differs from leptin receptor, the relation of the insulin BBB transporter to that of the insulin receptor remains unresolved, and insulin appears to act as its own counter-regulatory hormone after crossing the BBB (Banks, 2004). Insulin resistant states are characterized by persistently elevated levels of circulating anorectic peptides insulin and leptin. However, it remains unclear whether and how insulin resistant states affect the expression of insulin and leptin receptors, and thus the modulation of anorexigenic/orexigenic signaling in CNS.

**Study 2: Central regulation of food intake**

Effects in the brain areas involved in the central regulation of food intake, control of the body energy homeostasis, and/or in the brain reward centers (the arcuate nucleus, the dorsolateral hypothalamus, the dorsomedial nucleus) might be anticipated.

In metabolic syndrome as well as in the healthy volunteers lower insulin sensitivity is associated with decreased central serotonergic activity (Horáček et al., 1999; Muldoon et al., 2006; Herrera-Marquez et al., 2011). In dependence on the sensitivity of insulin receptors, insulin in the periphery augments plasma levels of tryptophan (serotonin precursor) on the account of large neutral amino acids, competing with tryptophan for transport across BBB (Fenstrom and Wurtman, 1972; Jamnicky et al., 1991, Fukagawa et al., 1987). Insulin also directly facilitates the transport of tryptophan across the BBB (Crandall et al., 1981; Fukagawa et al., 1986; Malone et al., 1993). Serotonin containing ventromedial and paraventricular hypothalamic nuclei regulate the appetite. Uremic anorexia is defined as “hyperserotonergic state of the brain” (Aguilera et al., 2007). The decrease of serotonin activity in raphe and hypothalamic nuclei may lead to a higher food intake with a consequent hypersecretion of insulin, which could finally contribute to insulin-resistance. It has been suggested that stimulation of the serotonergic...
neurotransmission enhances insulin sensitivity (Goodnick et al., 1995). Association between insulin resistant states and central expression of serotonergic receptors remains unclear. Post-mortem immunohistochemical and molecular biology investigation on the density and expression of the above mentioned receptors in regulating brain areas/nuclei in type 2 diabetic patients in regard to degree and duration of diabetes (insulin resistant state) could shed first light on the potential role and interaction of these systems in insulin resistant states. Insulin resistant vs. insulin sensitive controls deceased due to identified diagnosis should be investigated. Cohorts should be matched for gender, age, degree of obesity, treatment, and well biochemically characterized.

References:
Muldoon MF, Mackey RH, Korytkowski MT, Flory JD, Pollock BG, Manuck SB. The metabolic syndrome is associated with reduced central serotonergic responsivity in healthy community volunteers. J Clin Endocrinol Metab. 2006;91:718-21.
Collaboration in 2013 of the Institute of Cell Biology (Ukraine) with other institutions of the RECOOP-HST Association

Stoika R.S.
Institute of Cell Biology (ICB), NAS of Ukraine, 79005, Lviv, Drahomanov Str 14/16.
E-mail: stoika@cellbiol.lviv.ua

The list of institutions collaborating with different research teams of the Institute of Cell Biology and brief description of principal research achievements obtained in 2013 are presented.

**Institute of Macromolecular Chemistry (Prague, Czech Republic).** The biocompatibility of novel nano- and micro-scale polymeric and mineral (superparamagnetic) particles, as well as their potentials of application in biology and medicine have been estimated. Their targeting of the immune and tumor mammalian cells has been studied. Possibilities of their using as affinity sorbents in biological experiments are under evaluation. Articles for international journals were published and submitted. Proposals for Visegrad Foundation have been prepared and submitted.

**Big potentials also exist in the NanoBioTech-directed research** conducted in collaboration with: a) Lviv National Polytechnic University (Ukraine) in the development of novel perspective drug- and gene delivery systems; b) Taras Shevchenko National University in Kyiv (Ukraine) in using C60 fullerenes for biomedical purposes; c) Palladin Institute of Biochemistry (Kyiv, Ukraine) in application of novel fluorescent dyes in apoptosis study.

**Danylo Halitsky National Medical University (Lviv, Ukraine).** Suppression of tumor cell growth *in vitro* (cell culture) and *in vivo* (transplanted ascitic NK/Ly lymphoma and L1210 leukemia in mice) under the action of novel heterocyclic 4-tiazolidone derivatives was studied. The patent of Ukraine application was submitted. Article for the peer-reviewed international journal was prepared and will be submitted soon. Proposals to national scientific foundations that are based on the results of the above noted investigations have been submitted.

**Wroclaw University of Technology, Institute of Physics (Wroclaw, Poland)** in collaboration with Danylo Halitsky National Medical University (Lviv, Ukraine). NaGdF$_4$:Eu$^{3+}$ ultra-small nanocrystals possessing red light emitting fluorescence in the near-IR in the wavelength of potential application for imaging deep in the living tissues have been further modified to get functional groups on their surface. They were used for conjugation of specific glycan-recognizing proteins – the lectins, and the obtained lectin-conjugated nanocrystals were shown to be good markers for melanoma tumor visualization in the histological samples, and, in future, in animals with experimental melanoma tumor. Article for international journal and proposals based on the obtained results are under preparation.

**University of Defense, Faculty of Military Health Sciences (Hradec Kralove, Czech Republic)** in collaboration with the Institute of Hereditary Pathology (Lviv, Ukraine). The conducted research was addressed to evaluation of the role of anti-HSP90B1 autoantibodies as a novel potential biomarker to be used in diagnosis and prognosis of the recurrent miscarriage in women. Article for the international journal has been prepared.

**Slovakian Medical University (Bratislava), University Hospital in Hradec Kralove, Department of Obstetrics and Gynecology (Hradec Kralove, Czech Republic), School of Medicine, Department of Medical Biology (Osijek, Croatia),** and some other institutions of the RECOOP-HST Association. Collaborative research projects with these institutions have been discussed and expected for submission in future.
**Project title:** Combining Stem Cells and Biomaterials for Brain Repair – Unlocking the Potential of the Existing Brain Research through Innovative *In Vivo* Molecular Imaging  

**Project Acronym:** GlowBrain (www.glowbrain.hiim.hr)  

**Project Coordinator:** Srećko Gajović*, MD, PhD  

**Senior scientist:** Marija Lovrić*, PhD  

*Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Šalata 3, 10000 Zagreb, Croatia  
e-mail address: mlovric@hiim.hr*  

The main aim of GlowBrain is to unlock the existing research potential of University of Zagreb School of Medicine and respond to an emerging need for combined stem cells and biomaterials applications in brain repair. The already existing platform for mouse brain research is currently involved in research of molecular mechanisms underlying brain damage and repair. It includes the expertise and equipment to assess the brain at system, cellular and molecular levels. Through analysis of transgenic mouse models this is combined with competence to introduce the experimental brain damage in form of ischemic injury (MCAO), seizures or stereotaxic lesions.

The project proposes an innovative upgrade of existing facilities to enable simultaneous application of stem cells and biomaterials with the goal to enhance beneficial effects of stem cell therapy. By extending the platform with components for in vivo molecular imaging using bioluminescence and magnetic resonance, the insight in the underlying molecular mechanisms of stem cells and biomaterials applications would be achieved. Innovative multimodal combination of bioluminescent imaging (BLI) and magnetic resonance imaging (MRI) of the upgraded platform would enable to follow in real time, in living animals the molecular events in the mouse brain and fine tune consequences and efficiency of stem cell therapies. Together with already existing excellence in neuroscience research, the upgrade would result in a novel and complementary asset to leading European capacities.

Unlocking the research potential of University of Zagreb by assembling a unique platform at European Research Area level would have a high impact for the future cell therapies of the brain diseases. The leading edge research would boost innovations in regenerative medicine field and sustain the upgraded facility in the future.
Recent progress in light emitting inorganic nanocrystals growth, their functionalization and potential use in biomedicine.

A. Podhorodecki\textsuperscript{a*}, M. Banski\textsuperscript{a}, B. Sojka\textsuperscript{a}, A. Noculak\textsuperscript{a}, J. Misiewicz\textsuperscript{a}, J. Cichos\textsuperscript{b}, M. Karbowiak\textsuperscript{b}, R. Bilyy\textsuperscript{c}, T. Dumych\textsuperscript{c}, R. Stoika\textsuperscript{c}, M. Kuricova\textsuperscript{d}, J. Tulinska\textsuperscript{d}, A. Liskova\textsuperscript{d}, M. Bartusova\textsuperscript{d}, B. Zasonska\textsuperscript{e}, D. Horak\textsuperscript{e}

\textsuperscript{a} – Institute of Physics, Wroclaw University of Technology, Institute of Physics, Wroclaw, Poland
\textsuperscript{b} – Chemistry Department, Wroclaw University, Wroclaw, Poland
\textsuperscript{c} – Institute of Cell Biology NAS of Ukraine, Lviv, Ukraine
\textsuperscript{d} – Department of Immunology and Immunotoxicology, Slovak Medical University, Bratislava, Slovak Republic
\textsuperscript{e} – Institute of Macromolecular Chemistry of Academy of Sciences of the Czech Republic, Prague, Czech Republic

*Corresponding author: artur.p.podhorodecki@pwr.wroc.pl

Introduction: Optically active inorganic nanocrystals (quantum dots) are recently widely used in research related to bio-medicine as efficient optical markers. However, even if their excellent optical properties and their potential multi-functionality makes them much better probes than used so far proteins or molecular markers their clinical use is still only a future perspective. This is due to three, main disadvantages characterized these markers: low bio-degradability, high toxicity and hydrophobic nature of their surface.

Aim: The aim of this work was to find the optimal matrix composition of nanocrystals guarantee their low toxicity, emission or excitation in infrared spectral range and to find the way to control and change the hydrophobic nature of their surface to hydrophilic once.

Methods: To obtain these goals, with use of wet-chemistry approaches we synthesised different nanocrystals with optical activity, mainly in infrared spectral range (PbS, CdSeS, NaGdF\textsubscript{4}:Yb,Er, NaGdF\textsubscript{4}:Eu). In second step, we developed their efficient surface modifications with two approaches: silanization and ligand exchange. In addition, we succeed with their bio-functionalization via lectin-conjugation and perform immunotoxicity tests.

Results: Very high reproducibility and nanocrystals size control have been obtained for all synthesised by us nanocrystals. With a proper selection of nanocrystals size and matrix composition we were able to obtain nanocrystals emitting or excited in infrared spectral range. With the silanization approach we were able to obtain coatings with a controllable thickness and porosity. For NaGdF\textsubscript{4}:Eu nanocrystals we succeed with the ligand exchange approach, which allows us to attach to our nanocrystals proteins via –COOH groups. Moreover, it has been shown that these nanocrystals characterize by no immunotoxicity up to high nanocrystals concentrations. Finally, the potential use of obtained by us nanocrystals has been shown as optical markers used in melanoma cancer imaging.

Acknowledgement:
The presented research work would not have been possible without the support of 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).
Native and synthesized magnetic nanoparticles and their excitotoxic potential

T. Borisova, L. Kasatkina, R. Sivko, A. Borysov, N. Krisanova

Department of Neurochemistry, Palladin Institute of Biochemistry, NAS of Ukraine; 9 Leontovicha Street, Kiev, 01601, Ukraine

Magnetic nanoparticles attract increased attention because of their usage in magnetic resonance imaging, drug delivery, selective/local hyperthermia, tissue repair and cell separation. Ferritin is considered as model protein-coated nanoparticles, which can serve as good tools to investigate possible toxic properties of synthetic metal nanoparticles coated by polymer or dextran. Ferritins are composed of 24 subunits, which form a spherical shell with a large cavity where up to 4500 three-valent iron ions can be deposited as compact mineral crystallites resembling ferrihydrite. Ferritin cores exhibit superparamagnetic properties, which are inherent to magnetic nanoparticles.

The effect of exogenous ferritin and synthesized nanoparticles of magnetite on the key characteristics of glutamatergic neurotransmission was assessed in rat brain nerve terminals (synaptosomes). Exogenous ferritin (80 \( \mu \)g/ml, iron content 0.7 %) significantly increased the ambient level of L-[\(^{14}\)C]glutamate (0.200±0.015 versus 0.368±0.016 nmol/mg of protein) and endogenous glutamate (fluorimetric glutamate dehydrogenase assay) in the nerve terminals. This increase was not a result of augmentation of tonic release because the velocity of tonic release of L-[\(^{14}\)C]glutamate was not changed significantly in ferritin-treated synaptosomes as compared to the control. Ferritin caused a decrease in synaptic vesicle acidification that was shown using fluorescent dye acridine orange. Exogenous ferritin can provoke the development of excitotoxicity increasing the ambient level of glutamate and lowering synaptic vesicle acidification and glutamate uptake in the nerve terminals, however these effects are not completely iron-dependent. In contrast, Synthesized nanoparticles of magnetite, uncoated and coated by different polymers, did not affect significantly the functional state of nerve terminals and key characteristics of glutamatergic neurotransmission.

**Key words:** nanoparticles of magnetite, ferritin, apoferritin, glutamate, ambient level, uptake, synaptic vesicle acidification, rat brain nerve terminals
In vivo monitoring of transport of poly(L-lysine)-modified iron oxide nanoparticle-labeled macrophages in a rat

Daniel Horák¹, Martina Schmiedtová², Michal Babič², Rudolf Poledne², Vit Herynek²

¹ Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovský Sq. 2, 162 06 Prague 6, Czech Republic
² Institute of Clinical and Experimental Medicine, Vídeňská 1958/9, 140 21 Prague 4, Czech Republic

Coprecipitation of FeCl₂ and FeCl₃ with ammonium was used to prepare iron oxide nanoparticles dispersible in aqueous medium. Oxidation of the particles with sodium hypochlorite then yielded maghemite (γ-Fe₂O₃) nanoparticles which were coated with two types of coating, namely D-mannose and poly(L-lysine) (PLL) as confirmed by FTIR analysis. According to transmission electron micrographs, the particles were smaller than 10 nm. Hydrodynamic particle size according dynamic light scattering was ~ 180 nm. Viability and contrast properties of macrophages labeled with D-mannose-, PLL-coated and neat γ-Fe₂O₃ particles and commercial Resovist® serving as a control were assessed and compared. As a trade-off between viability and contrast of the labeled macrophages, PLL-coated γ-Fe₂O₃ nanoparticles were selected and then successfully used in in vivo experiments with rats. The labeled macrophages were both intraperitoneally and subcutaneously injected in animals which were up to 48 h in vivo monitored by magnetic resonance imaging (MRI). Transport of PLL-γ-Fe₂O₃ nanoparticle-labeled macrophages in a rat body was confirmed. Tracking of macrophages using newly developed particles can be prospectively used for monitoring of macrophage movements through adipose tissue and detection of inflammation and cell migration during cell therapies.

Financial support of Grant Agency of the Czech Republic (project P304/11/0731) is acknowledged.
**Project title:** Potential of Magnetic Resonance Imaging (MRI), Bioluminescent Imaging (BLI) and Fluorescent Imaging (FLI) for *In Vivo* detection of nanoparticles

**Project Acronym:** GlowBrain (www.glowbrain.hiim.hr)

**Project Coordinator:** Srećko Gajović*, MD, PhD

**Senior scientist:** Marija Lovrić*, PhD

* Croatian Institute for Brain Research, School of Medicine, University of Zagreb, Šalata 3, 10000 Zagreb, Croatia

e-mail address: mlovric@hiim.hr

The *in vivo* molecular imaging changes the use of animals in experiments. The strategy enables studying biological processes in living animals in real time.

The main goal of the GlowBrain project is based on combination of biomaterials and stem cell applications in the brain monitored by combined bioluminescent imaging (BLI), fluorescent imaging (FLI) and magnetic resonance imaging (MRI). This is expected to improve the stem cell delivery and integration in the brain and to evaluate the stem cells effects *in vivo*.

BLI and FLI vs. MRI are technologies at the opposite parts of the imaging spectrum. BLI and FLI can visualise sources of light emitted from luminescent enzymes or fluorofores. In BLI bioluminescence enzymes such as firefly luciferase emit light when provided with the appropriate substrate. In FLI fuorofores, either as products of transgenes or as introduced markers, are used to give a signal in the living animal. Both methods are very sensitive, BLI being the most sensitive *in vivo* system available, but have rather limited spatial resolution. The *in vivo* imaging system (IVIS Spectrum) from Perkin Elmer offers imaging of both modalities and is currently being used at Croatian Institute for Brain Research. MRI imaging is based upon the proton movement of the hydrogen atom, abundant as water in tissue. When caught in a strong magnetic field the protons align to produce a detectable magnetic field. MRI offers superior spatial resolution, and even magnetic nanoparticles can serve as molecular markers to be used in this technique. These three technics together enable comprehensive non-invasive, *in vivo* visualization of cellular and molecular events in normal and pathological processes. Combining these imaging modalities into multi-modal imaging, whereby the same animal is imaged simultaneously is expected to provide additional information not possible to obtain by either of the methods. These should be used to be able to detect biological properties of fluorescent and/or magnetic nanoparticles. In particular we expect that this approach will innovate translational research, through interdisciplinary application of nanoparticles, mouse transgenic models, imaging, and potential diagnostic and therapeutic approaches to establish possible future applications.
Dry throat swab sampling for enterovirus PCR diagnosis
Maria Borsanyiova, Sona Sarmirova, Shubhada Bopegamage

Enterovirus Laboratory, Slovak Medical University, Limbova 12, 83303 Bratislava, Slovak Republic.

Background: Traditionally throat swabs used in diagnosis of enteroviruses are collected in virus transfer medium. Techniques applied for detection of the viruses in the collected specimens range from tissue culture isolations, antigen detection to molecular analysis. PCR has increasingly replaced other methods in the routine diagnosis due to high sensitivity and rapid results. Enterovirus infections are common in humans, and often asymptomatic. These viruses can cause serious infections, and known to be stable in the virus transport medium at ambient temperatures.

Aim: Our aim was to test the applicability of the dry swab method and stability of enteroviruses in this method (without the transport medium) for PCR diagnosis.

Method: 10 fold serial dilutions of coxsackieviruses (CV), prototypes CVB4-JVB and CVA9-Griggs were prepared in phosphate buffered saline (PBS). Commercial sterile cotton swabs were allowed to soak for 5s in the diluted viruses and air dried at room temperature in a laminar box. 4 sets were prepared: dried swabs were vortexed in RNAse free water and frozen at -80°C (Set-I), whereas sets-II, III, and IV were processed after storing at 4°C for 1, 3, and 6 months. Throat swabs and saliva of 10 volunteers (5 from the Enterovirus laboratory and 5 control volunteers not professionally in contact with the viruses) were collected. Each throat swab set was dried and processed similar to the 4 sets described. Saliva was frozen at -80°C. Presence of virus was detected by the nested-PCR method.

Results: We observed fall of virus titer up to 4log\textsubscript{10} after 1 month of storage at 4°C. Viral RNA was detectable at one month depending on the original virus titer. In 7/10 volunteers viral RNA was detectable. Of this 5/7 were still positive after 1 month of storage at 4°C. 2/10 volunteers (not professionally linked to enteroviruses) showed positive saliva.

Conclusion: We conclude that the dry cotton swabs maybe utilized in routine sample collections. Best results were obtained when swabs are processed immediately. Storage at 4°C may be applied, but recommended only for short times.

Acknowledgements: Prof. Jochem Galama, MD., PhD., Nijmegen, the Netherlands, Sandor Vari, MD. Funds: NRC for Enterovirus Laboratory from Ministry of Health Slovak, , RECOOP HST Association, Norwegian Financial Mechanism, Mechanism EEA and Slovak Government and the State Budget of the Slovak Republic (SK 0082) ,Center of excellence of environmental health", ITMS No.24240120033,
The importance of estimating the correct gestational age and monitoring the pregnancy, in preterm birth

Cristian Poalelungi ¹,², Decebal Hudita ¹,², Iuliana Ceausu ¹,²

¹  "Carol Davila” University of Medicine and Pharmacy, Bucharest,
²  "Dr. I. Cantacuzino” Hospital, Department of Obstetrics and Gynecology, Bucharest, Romania
Ion Movila Street, no 5-7, Bucharest, Romania
Email: cristianpoalelungi@yahoo.com

Background: Preterm birth is one of the major problems encountered in obstetrics, having a major impact on individuals and society. The incidence of premature birth is increasing, despite the developments taking place in medical research. Neonatal mortality increases with decreasing gestational age. It is therefore important to establish the correct gestational age.

The incidence of the premature birth depends significantly by region and country. The highest incidence of prematurity is found in the less developed countries (11.8%), next being the moderately developed countries (11.3%), the lowest incidence existing in the high and very high developed countries (9.4% and 9.3%). The prenatal medical examination (including clinical examination, abdominal/transvaginal ultrasound (US) examination, vaginal examination, genetic and laboratory tests) is essential for the identification and prevention the risk factors associated with premature birth.

Study objectives: To highlight the importance of estimating the correct gestational age for preventing the preterm birth. We also assessed the correlation between the dispensarization incidence and the risk factors to women with preterm birth.

Materials and methods: This is a prospective study. It was conducted during 2011-2012 on a sample of 4078 pregnant women which gave birth at Department of Obstetrics and Gynecology,”Dr. I. Cantacuzino” Hospital. The data collection was under the guideline of the RECOOP HST Consortium Mother and Child Health Network.

In our study, we considered having a dispensarized pregnancy if the pregnant woman underwent the following exams: First trimester US, Biological markers, Screening tests, CVS, Amniocentesis, 2nd trimester US.

Results: From all 4078 births, 474 (11.62%) were preterm births (<37 weeks): 13 extremely preterm deliveries (0.32%), 118 (2.89%) very preterm, 115 (2.82%) at 32-34 weeks gestation and 228 (5.59%) were late preterm births.

Discussing the ultrasound scans performed during pregnancy, we found that 168 women with preterm birth where subjected to such examination in the first trimester, 163 during the second trimester and 144 have not been examined at all.
The closer is the preterm birth to a normal, term birth, the higher is the number of US examinations performed. There is a significant gap between the percentage of women who have not been subjected to US examination who give birth prematurely (in less than 28 weeks) and the similar percentage of women with term birth (46% to 11%, p<0.001). A similar difference exists for the other prematurity groups and the group of children born at term. We did not find any significant difference between the US examinations and pregnancy age at preterm births of 32-37 weeks. It is evident that for the lowest gestational age (less than 28 weeks) the absence of an US examination could affect the birth prognosis; this highlights the importance of an accurate calculation of the gestational age.

Prenatal testing was more associated with term birth (88.1%) than with preterm birth (70.3%, p<0.001). The odds ratio was 3.1 (95% CI, 2.3 to 4.2).

**Conclusion:** In our study, as expected, we found that the preterm birth incidence was influenced by the extreme age of the mother, by multiparty or smoking – all these being risk factors linked with non-dispenzarization. Apart from these factors the incidence of preterm birth is related to some diseases as: history of miscarriage, multiparty, vaginal bleeding in the first trimester of pregnancy, operated uterus, uterine incompetence, cerclaje, gestational diabetes, anemia, urinary infections during pregnancy and hypertension associated with pregnancy, all these being risk factors for a preterm birth (comparing with term birth).

The absence of a correct diagnosis of gestational age (estimated from patient’s history, from clinical and US examinations) signifies the lack of a proper antenatal surveillance with the consequence of placing the pregnant woman in the high risk group, having important health implications for both mother and fetus.

**Keywords:** prematurity, gestational age, high risk pregnancy
Murine cytomegalovirus defective in myeloid-cell dissemination functions exhibit decrease CNS invasiveness in newborn animals.

Seleme M.C. and Britt W.
Division of Infectious Diseases, Dept. of Pediatrics, Univ. of Alabama at Birmingham, Birmingham, Alabama, USA.
mariesel@uab.edu; wbritt@peds.uab.edu

Introduction: Congenital human cytomegalovirus (HCMV) is an important cause of central nervous system (CNS) infection throughout the world with up to 10% of infected infants exhibiting long term neurodevelopmental abnormalities. Understanding this CNS infection requires understanding mechanisms of neuroinvasion, a task limited to observational studies in humans. To extend our understanding of this infection, we have developed a murine model using the closely related murine CMV (MCMV) infection of newborn mice. Because newborn mice are neurodevelopmentally equivalent to a 2nd trimester human fetus, this model allows mechanistic studies of neuroinvasion and CNS disease. Using this system and mCMV mutant viruses we have initiated studies to determine pathways of neuroinvasion.

Methods and Results: Newborn Balb/c mice were inoculated intraperitoneally (ip) with wild type (WT) and mutant MCMV. Mutant viruses were constructed to include deletions of genes associated with macrophage tropism/chemokine activity (mck-2/UL129) and anti-apoptotic function (M38.5) as well as a double mutant. Viruses with mutations in these genes have been shown to have defects in cell associated hematogenous spread in adult animals. Mice received 50pfu of WT virus and larger inoculums of mutant viruses that provided similar levels of viral load in the liver and spleen. Whole blood, plasma, and spleen/liver/brain were harvested on various days and the amount of virus was quantified by qPCR.
When we adjusted inoculums to provide similar levels of virus replication in the liver/spleen, we also observed similar copy numbers of virus in the whole blood and plasma. Infection with 10 fold more mutant virus lacking the anti-apoptotic gene, m38.5 than WT virus resulted in 2-3 logs less virus in brain whereas the infection with the mck-2 mutant virus did not result in different amounts of virus in the brains of infected mice. In contrast, infection with 100fold more of the double (∆38.5/mck-2) viral mutant as compared to WT virus resulted in similar levels of virus in the liver/spleen and blood but 4-5 logs less virus in the brain of animals infected with the double mutant virus than WT infected mice. From these data it appears that MCMV dissemination through infection of myeloid cells is essential for efficient infection of the CNS and that the anti-apoptotic function of m38.5 plays a key role in spread of MCMV to the CNS.

Discussion: Because similar anti-apoptotic and chemokine functions are encoded by HCMV, by inference it can be argued that efficient HCMV infection of the developing CNS of the human fetus likely requires myeloid cell dissemination. Thus, protective innate and adaptive immune responses must have the capacity to eliminate HCMV infected myeloid cells during congenital HCMV infection. Current strategies for the development of prophylactic vaccines for congenital HCMV are directed at prevention of maternal infection and limiting disease in the developing fetus/newborn infant. These vaccine platforms depend on passively acquired antiviral antibodies providing protection as measured by the surrogate of virus neutralizing activity. Virus neutralizing activity alone could have limited efficacy in limiting cell associated virus spread in the infected fetus.
RECOOP M&CH Screening Mothers and Newborns for Cytomegalovirus Infection

Objective:

To identify infants with congenital CMV infection conclusively, infants’ saliva or urine should be tested for CMV by standard cell-culture methods during the first three weeks of life.

Retrospective identification of CMV by DNA polymerase chain reaction methods, using dried-blood spots collected from all newborns at birth, is also a method for identifying congenital CMV infection later, although the sensitivity and specificity for this method has not yet been established.

Project leader:
William J. Britt, University of Alabama- Birmingham, Alabama, USA
E-mail: wbritt@peds.uab.edu; wbritt@uab.edu

Participating organizations:
Sinisa Sijanovic, Department of Obstetrics and Gynecology, School of Medicine, University J. J. Strossmayer Osijek, Croatia
E-mail: sinisa.sijanovic@os.htnet.hr

Marian Kacerovsky, Department of Obstetrics and Gynecology, University Hospital in Hradec Kralove, Czech Republic
E-mail: marian.kacerovsky@gmail.com; Marian.Kacerovsky@seznam.cz

Tibor Ertl, Department of Obstetrics and Gynecologist, Medical Faculty, University of Pecs, Hungary
E-mail: tibor.ertl@aok.pte.hu

Iuliana Ceausu, Department of Obstetrics and Gynecology of “Dr. I. Cantacuzino” Hospital, “Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
E-mail: iulianaceausu2004@yahoo.com; iceausu@hotmail.com

Short summary:

CMV is the most common virus known to be transmitted in utero, affecting approximately 0.5%-1.5% of births. The risk of primary maternal CMV infection leading to congenital CMV infection is approximately 40%. Of neonates with congenital CMV infection, 85%-90% are asymptomatic at birth, yet 10%-15% eventually present with developmental, visual, hearing, or dental abnormalities in the first years of life. Of those who are symptomatic at birth, about half will present with some isolated findings, while the other half will present with cytomegalic inclusion disease. CMV disease in this group carries a mortality rate of around 30%; up to 80% of affected infants develop late complications, including developmental, visual, or hearing delay.

Patient group: newborns (term and preterm)
Protocol by William J. Britt, University of Alabama- Birmingham, Alabama, USA
Deadline December 21, 2012

Neonat consultant: Tibor Eről, Department Obstetric Gynecology, Medical School, University of Pécs

**Sample collection:**

a) Collect 200 consecutive saliva specimens from newborns, RECOOP will provide the swabs and it could be stored after drying it on room temperature or refrigerating at 4 °C.

b) Collect 200 serum specimens (0.5ml) from mothers for cross sectional seroprevalence for her maternal population


**References**


Cervical fluid interleukin-6: a possible predictor of early onset sepsis in pregnancies complicated by late PPROM

Marian Kacerovsky¹,², Ivana Musilova²

¹Biomedical Research Center, University Hospital Hradec Kralove, Czech Republic. ²Department of Obstetrics and Gynecology, Charles University in Prague, Faculty of Medicine Hradec Kralove and University Hospital Hradec Kralove, Czech Republic.

Introduction: A substantial proportion of preterm prelabor rupture of membranes (PPROM) cases (40-60%) occur between 34 and 37 weeks. Although newborns of mothers with PPROM between 34 and 37 weeks (late PPROM) are often incorrectly considered nearly physiologically mature, they represent a high-risk group with an increased rate of morbidities and a higher rate of hospital readmission in the first month of life when compared to full-term infants. Recent studies have evaluated the differences between expectant and active management of women with late PPROM with respect to short-term neonatal morbidity, especially for early-onset sepsis. Although these studies were well designed, they did not shed light on this problem, as they revealed no difference in the rate of early-onset sepsis between active and expectant management of pregnancies with late PPROM. Therefore, the optimal management of late PPROM remains a clinical dilemma because there is lack of evidence to justify either expectant or active management. This is in contrast to PPROM prior to 34 weeks, a condition for which expectant management is recommended.

The aim of the pilot study was to evaluate amniotic fluid interleukin (IL)-6 levels with respect to the presence and absence of selected aspects of severe neonatal morbidity. Material and Methods

This pilot study included 99 women with singleton pregnancies complicated by late PPROM. Amniocenteses were performed at the time of admission; amniotic fluid IL-6 concentrations were determined. Data regarding morbidity and mortality were reviewed for all newborns.

Results: Severe neonatal morbidity and early onset sepsis was observed in 25% (25/99) and 4% (4/99) of newborns, respectively. The presence of early-onset sepsis was associated with a higher IL-6 concentration by crude analysis and after adjustment for gestational age ($p<0.0001$). An amniotic fluid IL-6 concentration of 3353 pg/mL was found to be the best cut-off for the identification of early-onset sepsis (area under the receiver operator curve: 0.98; sensitivity: 100%; specificity: 98%; positive predictive value: 58%; negative predictive value: 100%; likelihood ratio: 32; $p<0.0001$).

Discussion: Our results suggest that pregnancies with late PPROM with amniotic fluid IL-6 concentrations < 3353 pg/mL are at a low risk of early-onset sepsis. This finding is clinically relevant, especially in light of the fact that previous studies comparing active and expectant management listed early-onset sepsis as a primary outcome. We are aware that an invasive amniocentesis must be performed to obtain amniotic fluid for analysis, which is critical to determining the inflammatory status of the intraamniotic compartment. Amniocentesis is also limited by the presence of severe oligohydramnios or anhydramnios, and this seems to be a major clinical limitation. Therefore, these results should be replicated for non-invasive samples such as cervical fluid. We believe that this approach can be successful because our preliminary results clearly showed a strong correlation between amniotic and cervical fluid IL-6 levels ($p<0.0001$). Therefore, the main aim of a validation study performed by RECOOP partners will be to evaluate cervical fluid IL-6 levels in late PPROM pregnancies with respect to the presence and absence of early onset sepsis.
Testing the Timing Hypothesis of Atherosclerosis Prevention in Women

Jan Pitha, Laboratory for Atherosclerosis Research, Institute for Clinical and Experimental Medicine (IKEM), Prague, Czech Republic.

In our previous work we proposed the hypothesis, that menopausal transition could be critical period for the progression of atherosclerosis especially in the presence of smoking. This progression could be mediated through several mechanisms including impaired vascular protection, impaired reverse cholesterol transport, and rapid changes of particular sex hormones. According to this, women in menopausal transition could be more vulnerable to environmental insults and more responsive to intervention of cardiovascular risk factors. This hypothesis is based on observational data. In the future project we would like to confirm this hypothesis in an interventional study using also epigenetic markers including measurements of circulating microRNAs. The group of ten non-smoking women and ten otherwise healthy smoking women in menopausal transition will undergo standardized intensive 8 week lifestyle intervention. Before and after the intervention the parameters will be analyzed as follows: body mass index, waist circumference, blood pressure, pulse pressure velocity and aortic stiffness, endothelial function, plasma lipids, hs C-reactive protein, endothelial progenitor cells, endothelial microparticles, and circulating microRNAs type 143/145. The same intervention will be done and the same parameters will be measured in ten non-smoking and in ten smoking premenopausal and postmenopausal women and in ten smoking and in ten non-smoking men of the similar age, body mass index and in the case of women of similar waist circumference. We will compare the differences of impact of intervention between women in menopausal transition and premenopausal, menopausal women, and men. The null hypothesis is that women in menopausal transition will be more sensitive to intervention than other study groups, especially when smoking. If confirmed, these results could be immediately spread into the population.

This research was supported by the project (Ministry of Health, Czech Republic) for the development of research organization 00023001 (IKEM, Prague, Czech Republic) – Institutional support.

REFERENCES:


Impact of adipose tissue and related inflammatory mechanisms on the atherosclerosis

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

Atherosclerosis is still the most common cause of death in developed countries. In addition to plasma lipids, it is driven by inflammatory cells, mainly macrophages. In our project we are searching for specific cell markers of proinflammatory status (phenotypes of macrophage subpopulation) in subcutaneous, perirenal and perivascular adipose tissue of patients with atherosclerotic disease. In addition, we are analyzing gene expression of proinflammatory cytokines in subcutaneous, perirenal and perivascular adipose tissues of subjects with advanced atherosclerosis. All these factors we are also analyzing in healthy control subjects. In addition, we are analyzing relations of characteristics of monocytes isolated from adipose tissues to traditional risk factors of atherosclerosis in groups of living kidney donors, in patients with atherosclerotic disease and in control subjects.

To characterize the macrophages eluted from adipose tissues, samples of adipose tissues (subcutaneous, perirenal and samples of perivascular fat surrounding renal artery) are obtained in two groups of subjects undergoing surgery procedures. Samples from group with advanced atherosclerosis are obtained during suprainguinal lower limb artery reconstructions for peripheral artery disease. Control samples are obtained from healthy living kidney donors during removal of the kidney. Samples of 3 grams of adipose tissue are processed as follows: 2.5 grams of adipose tissues are used for analyses of macrophages subpopulations. Stroma vascular fractions, including preadipocytes and endothelial cells in addition to immune cells are eluted from this sample. The remaining 0.5 grams are stored in -80°C for subsequent gene expression analyses. Our research should help to understand behavior and characteristics of macrophages in subcutaneous, perirenal and perivascular adipose tissues of patients with atherosclerosis compared to healthy subjects. In the future we would like to focus also on gender differences in these factors.

This research was supported by the project (Ministry of Health, Czech Republic) for the development of research organization 00023001 (IKEM, Prague, Czech Republic) – Institutional support.
Analysis of the data for most significant risk factors and gender differences at all sites in the RECOOP WH and CVD Retro Study

Dražen Mlinarević, M.D., Emergency Cardiology, Clinical Hospital Osijek and Department of Medical Biology, School of Medicine Osijek, J.Huttlera 4, 31000 Osijek

Background
Ischemic heart disease (IHD) is the biggest contributor to global morbidity and mortality, especially in developed countries and countries in transition. The most common cause of IHD is coronary artery disease (CAD), which is propagated by a high-fat and high-energy diet, obesity, a sedentary lifestyle and smoking.

Methods
In this multicenter retrospective study we collected data for 1138 IHD patients from Hungary (Debrecen), Czech Republic (Prague) and Croatia (Osijek and Split). This analysis focuses on traditional IHD risk factors – age, BMI, history of hypertension or diabetes and blood lipid levels on admission. We compared the data from the four centers and retained a special focus on gender-related differences, as well as the differences between three continental centers (Debrecen, Osijek, Prague) and one Mediterranean center (Split).

Results
We found statistically significant differences between the four centers in almost all investigated variables – age (p<0.001), BMI (p<0.001), cholesterol (p<0.001), HDL (p<0.001), LDL (p<0.001) and triglycerides (p<0.001). Gender-related differences were also present, most notably in HDL, LDL and triglyceride levels, where we found significant differences in male comparisons, but no differences in female comparisons.

Conclusions
We found significant differences in almost all observed clinical parameters between cohorts from three Central European countries. We also found significant differences between male and female patients’ data and finally between continental centers and the Mediterranean center.
Abstracts Review for CMJ
Lectin-conjugated nanocrystals as markers for melanoma tumor visualization.

T. Dumych\textsuperscript{a}, M. Lutsyk\textsuperscript{c}, M. Bansi\textsuperscript{b}, A. Yashchenko\textsuperscript{c}, B. Sojka\textsuperscript{b}, A. Lutsyk\textsuperscript{c}, R. Stoika\textsuperscript{a}, J. Misiewicz\textsuperscript{b}, A. Podhorodecki\textsuperscript{b,*}, R. Bilyy\textsuperscript{a,c,*}

\textsuperscript{*} - equally contributed as senior authors
\textsuperscript{a} – Institute of Cell Biology NAS of Ukraine, Lviv, Ukraine
\textsuperscript{b} – Wroclaw University of Technology, Institute of Physics, Wroclaw, Poland
\textsuperscript{c} – Lviv National Medical University, Lviv, Ukraine

Introduction
Melanoma belong to one of most dangerous tumours, with main treatment being surgical incision. Taking into account high metastatic potential of melanoma it is very important to have a precise visualization of tumor contours during its removal. Current available markers for melanoma visualization do not provide adequate signal strength for in vivo visualisation.

Aim
We focused on the creation of bright nanomarkers which can be used for visualization of melanoma in vivo and provide reversible binding, being easy removed from the organism after treatment. To ensure the signal strength we used NaGdF\textsubscript{4}:Eu\textsuperscript{3+} ultrasmall nanocrystals, which are able to be excited with red light emitting fluorescence in near-IR in the wavelength which could be potentially used for imaging deep into living tissues. These nanocrystals were further modified to have functional groups on their surface. To provide the specific but still reversible recognition of melanoma cells we screened a set of glycans-recognizing proteins – lectins – for their ability to bind to melanoma cells. Since usually the lectin affinity binding constant (~10\textsuperscript{-7} M) is two orders of magnitude lover than that of monoclonal antibodies (~10\textsuperscript{-9} M) their binding to tissue can be disrupted with the use of specific sugar (usually non-harmful) inhibitor.

Methods
We used B10F16 melanoma cells which were inoculated to black mice and melanoma tumor was allowed to grow for 15 days providing a tumor with 1-2 grams range. The tumor was removed and histological sections of tumors cells were prepared. They were screen for the ability to bind the following lectins with distinct glycans specificities: \textit{Laburnum anagyroides bark agglutinin}, LABA; \textit{Perca fluviatilis agglutinin}, PFA; \textit{Pisum sativum} lectin, PSL; \textit{Peanut agglutinin}, PNA; \textit{Helix pomatia agglutinin}, HPA; \textit{Sambucus nigra agglutinin}, SNA; \textit{Galanthus nivalis agglutinin}, GNA; \textit{Narcissus poeticus} lectin, NPL.

Among those lectin collections LABA, PFA, GNA were discovered or characterized in our laboratory and showed to have affinity towards altered, tumor-related glycoepitops, as well as NPL lectin being a good marker of dying cells. PNA, LABA, NPL and GNA lectins showed preferential binding with distinct parts of melanoma tumors. PNA lectin (specific to desialylated glycoepitops, namely to TF-antigen, is widely used in cancer research and diagnostic histopathology) was bound to tumor stroma; LABA, as demonstrated earlier, exposed high affinity to tumor microvascular; NPL and GNA lectins (specific to oligomannose-rich glycans) showed preferential binding with regions of melanin-producing cells Further, those lectins were conjugated to NaGdF\textsubscript{4}:Eu\textsuperscript{3+} - COOH nanoparticles via zero length cross-linking reaction, and
were used for further visualization of histological samples. The conjugates were tested for
cytotoxicity and were found to be non-toxic.

Results
The use of lectin-conjugated nanoparticles allowed us to clearly identify the contours of
melanoma tissue on histological sections using red excitation at 625-655 nm and near-IR
emission of 670-720 nm. The obtained results are of practical significance for the creation of
glycans-conjugated nanoparticles for in vivo visualization of melanoma tumor.

Acknowledgement:
The presented research study was completed with participating Cedars – Sinai Medical Center - RECOOP Research
Centers (CRRC) of the Association for Regional Cooperation in the Fields of Health, Science and Technology
(RECOOP HST Association)
Anti- HSP90B1 autoantibodies as a novel potential marker for diagnostic of the recurrent miscarriage (RM)

Starykovych Marina¹, Zastavna Danuta², Juraj Lenco³, Stoika Rostyslav¹, Kit Yuriy¹,

1 – Institute of Cell Biology, NAS of Ukraine
2-Institute of Herediatry Pathology, AMS of Ukraine
3- Faculty of Military Health Sciences, University of Defense, Hradec Kralove, Czech Republic

Recurrent miscarriage is the spontaneous loss of three or more consecutive pregnancies with the same biological father in the first trimester, and affects 1–2% of women, half of whom have no identifiable cause. Overall, 75% of affected women will have a successful subsequent pregnancy, but this rate falls for older mothers and with increasing number of miscarriages. Antiphospholipid syndrome, with anticardiolipin or lupus anticoagulant antibodies, is present in 15% of women with recurrent first and second trimester miscarriage. Since the maternal immune response towards the fetus is an important cause of secondary infertility, a search for novel auto-antigens appearing at the recurrent pregnancy losses is of great importance. Although an immunology-based etiology underlying, anexplained RM has been demonstrated (Baek et al., 2007, Blank et al.), the exact molecular mechanisms stay poorly understood. Chorionic tissue (8 specimens) was homogenized in detergent lysis buffer, centrifuged, and tested for the presence of specific auto-antibodies by the Western blotting with using HRP conjugates of the anti-human antibodies (Abs). When such auto-Abs were detected, the extracted chorion proteins were subjected to the chromatography on Protein G-Sepharose column, and the Abs were collected, biotinylated, and tested for the auto-antigen (AG) specificity towards the chorionic proteins. The biotinylated auto-Abs were incubated with the lysate of the chorionic tissue, and the auto-AG-auto-Ab complexes were isolated by using Avidin-coupled magnetic particles (BioMagCarboxyl, Polysciences, Inc). Proteins were isolated by "pool-down" affinity chromatography with auto-Ab (chorionic) on Sepharose (auto-Ab). IgG-antibodies conjugated Sepharose (sera-Ab) purified from blood serum of healthy human were used as control. Specific auto-AG proteins of cell lysates of human chorions were subjected to SDS-electrophoresis in 12% PAGE in the reducing conditions. 5 potential candidates for protein auto-antigens characteristic for the RM have been identified by mass-spectrometry. Among them, HSP90B1(Heat Shock Protein 90kDa Beta Member 1) is of particular interest since it may be involved in the implementation of immune response in RM patients. HSP90B1 is an abundant molecular chaperone, resident of endoplasmic reticulum. It plays critical roles in folding protein in the secretory pathway such as Toll-like receptors and integrins. It has been implicated as an essential immune chaperone to regulate both innate and adaptive immunity (Schild et al., 2000). Interestingly, that HSP90B1 can serve as endogenous activator for dendritic cells (Liu et al., 2003).

We suggest that the appearance of anti- HSP90B1 auto-Ab in blood serum of RM patients is linked to overexpression of some heat shock proteins in chorionic tissues. Their determination in blood serum can be used for diagnostic and prognostic aims.

Acknowledgement:
The presented research study was completed with participating Cedars – Sinai Medical Center - RECOOP Research Centers (CRRC) of the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association)
REDOX-MODULATING COMPOUNDS ENHANCE SELECTIVITY OF ACTION OF ANTICANCER DRUGS

Panchuk R.R.\textsuperscript{1}, Skorokhyd N.R.\textsuperscript{1}, Chumak V.V.\textsuperscript{1,2}, Lehka L.V.\textsuperscript{1}, Moiseenok A.G.\textsuperscript{3}, Heffeter P.\textsuperscript{4}, Berger W.\textsuperscript{4}, Stoika R.S.\textsuperscript{1,2}

\textsuperscript{1} – Institute of Cell Biology NAS of Ukraine, 79005, Lviv, Drahomanov Str 14/16, Ukraine
\textsuperscript{2} – Ivan Franko Lviv National University, Hrushevsky Str. 4, Lviv, 79005, Ukraine
\textsuperscript{3} – Center of Food, NAS of Belarus (Grodno Branch), BLK-50, Grodno, Belarus
\textsuperscript{4} – Institute of Cancer Research, Vienna Medical University, 1090 Vienna, Borschkegasse 8A, Austria

E-mail: rpanchuk@ukr.net

Low selectivity of action is a significant drawback of many anticancer drugs that lead to serious side effects in the organism of cancer patients. Production of reactive oxygen species (ROS) is one of the main reasons for that, since they are extremely toxic for heart and kidney cells (Berndtsson et al, 2007; Minotti et al, 2004). That is why using specific antioxidant agents capable of reducing generation or action of free radicals induced by the anticancer drugs, could be of extreme importance in cancer chemotherapy.

We conducted studies of the effect of sodium selenite, seleno-methionine and D-panthetin towards human leukemia and carcinoma cell lines with multi-drug resistance (MDR) phenotype that are characterized by the overexpression of P-glycoprotein or knockout of Bax gene involved in apoptosis regulation. It was found that sodium selenite possessed a pronounced cytotoxic effect towards tumor cells in 10 µM dose, whereas seleno-methionine was much less toxic (100 µM) and D-pantetin (vitamin B5 analogue) demonstrated antineoplastic activity in a concentration range of 100-1000 µM depending on the origin of tested cell lines. The effect of these antioxidants in physiologically harmless concentrations in combination with semi-lethal (LC\textsubscript{50}) dose of anticancer drugs cisplatin or doxorubicin was also studied. It was shown that all studied antioxidants enhanced cytotoxic effect of cisplatin towards malignant cells, and such effect was particularly pronounced in tumor cells with the MDR phenotype. Sodium selenite used in low concentrations inhibited antineoplastic activity of doxorubicin by 20-25%. This action was accompanied by a complete inhibition of production of toxic superoxide radicals which were induced by doxorubicin. The detected protective effect of sodium selenite at the action of anticancer drugs might be extremely important at planning further pre-clinical trials of this combinatory regiment, since it can protect normal cells, specifically cardiomyocytes, from toxic effects of doxorubicin in treated organism.

This work was initiated by the RECOOP-HST addressed to involvement of new partners of this Association from the non-member countries, such as Belarus.
ADENOVIRAL VECTORS AS HIGHLY EFFICIENT TOOLS for GENE DELIVERY to PRIMARY MAMMALIAN CELLS

Olexandr Korchnyki\textsuperscript{1,2,3}

\textsuperscript{1} - Institute of Cell Biology, NAS of Ukraine, Lviv, Ukraine (current address).
\textsuperscript{2} - University of North Carolina at Chapel Hill, Chapel Hill, NC, U.S.A.
\textsuperscript{3} - Virginia Commonwealth University, Richmond, VA, U.S.A.

E-mail: olexkor@hotmail.com

Tissue cell culture is a widely used and convenient technique in modern biology and drug discovery. Multiple cell lines derived from normal and neoplastic tissues are used world-wide. However, all established cells lines, including pseudonormal ones, are at least minimally transformed and frequently cannot properly re-capitulate the \textit{in vivo} situation. Many of these limitations can be circumvented by using primary cells. Unfortunately, these cells are frequently difficult to transfect. The E1/E3-deficient recombinant adenoviruses (rAdv) shown to be highly efficient gene delivery tools that allow successful work with multiple types of difficult to transfect cells in different \textit{in vivo} applications. Generation of the recombinant adenoviral constructs includes sub-cloning of the insert into a shuttle vector with convenient polycloning site with subsequent homologous recombination of the shuttle vector with an adenoviral backbone. He et al. (1998) developed a simplified AdEasy system that allows homologous recombination to be performed in the bacteria and to generate rAdvs in less than one month. Here we describe a generation of more than 80 new rAdvs including expression, luciferase reporter and small hairpin interfering RNA (shRNA) vectors by using the AdEasy system. The generated rAdvs can be used in multiple biological applications and in combination with proper maintenance protocol allow for 100\% efficiency of transduction of difficult to transfect cells including primary mouse keratinocytes, human bone marrow (BM)-derived primary mesenchymal stem cells (hMSC) and other types of primary mammalian cells.

This project was discussed with potential RECOOP-HST partners Peter Boor, MD., PhD (RWTH University Aachen, Germany) and Marija Heffer, MD., PhD (School of Medicine Osijek, Croatia) during 8\textsuperscript{th} Annual RECOOP-HST Meeting as a basis for future collaboration within RECOOP-HST network.
ALTERATION OF CHOLECALCIFEROL METABOLISM IN HEPATOCYTES ASSOCIATED WITH PREDNISOLONE ADMINISTRATION


O.V. Palladin Institute of Biochemistry of NAS of Ukraine, Leontovich street 9,
Kyiv, 01601, tel./fax (38)044-279-63-65
e-mail: veliky@biochem.kiev.ua

Background. Growing evidence suggests that glucocorticoid therapy associated side effects could be ascribed to inhibition of vitamin D₃ turnover, particularly due to abnormal processes of hydroxylation and 25OHD₃ formation in hepatocytes. Changes in cholecalciferol hydroxylation can be linked to prednisilone-induced impairments of structural and functional state of hepatocytes. The study was designed to find out the role of hepatic vitamin D₃ 25-hydroxylase isoforms (CYP27A1 and CYP2R1) in changes of vitamin D₃ hydroxylation in relation to hepatocytes viability and prooxidation processes in liver tissue, induced by synthetic glucocorticoid – prednisolone.

Methods. Female Wistar rats (100±5 g) received prednisolone at dose 5 mg per kg of b. w. per os (daily for 30 days). The content of 25OHD₃ in serum was measured by ELISA. Vitamin D₃ 25-hydroxylase activity was assayed in vitro in isolated hepatocytes by the method of radio-competitive binding of [³H]-25OHD₃. The levels of CYP27A and CYP2R1 in liver tissue were measured by Western blot analysis. Expression of Bax and Bcl-2 in hepatocytes was assessed by immunocytochemistry. Reactive oxigen species (ROS) production and cell viability were determined using flow cytometry with DCF-DA and propidium iodide (PI) respectively.

Results. It was shown that glucocorticoid administration lowered the level of 25OHD₃ (by 70%) in blood serum and inhibited two-fold the total activity of vitamin D₃ 25-hydroxylase in hepatocytes vs. control. Prednisolone reduced the content of mitochondrial (CYP27A1) and microsomal (CYP2R1) isoforms of 25-hydroxylase by 78% and 27% respectively. Administration of prednisolone led to disruption of the integrity of hepatocytes triggering destructive changes in these cells and thus reduces the number of functionally active hepatocytes. These cytological data were further confirmed by significant increase in the number of hepatocytes capable to accumulate PI that is associated with necrotic cell death. In contrary, apoptotic index Bax/Bcl-2 was found to be markedly reduced suggesting pro-apoptotic signaling down-regulation. Glucocorticoid therapy caused the increase in prooxidant status of liver as it is evident from accumulation of ROS, carbonylated proteins and thiobarbituric acid reacting products. In parallel, it was shown the decrease in the activity of enzymatic antioxidants superoxide dismutase, catalase and glutathione peroxidase as well as the content of free low molecular SH-containing compounds, indicative of reduced capacity of antioxidant system.

Conclusions. Our findings indicate that glucocorticoids inhibit 25-hydroxylation of cholecalciferol by decreasing tissue levels of both CYP27A1 and CYP2R1. Disturbances in cholecalciferol metabolism can be associated, at least in part, with increased prooxidant status of hepatocytes, impairment of their structure and necrotic death enhancement.
Synthetic polymer-covered nanoparticles of magnetite and native protein-covered magnetic nanoparticles for usage in nanoneurotechnology

N. Krisanova¹, O. Brik², N. Dudchenko², L. Kasatkina¹, A. Borysov¹-³, R. Sivko¹, L. Ostapchenko³, T. Borisova¹*

¹Palladin Institute of Biochemistry NAS of Ukraine NAS of Ukraine; ²Semenenko Institute of geochemistry, mineralogy and ore formation NAS of Ukraine; ³Educational and Scientific Center «Institute of Biology», Taras Shevchenko National University in Kiev

AIMS: Magnetic nanoparticles attract increased attention because of their usage in magnetic resonance imaging, drug delivery, selective/local hyperthermia, tissue repair and cell separation. The aim of this work was to study interaction of synthesized polymer-covered nanoparticles of magnetite and native protein-covered magnetic nanoparticles with brain nerve terminals, and to analyze their effects on the key characteristics of glutamatergic neurotransmission. Glutamate is the main excitatory neurotransmitter involved in most aspects of normal brain function, whereas disturbances in glutamate homeostasis contribute to the pathogenesis of major neurological disorders.

METHODS: Superparamagnetic nanoparticles covered by dextrane, hydroxyethyl starch, oxidized hydroxyethyl starch, chitozan and silica were synthesized at Semenenko Institute of geochemistry, mineralogy and ore formation (Kiev, Ukraine). Binding of nanoparticles with rat brain nerve terminals (synaptosomes) was studied by photon correlation spectroscopy, flow cytometry and radiolabeled assay. Spectrofluorimetry with potential-sensitive and pH-sensitive fluorescent dyes were applied for characterization of functional state of nerve terminals.

RESULTS: Nanoparticles administration resulted in an increase in nerve terminals size that indicated the binding of synthesized nanoparticles with nerve terminals, radiolabeled assay revealed different efficiency of nanoparticles binding that depended from coating type. There was no significant influence of nanoparticles on the potential of the plasma membrane and acidification of synaptic vesicles in nerve terminals. Also, nanoparticles did not affect active transport of L-[¹⁴C]glutamate and the extracellular level of the neurotransmitter in nerve terminals. In contrast, native protein complex with magnetic nanoparticles, ferritin, significantly altered L-[¹⁴C]glutamate uptake as well as the extracellular level of the neurotransmitter.

CONCLUSIONS: Synthesized nanoparticles did not affect significantly the functional state of nerve terminals and key characteristics of glutamatergic transmission. Based on the experimental data concerning binding of synthesized nanoparticles with the nerve terminals, certain types of covering polymers were selected for manipulation of nerve terminals by externally applied magnetic field.

KEYWORDS: polymer-covered magnetic nanoparticles, ferritin, brain nerve terminals.
The diabetes-induced increase in the level of cholesterol of synaptosomal plasmalemma inhibits the fusion with synaptic vesicles in vitro.

Vitaliy Gumenyuk², Irene Trikash², Tamara Kuchmerovska¹
¹Departments of Coenzymes and ²Neurochemistry Palladin Institute of Biochemistry of NAS, Kyiv, Ukraine

Alterations in neurotransmission processes may directly or indirectly contribute to pathophysiology associated with several diseases including diabetes. Regulated exocytosis forms the basis for many intracellular signaling processes, for example, neurotransmitter release. The Ca^{2+}-triggered fusion of synaptic vesicles (SVs) with the presynaptic plasma membranes (PM) is regarded as a final step of exocytosis. Disturbances of calcium-dependent SVs fusion may be a pathophysiological mechanism in diabetes. It is known that effect of anticonvulsant drug gabapentin can be realized via regulation of GABA, glutamate and others neurotransmission. However, it should be recognized that the probable mechanisms underlying the action of AEDs remain little studied.

The highly enriched in cholesterol microdomains of neuronal plasmalemma have important role in synaptic function. It was expected that modulating the cholesterol levels in synaptosomal plasma membrane by methyl-b-cyclodextrin (MCD) would affect the course of the membrane fusion in model experiments.

The aim of this study was to investigate the diabetes-induced alterations in cholesterol level of synaptosomal plasmalemma and in the process of Ca^{2+}-triggered fusion of SVs with plasma membranes of synaptosomes. The effect of gabapentin on Ca^{2+}-induced fusion of synaptosomal membrane structures isolated from rat brain with experimental diabetes was studied too.

Streptozotocin-induced (60 mg/kg of body weight, i.p.) diabetic rats were treated with gabapentin (50 mg/kg, i.p. 5 times per week) for one month following 4 weeks of untreated diabetes. Synaptic vesicles and synaptosomal plasma membrane were isolated from rat brain synaptosomes by step-wise centrifugation. Fusion experiments were performed in the cell-free model system using fluorescent dye octadecylrhodamine B (R18), which was incorporated into SVs membranes at self-quenching concentration. The fusion of SVs, containing marker R18, with target membranes was detected by dequenching of the probe fluorescence. The Ca^{2+}-dependent SVs fusion was carried out on heterotypic and homotypic membrane systems in synaptosomal cytosolic proteins media. PM were overloaded with cholesterol by methyl-β-cyclodextrin (MCD).

It was found that in diabetic rats the rate of SVs fusion with PM (heterotypic fusion) in the presence of Ca^{2+} and synaptosomal cytosolic proteins was decreased as it is evident from lowering of fluorescence from 23% in control to 14.5% in diabetes, p<0.05. Diabetes-induced reduction in fusion is likely to be associated with alteration of plasma membrane cholesterol, which can play a critical role in this process. To verify this idea we have overloaded the PM by cholesterol using MCD:cholesterol mixture. PM cholesterol level in control was 0.67±0.06 μM/mg of protein as compared with 0.75±0.07 μM/mg of protein in diabetes, both p<0.05. After overloading PM by cholesterol its level in control was 0.88±0.07 μM/mg of protein, p<0.05. The
fusion ability of cholesterol-saturated PM was shown to be reduced to the same extent with PM from diabetic brain. The opposite effect has been shown in the rate of SVs fusion with each other (homotypic fusion): in diabetes the fluorescence signal was achieved up to 30% vs 25% in control, p<0.05. Following gabapentin treatment the rate of SVs fusion with target membranes was partially normalized. The findings suggest that diabetes may cause physiologically drastic failure in realization of exocytosis last step – membrane fusion. These alterations may be associated with increased cholesterol content of synaptosomal PM that modifies the structure and function of membrane-bounded proteins and affects membrane fluidity and fusibility. Our data imply that gabapentin is involved in the exocytosis more likely acting on proteins that provide synaptic vesicles fusion. Thus, the presynaptic fusion machinery may be considered as a viable therapeutic target for the development of new drugs for the treatment of CNS abnormalities related to diabetes.
Perinatal hypoxia: Different effects of the inhibitors of GABA transporters GAT-1 and GAT-3 on the initial velocity of $[^3]$H$ \text{GABA}$ uptake by cortical, hippocampal and thalamic nerve terminals

N. Pozdnyakova, M. Dudarenko, L. Yatsenko, T. Borisova, N. Himmelreich

Department of Neurochemistry, Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine, Leontovicha Str. 9, Kiev, 01601, Ukraine, e-mail: tborisov@biochem.kiev.ua

Perinatal hypoxia leads to multiple chronic neurological deficits including mental retardation, learning and memory disability, behavioral abnormalities and epilepsy. In the pathophysiology of epilepsy, GABAergic system is thought to play a pivotal role in controlling neuronal excitability and maintaining balance between excitation and inhibition. The ambient level of the inhibitory neurotransmitter GABA was controlled by high-affinity Na$^+/Cl^-$-dependent GABA transporters. There are four types of GABA transporters, that is, GAT-1, GAT-2, GAT-3, BGT-1, which differ in localization, affinity to substrate and sensitivity to inhibitors. The predominant types of transporters expressed in the neuronal cells are GAT-1 and GAT-3, which are considered as the main regulators of the extracellular GABA concentration in brain, whereas their overexpression is associated with seizure activity.

Using model of perinatal hypoxia (Pozdnyakova et al., 2011, Yatsenko et al., 2012), rats underwent hypoxia and seizures at the age of 10-12 postnatal days and GABA transporter activity in cortex, hippocampus and thalamus was measured 8-9 weeks after hypoxic stress. The effects of specific non-substrate inhibitor GAT-1, that is, NO711, and substrate inhibitor GAT-3, that is, β-alanine on the initial velocity of $[^3]$H$ \text{GABA}$ uptake by cortical, hippocampal and thalamic nerve terminals (synaptosomes) were analyzed. It was shown that NO711 completely inhibited $[^3]$H$ \text{GABA}$ uptake by cortical and hippocampal synaptosomes and reduced uptake $[^3]$H$ \text{GABA}$ by 90% in the thalamus. The effect of NO711 on $[^3]$H$ \text{GABA}$ uptake was the same in control and perinatal hypoxia.

Substrate inhibitor of GAT-3, i.e. β-alanine, similarly to GABA, is transported to the nerve terminals by GABA transporters, so GABA can effectively compete with β-alanine for substrate binding site in GABA transporters and vise versa. However, the affinity of transporters to GABA and β-alanine is different. Preliminary incubation of control nerve terminals with β-alanine decreased the initial velocity of $[^3]$H$ \text{GABA}$ uptake from 187 ± 14 to 162 ± 10 pmol x min$^{-1}$ x mg protein$^{-1}$ (13.5%) in the cortex, from 183 ± 15 to 155 ± 8 pmol x min$^{-1}$ x mg protein$^{-1}$ (15.3%) in the hippocampus and from 155 ± 12 to 114 ± 9 pmol x min$^{-1}$ x mg protein$^{-1}$ (26.5%) in the thalamus. Therefore, the process of $[^3]$H$ \text{GABA}$ uptake in the thalamus was more sensitive to β-alanine.

In animals, which were subjected to perinatal hypoxia, inhibition of $[^3]$H$ \text{GABA}$ uptake by β-alanine in cortex and hippocampus was similar to the control ones, whereas in the thalamus it was increased from 26.2% to 35.2%. This increase in the sensitivity of $[^3]$H$ \text{GABA}$ uptake to β-alanine allow to suggest that cell surface expression of GAT-3 was increased after perinatal hypoxia, thereby reducing the high level of ambient GABA shown under these conditions (Pozdnyakova et al., 2011).
LE is an experimental anticancer drug active as a dual topoisomerase I/II poison

Alla Korynevska ¹, Petra Heffeter ², Bohdan Matselyukh ³, Walter Berger ²

¹- Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine
²- Institute of Cancer Research, Department of Medicine I, Medical University Vienna, Borschkegasse 8a, 1090 Vienna, Austria
³ -Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kyiv, Ukraine

It is of special interest to discover structurally novel families of natural compounds that display strong anticancer activity, since they might fight multidrug resistant cancers and/or operate via a novel mechanism (thus establishing a new drug target and inspiring new rounds of research).

Understanding of the mechanisms of cytotoxic properties of the anthracycline-related angucycline landomycin E (LE) produced by Streptomyces globisporus towards a mammalian tumor cell lines is necessary for adequate using as potential antitumor drug. That is why our attention were concentrated on the action of LE according to topoisomerase I (Topo I) and topoisomerase II (Topo II). The multiple functions of these enzymes are important as they play a role during replication, transcription, recombination, repair and chromatin organisation [Salermo, 2010 ].

In the concentration of 10-100 µM LE supercoiled pGEM DNA in a presence of Topol was fixed. We can suggested that the activity of LE is might be similar to camptothecin action - taget topoisomerase I through the cleavage complex stabilization.

Inhibition of topoisomerase II was studied with a decatenation assay. Complete inhibition of decatenation was induced by concentration higher than 10µM of LE, and this effect was similar to that for the known topoisomerase II inhibitor doxorubicin used as reference.

Our results provide strong evidence that LE exhibit antiproliferative properties against human cancer cells by inhibiting the activity of topoisomerase I and II cleavable complexes.

A single compound able to inhibit both Topo I and Topo II may present the advantage of improving antitopoisomerase activity, with reduced toxic side effects, with respect to the combination of two inhibitors.
All About Health Care Data
Linn Defensor
Office of Research Compliance and Quality Improvement, Cedars-Sinai Medical Center, Los Angeles, CA, USA and RECOOP HST Consortium CTSMN Project Leader

What is data?
• Dictionary: “Factual information, especially information used for analysis or used to reason or make decisions”
• May be in written, printed, electronic, photographic, audio media or other form for later reference
(Chesney, 2009)
  • In scientific research, data include the materials, products, procedures and other data sources that are part of the research project.
(Clinical Tools, 2006)

Data Management
Source: Clinical Tools (2006). Funded by Office of Research Integrity, USDHHS

• One of core areas addressed by the Office of Research Integrity (ORI) in its responsible conduct of research initiatives
• Requires a significant investment of time and effort by the Principal Investigator (PI)
• Ensures that that every member of research project team is involved in the planning, implementation and maintenance of data management and policies

Guidelines for the Responsible Data Management in Scientific Research

- *Data Ownership – Legal right
- *Data Collection – consistent, systematic manner (reliability); establish a system for evaluating and recording changes (validity)
- *Data Storage – amount of data
- *Data Protection- protection from physical damage (data integrity) including tampering or theft
- *Data Retention – how long, includes secure destruction of data
- Data Analysis – how raw data are chosen, evaluated and interpreted
- Data Sharing – how data and research results are disseminated
- Data Reporting – publication
Re: Research – Material Transfer Agreement

Dear [NAME]:

This is to acknowledge your request for ______________________ (“Material”) which will be provided to you by __________________________ of the Medical Center’s __________________________ for your use in non-commercial scientific research only, under the following conditions:

1. The Material covered by this Agreement includes not only the Material, but also any additional progeny or unmodified derivatives which could not have been made but for the Material and any related information and know-how which will be received under this agreement.

2. The Material will be used only by you and by individuals working under your direct supervision in your institution, and will not be transferred, distributed or released to any other person without the prior written approval of Cedars-Sinai Medical Center (“Medical Center”). You understand that no other right or license to this material or to its use is granted or implied as a result of our sending the Material to you. This Agreement constitutes a limited license to you to use the Material as permitted hereunder solely for academic and not-for-profit purposes. The Material shall not be used in research that is subject to consulting or licensing obligations to any corporation, institution, or individual unless specific prior written permission is obtained from Medical Center.

3. You will inform the Medical Center in confidence of research results related to the Material by personal communication or by providing the Senior Vice President for Academic Affairs with a manuscript describing the results of such research.
at least thirty (30) days prior to submitting the manuscript for publication. If publication results from research using the Material, acknowledgment of and/or credit will be given to the Medical Center, as scientifically appropriate. Any derivative of the Material made in your laboratory will be made available to the Medical Center upon request. If the research which involves the Material results in an invention or substance that may be commercially useful, then you will promptly disclose the invention or substance to your institution and notify it of Medical Center’s role as a supplier of the Material used and the rights reserved hereunder. To the extent that is permissible under applicable law, the Medical Center’s investigators will be recognized for their contribution.

4. The Material is made available for investigational use only in laboratory animals or in in vitro experiments and will not be used in humans or for any other purpose.

5. All characteristics of the material are not fully understood and its use may involve risks or dangers that are not known or fully appreciated. The Material will be used and disposed of by you in compliance with all laws and regulations including current National Institutes for Health guidelines. THE MATERIAL IS EXPERIMENTAL IN NATURE AND IS PROVIDED WITHOUT ANY WARRANTY, EITHER EXPRESS OR IMPLIED, AS TO ITS SAFETY OR FITNESS FOR ANY PARTICULAR PURPOSE OR USE. Acceptance of the material will constitute your acceptance of liability for any damages or injuries resulting from your possession or use of the Material. The Medical Center makes no representation that the use of the Material will not infringe any patent or other proprietary rights of third parties.

6. You and your institution will indemnify and hold the Medical Center and its agents and employees harmless from any loss, claim, damage or liability, of any kind, which may arise from or in connection with this Agreement over the use, handling or storage of the Material. In no case shall the Medical Center be liable for any use by you, by individuals working under your direct supervision, or by your institution, of the Material for any loss, claim, damage or liability, of any kind, which may arise from or in connection with this Agreement or the use of handling or storage of the Material.

7. You understand that no other right or license to this Material or to its use is granted or implied as a result of our sending the Material to you.

8. Upon the request of the Medical Center, any unused Material will be returned to the Medical Center or destroyed.

9. This Agreement shall be governed by and interpreted according to the laws of the State of California.
If you agree to accept the Material under the above conditions, please sign the Agreement, have it signed by an authorized representative of your institution and return it to:

Cedars-Sinai Medical Center
8700 Beverly Boulevard
Los Angeles, California  90048-1865
Attn:  Senior Vice President for Academic Affairs
with copy to:  Senior Vice President for Legal Affairs and General Counsel

The Material will be sent to you as soon as possible after we receive a copy of the signed Agreement from you.

Sincerely yours,

CEDARS-SINAI MEDICAL CENTER

By: ___________________________
Name: ___________________________
Title: ___________________________

By: ___________________________
Name: ___________________________
Title: ___________________________

ACCEPTED:

[REQUESTING INSTITUTION]

By: ___________________________
Name: ___________________________
Title: ___________________________
Date: ___________________________
Welcome to Split

You have to take care of your airport transfer and local transportation!

Information package

1. Getting to Split
2. Getting to your hotel from the airport
3. Getting to your hotel from the train and bus station in the city port
4. Walking from hotel Dujam/Art to the city center
5. What to see and do in Split
6. Public transport in Split
7. Taxis
8. Practical tips

1. Getting to Split
Split is served by airport located in the city of Kaštela (about 25 km outside of Split), ferry and bus transport (last station of both train and long-haul buses is located in the city center, at the port). You can also comfortably arrive by car; Croatia has built an excellent network of highways in recent years.
Alternatively, there is an airport in Zadar ([http://www.zadar-airport.hr/en/](http://www.zadar-airport.hr/en/)), which is served by Germanwings, Ryanair and Danube wings. If you decide to fly to Zadar, you can take airport bus to get to the bus station for long-distance buses in Zadar, and take another bus from there to Split – drive from Zadar to Split is about 2 hrs long.

### 2. Getting to your hotel from the airport

**By airport bus:** There is official airport bus, located immediately at the front of the airport building. When you exit the airport, turn to the right. Bus schedule: [http://www.plesoprijevoz.hr/split.html](http://www.plesoprijevoz.hr/split.html) (departures from Split airport). You can buy ticket at the driver. One-way bus ticket costs 30 HRK (about 4 Eur). The driver does not accept Euros so you should change currency at the airport. This bus does not have any stops before Split, and it takes about 30 min to get to Split. Ask the driver to stop for you on the bus station in front of the Atrium hotel in Split. From that bus station, it is a short walk to hotel Dujam (Map 1) and hotel Art (Map 2).

**By local intercity bus:** The official airport bus has limited departures. If it happens that the bus is not leaving for Split around your arrival time, you can take local bus #37 that drives from Trogir to Split. The local bus stop is just 100 m below the airport, on the road. When you cross the airport parking, cross the road and the station will be right there. You can buy ticket at the driver (you should enter the front door). The bus ticket costs about 20 HRK (around 3 Eur). The driver does not accept Euros so you should change currency at the airport. This bus has numerous stops along the way, and it will take about 50-60 min to get to Split. Ask the driver, or fellow passengers on the bus, to warn you when the bus will stop in front of the Atrium hotel in Split.

**By taxi:** Multiple taxis are available in front of the airport. It costs at least 200 HRK (about 30 Eur). Please check the price before entering the taxi. The drive will take about 30 min to get to the meeting venue.

### 3. Getting to hotel Dujam from the train and bus station in the city port

To get to the hotel Dujam from the central train/bus station in the city centre: after exiting the railway/bus station, one needs to turn left, and walk for about 200 m to get to the station of the local bus #9. Take the bus #9 and exit the 6th station (you can also ask the driver to warn you about the station of the Dujam hotel). Bus ticket can be bought inside the bus from the driver, and it costs 12 kn (about 1.5 Eur). The driver does not accept Euros so you should change currency at the station. One can also walk from the railway station to the hotel Dujam – the walk is 1.8 km long and it could take 25-30 min (Map 3).

### 4. Walking from hotel Dujam/Art to the city center

The hotels are located about 20 min of slow walk from the city center. If you would like to take the local bus, you can take bus #9 near the hotels. Please refer to Maps 1 and 2 to see how to get to the nearest local bus station.

### 5. What to see and do in Split

Split is a historical city, 1700 years old. There are plenty of things to see and do. **Diocletian’s palace** was built by Roman emperor in the 4th century AD, and the city grew around the palace. The palace is the very center of the city, so do not expect to visit some isolated and empty palace. Many people live in the palace, and it is full of shops and restaurants.
Cathedral and Bell Tower of St. Domninous are right in the center of Diocletian’s palace. You can walk all the way to the top of the bell tower and the entrance ticket is 30 kn (about 4 Eur). Not recommended for people afraid of heights and open spaces, as the bell tower is quite ‘airy’. Riva is a promenade at a seaside front of the Diocletian’s palace, facing the city. Riva is the living room of Split. We go there to see and been seen. Take a stroll through Marmont street and along Riva, and then have a coffee at one of numerous cafés.

West coast is a recently renovated and expanded part of the promenade that goes from the end of Riva to the Sustipan. Very nice and relaxing walk, highly recommended. There are several cafés and one restaurant right in the middle of the West coast.

Coffee culture is very strong in Split and you will see numerous people sitting in cafés. People from abroad always ask us is anyone working at Split, when there are so many people in cafés. Considering our high unemployment rates in Croatia, the most accurate answer is – very few people actually work. The price of beverage in cafés is the same if you sit or if you drink at the bar. To give you a rough estimate of prices to expect in cafés – plain espresso coffee is around 8 kn (cca 1 Eur), coffee with milk or espresso around 10 kn (cca 1.5 Eur), soft drink around 12 kn (cca 1.7 Eur), small beer about 15 kn (cca 2 Eur).

Marjan hill is a small hill (highest point 178 m) with forest and recreational facilities. It is highly recommended to go to Vidilica – the observation point above Split. It is about 15 min walking uphill from the city center. When you get there, you will be rewarded with fantastic views of Split, sea and islands. And, of course, there is a café there.

Meštrović gallery houses works of a world renowned Croatian sculptor Ivan Meštrović. The gallery and surrounding park are truly worth a visit. Take the bus #12 from Riva to get there.

Bačvice beach is only 10 min walking away from the city center. It is a beautiful sandy beach, with a Blue Flag. This is not one of the beach resorts, and it does not have any mega hotels. A good place to relax, have an ice cream, or – a drink in one of numerous bars along the beach.

Islands Brač and Šolta are one hour away with a ferry from Split. Island Hvar is two hours away. Going to islands would require a day trip and staying in Split more days before or after the meeting.

Cities of Trogir and Omiš are one hour away from Split by local buses. Those are beautiful historical cities, pearls of Adriatic. 

Popular tourist attractions further away from Split are national parks Krka and Plitvice Lakes, city of Dubrovnik and city of Mostar in Bosnia and Herzegovina. Visiting those places requires at least a full-day trip.

6. Public transport in Split

Split has bus lines numbered from 1 to 19. Day buses 1 through 18 run from 05:00 to 23:00. There is only one night bus, number 19, which runs on Fridays and Saturdays. Maps and schedules for each line can be found at their respective stops. Tickets can be purchased on the bus for 11 kn or from kiosks near each bus stop for less. The company that operate Split's buses is called Promet Split, so make sure the kiosk has that name on it before trying to buy a ticket. Split is covered by one zone, so a ticket is good for one trip anywhere in the city. Sukkošanska is the main station from which you can catch buses for Trogir, Omiš, the airport and other destinations outside of Split. Sukkošanska's ticket office operates from 06:00 to 20:00 on weekdays, 06:00 to 12:00 on Saturdays and is closed on Sunday. To contact the Sukkošanska station, dial (021) 48 06 56. For general information regarding bus services, call (021) 40 79 99.

http://www.promet-split.hr/
7. Taxis
The simplest way to call a taxi is to dial 970. The starting fee for a taxi trip is 20 kn, with a 10 kn fee added per kilometer and 2.5 – 10 kn added per each piece of luggage. There is no additional charge for traveling at night. Taxis wait in front of most major hotels, Firule and Križine hospitals, at the ferry port, at the main bus station and near the Riva.

8. Practical tips
Tap water in Split is potable. Numerous public places have water fountains with potable water as well.
Currency is Croatian kuna (HRK). The exchange rate is approximately 1 Eur = 7.5 HRK. There are many currency exchange offices around Split and your hotel might also provide this service. Split is generally a safe city, but exercise caution and common sense. Protect your valuables.
Official language is Croatian.
Majority of population is Croatian (90.4%), Catholic and conservative. Population of Croatia is 4.2 million.
Croatia joined EU on July 1, 2013.
Useful links:
http://www.split.info/
www.croatia.hr
www.visitsplit.com
Map 1. Walking from bus/train station to hotel Dujam (1.8 km, cca 25 min)
Map 2. Walking from hotel Dujam/Art to the city center (Diocletian’s palace)
Participants of the 4th RECOOP TriNeT Annual Project Review Meeting on October 10-13, 2013 in Split, Croatia.

Participants are the Cedars-RECOOP Research Centers’ (CRRC) project leaders and their young scientists

Croatia

School of Medicine, University of Split

Dr. Livia Puljak, MD., Ph.D.,
Assistant Professor
Department of Anatomy, Histology and Embryology
School of Medicine, University of Split
Soltanska 2 HR-21000 Split, Croatia
Phone (office): +385-21-557-807
Mob: +385-99-688-0260
Fax: +385-21-557-811
e-mail: livia.puljak@mefst.hr; livia.puljak@gmail.com

Antonia Jelicic Kadic, MD
Laboratory for Pain Research,
Department of Anatomy, Histology and Embryology
School of Medicine, University of Split
Soltanska 2 HR-21000 Split, Croatia
Phone (office): +385 2 155 7801
Mob: +385 95 914 1119
E-mail: jelicic.antonia@gmail.com

Matija Boric, MD
Laboratory for Pain Research,
Department of Anatomy, Histology and Embryology
School of Medicine, University of Split
Soltanska 2 HR-21000 Split, Croatia
Phone (office): +385 2 155 7801
Mob: +385 91 726 3964
E-mail: matija.boric.st@gmail.com

Prof. Ana Marusic, MD, PhD
Chair, Department of Research in Biomedicine and Health
University of Split School of Medicine
Soltanska 2, 21000 Split, Croatia
Phone +385 21 557 812
Fax +385 21 557 811
E-mail: ana.marusic@mefst.hr
Prof. Matko Marusic, MD, PhD  
Department of Research in Biomedicine and Health  
School of Medicine, University of Split  
Soltanska 2  
21000 Split, Croatia  
Phone +385 21 557 820  
Mob: 385 99 261-1161  
Fax +385 21 557 811  
E-mail: matko.marusic@mefst.hr

Natasa Mrduljaš – Dujić, MD  
md.natasa@gmail.com

Ivancica Pavlicevic, MD  
jpavlicevic@gmail.com  
Davorka Vrdoljak, MD  
davorka.vrdoljak@mefst.hr

University of Zagreb School of Medicine, Croatia

Marija Lovrić, PhD  
Senior Scientist on GlowBrain project  
University of Zagreb, School of Medicine  
Šalata 3, HR-10000 Zagreb, Croatia  
tel: +385 1 459 6836,  
mob: +385 91 535 0856  
fax: +385 1 4566 795  
e-mail: mlovric@hiim.hr

Lejla Ferhatovic, PhD  
postdoc on EU FP7 Project GlowBrain  
University of Zagreb School of Medicine  
Šalata 3, HR-10000 Zagreb, Croatia  
tel: +385 1 459 6836  
mob: +385 921111158  
fax: +385 1 4566 795  
e-mail: lferhatovic@hiim.hr

School of Medicine University Josip Juraj Strossmayer Osijek

Marija Heffer, MD, PhD  
Department of Medical Biology  
School of Medicine Osijek
RECOOP Annual Project Review Meeting in Split Croatia on October 10 -13, 2013

Huttlerova 4
31 000 Osijek
Croatia
Phone: +385 31 512 845
Mob. 385 91 504 36 77
E-mail: mheffer@mefos.hr; marija.heffer@gmail.com;

Barbara Viljetić, Mr. sc.
Department of Chemistry,
Biochemistry and Clinical Chemistry,
School of Medicine,
Josip Juraj Strossmayer University of Osijek, Croatia
Phone: +385 31
Mobile phone: + 385 91 586 33 08
Fax. +385 31 505 615
E-mail: bviljetic@mefos.hr

Marta Balogh
Department of Medical Biology,
School of Medicine Osijek,
Huttlerova 4, 31000 Osijek,
Phone: +385 98 655 705
E-mail: marthab007@gmail.com

Dražen Mlinarević, M.D.,
Emergency Cardiology, Clinical Hospital Osijek and
Department of Medical Biology, School of Medicine Osijek,
J.Huttlera 4, 31000 Osijek
E-mail: dmlinare@gmail.com

Department of Biology, University J.J. Strossmayer

Professor Elizabeta Has-Schön, PhD,
Department of Biology,
University J.J. Strossmayer
Osijek, 31000 Osijek, Cara Hadrijana 8A, Croatia
Land line: +385-31-399933;
Mobil: +385-91-224 1413; +385-91-530 3066
Fax:+385-31-399339
E-mails: hasschon@biologija.unios.hr; hasschon.elizabeta@gmail.com;

Rosemary Vuković Ph. D.
2013. University J.J. Strossmayer in Osijek
Cara Hadrijana 8/A, Osijek
Tel: ++385-31-399-913
Fax: ++385-31-399-939
E-mail: rosemary@biologija.unios.hr

Senka Blažetić
Department of Biology
University J. J. Strossmayer Osijek
Cara Hadrijana 8/A
Osijek
Tel: +385 91 784 24 85
E-mail: senka.00@gmail.com

Czech Republic

IKEM

Jan Pitha M.D., Ph.D.
Head of Laboratory for Atherosclerosis Research,
Centre for Experimental Research
Institute for Clinical and Experimental Medicine (IKEM)
Videnska 1958/9 140 21 Prague 4, Czech Republic
Tel.: +420 26 136 3069
Fax: + 420 24 1721 574
Mob: + 420 60 764 3119
E-mail: japi@ikem.cz;

Anna Králová (Mgr.)
Laboratory for Atherosclerosis Research,
Centre for Experimental Research
Institute for Clinical and Experimental Medicine (IKEM)
Videnska 1958/9 140 21 Prague 4, Czech Republic
Tel.: +420 26 136 3064
Fax: + 420 24 172 1574
Mob: + 420 72 305 1827
E-mail: annaurban@seznam.cz

Participants Institute of Macromolecular Chemistry

Professor Daniel Horák, Ph.D.
Head of the Department of Polymer Particles
Institute of Macromolecular Chemistry AS CR
Heyrovského nám. 2, 162 06 Praha 6
Czech Republic
Tel. 420-296809260
Fax. 420-296809410
Mobil 420-775426744
E-mail: horak@imc.cas.cz

Participants Hradec University Hospital

Dr. Marian Kacerovsky, MD
Department of Obstetrics and Gynecology
University Hospital in Hradec Kralove
Sokolska 581
Hradec Kralove, 500 05
Czech Republic
Tel: + 420 49 583 3743
mobile: + 420 77 765 7991
E-mail: marian.kacerovsky@gmail.com; Marian.Kacerovsky@seznam.cz

Martin Stepan MD
Department of Obstetrics and Gynecology
University Hospital Hradec Kralove
Sokolska 581
500 05 Hradec Kralove
Cellular phone +420 606 308 680
E-mail: mstepan@post.cz

Hungary

University of Debrecen

Zoltan Papp, M.D., Ph.D., D.Sc.
Institute of Cardiology, Clinical Physiology Department
University of Debrecen
Móricz Zsigmond krt. 22. H-4032 Debrecen, Hungary
Tel: 36 52 411-600 [operator] / 54329 extension
E-mail: pappz@dote.hu

Attila Borbely, M.D., Ph.D.
Cardiologist
Institute of Cardiology, Medical and Health Science Center
University of Debrecen
Móricz Zsigmond krt. 22.
H-4032 Debrecen, Hungary
Tel: 36 52 255 928
Mob: 36 70 316 7810
E-mail: borattila@freemail.hu

Judit Kalász
Ph.D student (25ys)
University of Debrecen MHSC,
Institute of Cardiology
Division of Clinical Physiology
E-mail: kalaszjudit@hotmail.com

University of Pecs Participant

Timea Kvárik, MD
PhD student
Department of Obstetrics and Gynaecology, Medical School, University of Pécs, Pécs, Hungary
Édesanyák útja 17., Pécs, 7624, Hungary
Mob: +3630/4088076
e-mail: kvarik.timi@gmail.com

Barbara Mammel, MD
PhD student
Department of Obstetrics and Gynaecology, Medical School, University of Pécs, Pécs, Hungary
Édesanyák útja 17., Pécs, 7624, Hungary
Mob: +3670/4663457
e-mail: mammel.barbara@gmail.com

University of Szeged

Robert Gaspar PhD
Head, associate professor
Dept. of Pharmacodynamics and Biopharmacy
University of Szeged, Hungary
Tel.: +3662341971
Mobile: +36309755401
Fax: +3662545567
E-mail: gaspar@pharm.u-szeged.hu

Arpad Marki PhD
Department of Pharmacodynamics and Biopharmacy,
University of Szeged, Hungary
E-mail: marki@pharm.u-szeged.hu

Semmelweis University, Budapest

Éva Szökő, PhD, DSc.,
Department of Pharmacodynamics,
Faculty of Pharmacy, Semmelweis University
Nagyvárad tér 4, Budapest, Hungary H-1089
Tel: (36-1) 210 2930/ 56324,
Fax: (36-1) 210 4411
E-mail: eva.szoko@net.sote.hu
Tamás Tábi PhD
assistant professor
Semmelweis University, Department of Pharmacodynamics
Nagyrád tér 4., Budapest, H-1089 Hungary
Tel.: +36-1-210-2930/56412
Mob: 36 20 920 6408
Fax: +36-1-210-4411
E-mail: tamas.tabi@net.sote.hu

Miskolc, Medical Informatics

Mr. Gyula Markovics
Praxinfo
Miskolc, Aulich Lajos u. 13. Hungary, H-3529
Tel: 56 46 560-417
E-mail: markovicsgyula@t-online.hu

Poland

Wrocław University of Technology

Artur Podhorodecki
Institute of Physics, Wrocław University of Technology
Wybrzeże Wyspianskiego 27, 50-370 Wrocław, Poland
Phone:+(48) 713202358
Mobile:
E-mail: artur.p.podhorodecki@pwr.wroc.pl

Anna Zelazo
Institute of Physics, Wrocław University of Technology
Wybrzeże Wyspianskiego 27, 50-370 Wrocław, Poland
Phone: + 48 71 320 23 58
E-mail: 193991@student.pwr.wroc.pl

Romania, Bucharest

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
Ion Movila Street, no 5-7, sector 2, Bucharest 70266 Romania
Tel: +40 1-210 2806
Fax: +40-1-310 1213; +40-1-210 6435,
Mobile: +40-7223-790-093
E-mail: iulianaceausu2004@yahoo.com; iceausu@hotmail.com

Cristian Poalelungi, MD
Junior Research Fellow, 3rd year Resident in Obstetrics-Gynecology
Obstetrics-Gynecology Department, Hospital "Dr.I.Cantacuzino",
“Carol Davila” University of Medicine and Pharmacy, Bucharest, Romania
5-7, Ion Movila, sector 2, Bucharest, Romania
Phone: 004-0212102806
Cell: 004-0722453515
e-mail: cristianpoalelungi@yahoo.com

Slovak Medical University

Doc. MUDr. Martin Gajdoš, PhD.
Vice-rector for Research Affairs
Slovak Medical University
Limbova 14, 833 03 Bratislava
Office: +42125 937 0250
mobile: +421 90 347 0989
Reception: +421259370251
E-mail: martin.gajdos@szu.sk

Shubhada Bopegamage, MD, PhD
Head, Entervirus Laboratory
Virology Department
Slovak Medical University
Limbova 12, 83303 Bratislava, Slovak Republic
E-mail: shubhada.bopegamage@szu.sk; s.bopegamage@gmail.com

MUDr. Jana Tulinska, PhD.
Laboratory of Immunotoxicology
Slovak Medical University
Limbova 14, 833 03 Bratislava, Slovakia
tel: +421 25 937 0244
fax: +421 259 370 388
email: jana.tulinska@szu.sk

Maria Borsanyiova, MSc., PhD
Junior Research Scientist, age 38.
Enterovirus Laboratory
Medical Faculty
Slovak Medical University
Limbova 12, 83303 Bratislava, Slovak Republic
E-mail: maria.borsanyiova@szu.sk
Patricia Kramarova, MSc.,
PhD Student
Slovak Medical University, Medical Faculty,
Dept. of Clinical and Experimental Pharmacotherapy
Limbova 12, 83303 Bratislava, Slovak Republic
E-mail: patriciakramarova@gmail.com

Comenius University, Bratislava

Katarina Sebekova, MD, PhD,
Institute of Molecular BioMedicine
Medical Faculty, Comenius University
Sasinkova 4
811 08 Bratislava
Tel: +421-25 935 7429
M: +421 90 779 4093
E-mail: kata.sebekova@gmail.com

Radana Kollarova, MSc
PhD student
Institute of Molecular BioMedicine
Faculty of Medicine
Comenius University
Sasinkova 4
811 08 Bratislava Slovakia
Mob: +421 – 90 – 753 9253
E-mail: radana.kollarova@gmail.com

Ukraine

Palladin Kiev Ukraine

Dr. Tatiana Borisova, PhD
Foreign Secretary, Ukrainian Biochemical Society
Senior Scientist, Department of Neurochemistry,
Palladin Institute of Biochemistry NAS of Ukraine
9 Leontovicha str., 01601 Kiev, Ukraine
Tel.: 380 44 234-32-54
Fax 380 44 279-63-65
E-mail: tborisov@biochem.kiev.ua
ICB Lviv Ukraine

Professor Rostyslav Stoika, PhD, D.Sc.
Head and Professor
Department of Regulation of Cell Proliferation and Apoptosis
Institute of Cell Biology, National Academy of Sciences of Ukraine
Drahomanov Street 14/16 79005, Lviv, UKRAINE
phone/fax: +38 032 261 22 87
Mobil: 380-66-303-2152
E-mail: stoika@cellbiol.lviv.ua; stoika.rostyslav@gmail.com

US participants

University of Alabama- Birmingham

Professor William J. Britt, M.D.,
University of Alabama- Birmingham
Department of Pediatrics
1600 7th Ave South, Chb 107
Birmingham, Al 32594
Birmingham, AL 35233-1711
Phone #: (205) 996-7762
Mob: 1 205 834 5146
E-mail: wbritt@peds.uab.edu; wbritt@uab.edu

Cedars-Sinai Medical Center

Sandor G. Vari, MD
Director, International Research and Innovation Management Program
Cedars-Sinai Medical Center &
President of the RECOOP HST Association
6420 Wilshire Blvd., Ste. 300, Los Angeles, CA  90048-5502
Tel: 1 323-866-8122; 1 323-866-6824
Mob: 1 818 398-2642
E-mail: vari@cshs.org,

Chander P. Arora, PhD
Research Project Adviser
International Research and Innovation Management Program,
Cedars-Sinai Medical Center and RECOOP HST Association Research Project Management
8635 West 3rd Street, Suite 160W
Los Angeles, CA 90048
Tel: 1 (310) 423-7709
Mob: 1 (818) 510-2039
E-mail: Chander.Arora@cshs.org; prabhaarora5@gmail.com;

Linn Defensor RN, CCRP
Office of Research Compliance and Quality Improvement
Cedars-Sinai Medical Center
RECOOP HST Consortium CTSMN Project Leader
Phone  (310) 423-3783
Fax    (310) 423-4195
E-mail: defensor@cshs.org