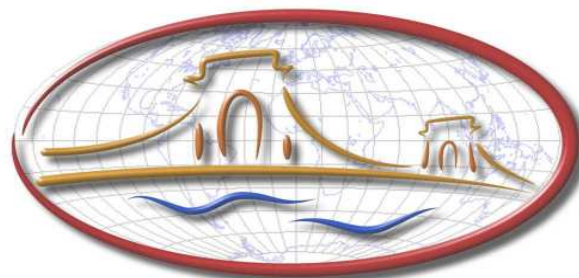




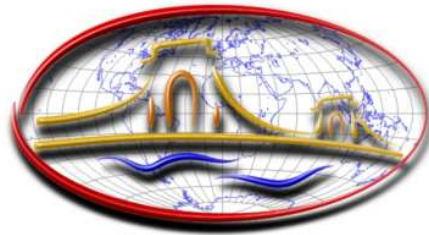
RECOOP 5th TriNet Meeting

Wroclaw on October 17 – 19, 2014



RECOOP HST ASSOCIATION

**Cedars - Sinai Medical Center -
RECOOP HST Association Research Center**



CRRC

RECOOP 5th TriNet Meeting in Wroclaw on October 17 – 19, 2014

Venue: VEGA HOTEL <http://www.hotelvega.pl/en>

Arrival on October 17 until 17:00,
Departure on October 19, 2014

Number of participants: 40

Agenda on October 17, 2014

Arrival until 17:00 pm

14:00 – 16:00 Sightseeing

16:00 – 17:00 Lab visit

18:00 – 22:00 Boat Tour - Dinner

Agenda

October 18, 2014

08:30 – 10:30 Plenary Session – CRRC manuscript review for CMJ publication April 2015

08:30 – 8:45 Summary of the submitted abstracts by organizations and topics
Sandor G. Vari, CSMC - RECOOP

08:45 – 9:15 Review Process – criteria – mechanism
Srećko Gajović, Editor-in-Chief, Croatian Medical Journal

- 09:15 – 9:45 Selection of RECOOP Review Board
Sandor G. Vari, CSMC – RECOOP
- 09:45 – 10:00 CMJ – RECOOP Review Time Table
Sandor G. Vari, CSMC – RECOOP
Srećko Gajović, Editor-in-Chief, Croatian Medical Journal
- 10:00 – 13:00 Plenary Session - RECOOP Research Plan for the next five years
- 10:00 – 10:30 RECOOP Strategy for Common Mechanism of Diseases
Sandor G. Vari, CSMC – RECOOP
- 10:30 – 11:00 Coffee break
- 11:00 – 13:00 Plenary Session - RECOOP Strategy for Common Mechanism of Diseases
- 11:00 - 11:15 Gender difference in expression of estrogen receptor β and leptin receptor in adrenal gland after chronic and acute stress
Research Team: University J. J. Strossmayer Osijek, University of Szeged, Slovak Medical University
Marija Heffer, Marta Balog, University J. J. Strossmayer Osijek
- 11:15 – 11:30 Stress ovarian-derived sex hormones alter cardiomyocyte contractile function in ovariectomized rats
Research Team: University of Debrecen, University J. J. Strossmayer Osijek, University of Szeged
Attila Borbely, University of Debrecen
- 11:30 – 11:45 Effects of high fat diet, ovariectomy and physical activity on leptin receptor expression in rat brain and white fat tissue
Research Team: Slovak Medical University, University J. J. Strossmayer Osijek, Zora Krivosikova, Slovak Medical University
Elizabeta Has-Schon, Marija Heffer and Radivoje Radić, University J. J. Strossmayer Osijek, University of Szeged
Robert Gaspar, University of Szeged
- 11:45 – 12:00 Impact of ovariectomy, high fat diet and lifestyle modifications on oxidative/antioxidative status in rat liver
Research Team: University J. J. Strossmayer Osijek, University of Szeged, Slovak Medical University
Elizabeta Has-Schon, Marija Heffer and Radivoje Radić, University J. J. Strossmayer Osijek, University of Szeged
Zora Krivosikova, Slovak Medical University

- 12:00 – 12:15 Nanoparticles for targeted drug delivery
Research Team: Institute of Cell Biology Lviv, Institute of Physics, Wroclaw University of Technology, Institute of Macromolecular Chemistry Prague, Palladin Institute of Biochemistry Kiev, Danylo Halatsky Lviv National Medical University, Slovak Medical University, University of Zagreb School of Medicine, Cedars – Sinai Medical Center, University of Copenhagen, Faculty of Pharmacy
 Rostyslav Stoika, Rostyslav Bilyy, *Institute of Cell Biology Lviv*
 Daniel Horak, *Institute of Macromolecular Chemistry Prague*
 Artur Podhorodecki, *Institute of Physics, Wroclaw University of Technology*
 Tatiana Borisova, , *Palladin Institute of Biochemistry Kiev*
 Roman Lesyk, *Danylo Halatsky Lviv National Medical University*
 Jana Tulinska, *Slovak Medical University*
 Srecko Gajovic, *University of Zagreb School of Medicine*
- 12:15 – 12:30 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
Research Team: Comenius University, Semmelweis University, University J. J. Strossmayer Osijek,
 Katarina Sebekova, Comenius University
- 12:30 – 12:45 Change in cardiomyocyte contractile function during age and gender
Research Team: University of Debrecen, University of Pecs
 Zoltan Papp, Attila Borbely, University of Debrecen
 Tibor Ertl, University of Pecs
- 12:45 – 13:00 Assessing the contribution of systemic to brain insulin resistance across neurodegenerative diseases
Research Team: Cedars – Sinai Medical Center, Semmelweis University, University of Osijek, Comenius University, University of Zagreb, School of Medicine, University of Copenhagen, Faculty of Pharmacy
 Konrad Talbot, Sandor G. Vari, Cedars-Sinai Medical Center
- 13:00 – 14:00 Lunch
- 14:00 – 16:00 Plenary Session - RECOOP Strategy for Common Mechanism of Diseases
- 14:00 – 14:15 Bioseparation of glycans, lipids and fatty acids to understand the common mechanisms of diseases
Research Team: University of Debrecen, Institute of Cell Biology Lviv, Semmelweis University, University J. J. Strossmayer Osijek
 Andras Guttman, *University of Debrecen*
 Martina Mihalj, *University J. J. Strossmayer Osijek*
 Tamas Tabi, , *Semmelweis University*

- 14:15 – 14:30 Study pathogenesis and role of transcription factors in adipose tissue inflammation, in chronic kidney diseases
Research Team: Semmelweis University, University of Szeged, University J. J. Strossmayer Osijek, University of Pecs, Institute of Cell Biology Lviv, University of Debrecen,
 Eva Szoko, Imre Fehrevari, Tamas Tabi, Semmelweis University
 Edit Szederkenyi, University of Szeged
 Rostyslav Stoika, Oleksandr Korchynskyy, Institute of Cell Biology Lviv
 Andras Guttman, University of Debrece,
 Marija Heffer, University J. J. Strossmayer Osijek
 Marianna Pap, University of Pecs
- 14:30 – 14:45 Risks of obesity during pregnancy
Research Team: University of Pecs, Semmelweis University, University of Szeged, University J. J. Strossmayer Osijek, Institute of Cell Biology Lviv, University of Debrecen,
 Jozsef Bodis, Tibor Ertl, Marianna Pap, University of Pecs
 Eva Szoko, Tamas Tabi, Semmelweis University
 Rostyslav Stoika, Oleksandr Korchynskyy, Institute of Cell Biology Lviv
 Andras Guttman, University of Debrece,
 Marija Heffer, University J. J. Strossmayer Osijek
- 14:45 – 15:00 Lifestyle Intervention:
 Testing Perimenopause as a Sensitive Period for Atherosclerosis Prevention
Research Team: Institute for Clinical and Experimental Medicine, Prague
 Jan Pitha, IKEM
- 15:00 – 15:15 Shared decision making in lifestyle and nutrition for intervention in women with risk factors in cardiovascular health
Research Team: Department of Family Medicine, University of Split School of Medicine, Institute for Clinical and Experimental Medicine, Prague
 Ivancica Pavlicevic, Mario Malički, Department of Family Medicine, University of Split School of Medicine
 Jan Pitha, *Institute for Clinical and Experimental Medicine, Prague*
- 15:15 – 15:30 Cross talk between skeletal muscle, adipose tissue and inflammation in the cohort study of assessment health condition among professionals of informational technology (IT) software industry
Research Team: Danylo Halytsky Lviv National Medical University
 Oksana Zayachkivska, Department of Physiology, Lviv National Medical University
- 15:30 – 16:00 Monitoring Plan for Clinical Research
 Linn Defensor, Office of Research Compliance & Quality Improvement (ORCQI), Cedars-Sinai Medical Center

16:00 – 16:30 Coffee Break

16:30 – 17:00 New projects

16:30 – 16:45 Screening for congenital CMV infection and identification of biomarkers predictive of long-term outcome in RECOOP
Research Team: University of Alabama- Birmingham, Cedars – Sinai Medical Center, Slovak Medical University, University of Pecs, Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania, Charles University in Prague, Faculty of Medicine Hradec Kralove, University Hospital Hradec Kralove William J. Britt, University of Alabama- Birmingham, Department of Pediatrics

16:45 – 17:00 Regulation of inflammatory responses of the digestive system
Research Team: Danylo Halytsky Lviv National Medical University Oksana Zayachkivska, Department of Physiology, Lviv National Medical University

17:00 – 18:00 Plenary – Discussion of RECOOP HST Association’s CMD research strategy
Moderator: Sandor G. Vari

18:00 – 18:30 Publication of RECOOP retrospective studies

W&CVD RN -Working title - Gender-related differences in coronary artery disease risk factors in Croatia, Czech Republic and Hungary
Dražen Mlinarević, School of Medicine, Josip Juraj Strossmayer University of Osijek

Preterm Birth, the risk factors, disparities and discrepancies in data from six Central and Eastern European Centers
Chander P. Arora and Sandor G. Vari

18:30 – 19:00 Review Stage 1 proposal for FP8 EU HORIZON2020 call “Health, demographic change and well-being” PHC – 1 **Understanding health, ageing and disease**
PHC-03-2015 Understanding common mechanisms of diseases and their relevance in co-morbidities submitted on October 14, 2014 and Stage 2 will come on February 24, 2015
Sandor G. Vari,

19:30 – 22:00 Dinner

October 19, 2014 Departure

RECOOP Strategy for Common Mechanism of Diseases

Sandor G. Vari,

CSMC – RECOOP HST Association has research activities in the areas of cell signalling, stress, obesity, metabolic disorders (diabetes), inflammatory process, cardiovascular disease (CVD) and neurodegenerative diseases (NDGD). RECOOP is profoundly involved in investigating the role of gender, age and lifestyle in different medical conditions, also implementing studies for lifestyle intervention to reduce the risk factors thus contributing to the prevention of major public health problems worldwide. Interpretation of the molecular mechanisms that underlie a range of common human disorders (obesity, CVD and NDGD) and exploration of possible research methods to study disease mechanisms, common pathways and gender differences (ND) are the main focuses of RECOOP.

Adipose tissue is capable of expanding many-fold during adulthood that is evaluated by waist - hip ratio (WHR). Accumulation of adipose tissue in individuals with obesity is associated with a state of persistent low-grade inflammation that seems to play a pivotal role in the pathogenesis of obesity-linked insulin resistance, diabetes, CVD and NDGD diseases.

Different role and contribution of subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) to these pathological processes are suggested. After menopause, as estrogen supplies dwindle, women start storing fat around their abdomen, will change from pear - shaped to more apple-shaped obesity and during this period, their risk for CVD disorders is highly increased.

The severity of insulin resistance can be assessed by glucose tolerance test (GTT) using glucose and insulin data to calculate systemic insulin resistance index (HOMA-IR). The involvement of adipose tissue in the inflammatory processes is well known and associated with production of a wide range of inflammatory mediators especially in abdominal type (apple) obesity.

RECOOP investigators already study SemiCarbazide Sensitive Amine Oxidase (SSAO)/ Vascular Adhesion protein (VAP-1) activity and expression in the adipose tissue as well as the correlation of inflammation and insulin resistance. Scientists in RECOOP will investigate the role of the regulatory pathways and transcription factors: Nuclear Factor κ B (NF- κ B), peroxisome proliferator-activated receptor γ (PPAR γ), cAMP response element-binding factor (CREB), repressor element 1-silencing transcription factors (REST), Wnt- β -catenin signalling in adipogenesis and adipose tissue inflammation. In further studies, they explore the role of Insulin Growth Factor (IGF-1) in adipogenesis and insulin resistance. The liver produced IGF has been shown to play roles in the promotion of cell proliferation and the inhibition of cell death (apoptosis). The grade of inflammation is to be characterised by tumor necrosis factor α (TNF α) Interleukin 6 and 10 (IL-6, IL-10) and VAP-1. Core features of the metabolic syndrome are the inflammation, the endoplasmic reticulum and oxidative stress, dyslipidemia; all associated with obesity. Localization and quantification of the estrogen receptor β (Er β), leptin receptor (Ob-R), and progesterone receptor (PR) in the adipose tissue and the Central Nerve System (CNS) helps understand the alterations in obesity compared to normal weight as well as changes in acute and chronic stress also reflecting gender differences. RECOOP investigators would study the Fat Mass and Obesity-Associated Protein (encoded by the FTO gene). FTO protein also known as alpha-ketoglutarate-dependent dioxygenase is an enzyme and certain variants of its gene have

been correlated with obesity. The alteration of the fatty acid composition in SAT and VAT will be evaluated with Micellar Electrokinetic Chromatography (MECK), correlated to insulin resistance and adipose tissue inflammation to assess its role in CVD and NDGD. RECOOP scientists concluded that the brain insulin resistance might be mediated by factors outside the CNS and may be related to the systemic (peripheral) insulin resistance. Scientist investigate the proposed connections between systemic insulin resistance, the metabolic syndrome, and obesity with various NDGD diseases, most notably Alzheimer's disease (AD). Nevertheless studies to date are mainly on rodents and do not test the possibility that brain insulin resistance may be a common mechanism for the pathologies and symptoms of NDGD diseases as a group. RECOOP scientists will investigate the association of Homeostasis Assessment Model - Insulin Resistance (HOMA-IR) and cognitive status with cytokines in the cerebrospinal fluid (CSF)-adiponectin, leptin both in adipose tissue and blood, Amyloid-beta A β , phosphorylated tau, synuclein, and TDP-43 in different diagnostic groups. The investigators will calculate the correlation between cognitive status and these parameters.

The progress in targeted drug delivery and personalized medicine is enhanced by nanoparticles engineered to be attracted to pathological cells. This technique strongly reduces damage to healthy cells in the body and allows earlier detection as well as targeted treatment of AD. The goal of RECOOP CMD is to test these nanoparticles for drug delivery to the brain through the Blood – Brain Barrier (BBB) in already established animal models and small animal imaging systems (CT, MRI).

RECOOP scientists will study the regulation of CMD with **Transcription Factors:** NF- κ B, PPAR γ , CREB, NRSF, REST, Wnt- β -catenin signalling.

They will investigate biomarkers already used and will identify new markers and investigate their roles in CMD:

Macrophage Inflammatory Proteins and Pro-inflammatory Cytokines: VAP-1, TNF α , IL-6, IL-10;

Stress Indicators: GTT, Er β , Ob-R, PR, SEMG, Cardiomyocyte contractility, Cortisol;

Obesity and Metabolic Markers: Waist -hip ratio, SAT, VAT, Body fat, SSAO, FTO, Leptin, Adiponectin, Insulin, Insulin receptors, IGF-1, lipid composition;

Biomarkers of Brain Insulin Resistance and Neurodegeneration IRS-1 pS and SOCS3, A β , phosphorylated tau, synuclein, and TDP-43.

RECOOP has the know-how, the technology transfer skills to bring novel biomedical developments from bench to bed and invent new biotechnologies.

RECOOP HST Association's research strategy in key words for common mechanisms of diseases

Primary key words have to correlate to understanding common mechanisms of diseases and their relevance in co-morbidities

Primary key words related to common mechanisms of diseases:

Regulation, inflammatory process, stress, obesity, metabolic disorders (diabetes), chronic disease, neurodegenerative diseases, cardiovascular diseases, cancer, nanobiomedicine, lifestyle, intervention, gender, age

Related key words to understanding common mechanisms of diseases and their relevance in co-morbidities:

Regulation: molecular biology, genetics, transcription, transcription factors, upregulation, downregulation, coactivator, corepressor

Inflammatory process: inborn immune system, immune cell homeostasis, immune activation, inflammation, inflammatory cell infiltration, SSAO/Vascular Adhesion Protein-1 (VAP-1), glycosylation, receptors, receptor-ligand signaling by glycosylation, viral surface glycoprotein, Cocksackie B viruses (CB), human cytomegalovirus (HCMV) congenital infections, screening, biomarkers, interleukins

Stress: adrenal gland, androgen receptor, steroid hormones, steroid receptors, adrenoceptors, smooth muscle electromyography (SEMG) cardiomyocyte contractility

Obesity: diet, food supplies, body fat, overweight, degree of obesity, body mass index (BMI), pregnant women, premenopausal and menopausal women, osteoporosis, bone mass density, bone mineral content, energy homeostasis, leptin receptor, serotonergic receptors, brain reward center (the arcuate nucleus, the dorsolateral hypothalamus, the dorsomedial nucleus), satiety-related signaling (c-Fos expression), orexigenic - anorexigenic receptors, Semmcarbazine Sensitive Amine Oxidase (SSAO), adipogenic factors, Wnt/ β -catenin signaling, adipocyte differentiation, PPRgamma, obesity-associated (FTO) gene, cAMP responsive element-binding protein (CREB), diabetes-mediated CREB dysfunction, oxidative damage, adipose tissue, intra - abdominal (mesenteric), subcutaneous (white), bioseparation, fatty acids, micellar electrokinetic chromatography (MEKC)

Metabolic disorders (diabetes): type 2 diabetes, insulin, insulin receptors, insulin sensitivity, insulin resistance, serotonergic neurotransmission, glucose transporters, Semmcarbazine Sensitive Amine Oxidase (SSAO), cAMP responsive element-binding protein (CREB), blood-brain barrier, gestational diabetes mellitus (GDM)

Chronic diseases: chronic kidney disease (CKD), hydromineral homeostasis, arginine vasopressin (AVP) transcription, cAMP responsive element-binding protein (CREB), cellular

differentiation, neurons, β -cells, adipocytes and smooth muscle cells; upstream regulation, mitochondrial biogenesis, SSAO/VAP-1, dysregulation, Wntk/ β -catenin signaling,

Neurodegenerative diseases: neuroprotective effect, neurogenesis, neuronal differentiation, brain-derived neurotrophic factor (BDNF), cerebral ischemia, oxygen-glucose deprivation (OGD), membrane potential, the proton gradient of synaptic vesicles, cAMP responsive element-binding protein (CREB), RE1-Silencing *Transcription* factor (REST), leptin-induced synaptogenesis, hippocampus, cognition, depression, anxiety, Alzheimer's and Parkinson's diseases and other cognitive disorders

Cardiovascular diseases: cardiometabolic risk factors; premenopausal and menopausal women, epigenetics, heart failure, coronary artery disease, atherogenesis, low grade inflammation, pro-inflammatory mediators, endothelial barrier, aberrant accumulation of lipid molecules, estrogen receptors ($ER\alpha$, $ER\beta$), vascular gene expression, anti-atherogenic effects, CREB, endothelial nitric oxide synthase (NOS), myocardin, CREB-binding protein, cardiac hypertrophy, systolic and diastolic dysfunctions, cardiomyocyte contractile force, myofilament proteins,

Cancer and Nanobiomedicine: nanoparticles, nanobiopolymers, semiconductor nanocrystals, maghemite, polysaccharide-coated nanoparticles of magnetite, poly (ethylene glycol), immunosafety of nanocrystals, immunotoxicity of newly developed nanoparticles, phagocytic activity change, tumor biology, immune system, long-circulating nanoparticles, complement-dependent tumour growth, C5a receptor, nanoscale polymeric drug carrier, doxorubicin, KP1019, 4-tiazolidinones, cancer cell lines, drug resistance, experimental tumor models *in vivo*, systemic toxicity, optical markers, molecular markers imaging, multi-targeting, inhibition, nanodrugs, melanoma, brain, breast, prostate cancers

Lifestyle intervention: Cardiovascular diseases (CDV) risks, lifestyle modifications, balanced diet, micronutrient intake, exercise, mitochondrial biogenesis

Gender difference in expression of estrogen receptor β and leptin receptor in adrenal gland after chronic and acute stress

Research Team: University J. J. Strossmayer Osijek, University of Szeged, Slovak Medical University

Marija Heffer, Marta Balog, University J. J. Strossmayer Osijek

Plasma glucose, cholesterol and urates level combined with the weight monitoring and glucose tolerance test (GTT) are good markers for metabolic syndrome caused by stress. Leptin has the main role in body weight maintenance but it also lowers the hypothalamus-pituitary axis stress response by inhibition of steroid-hormone secretion from the adrenal cortex. Appearance of strong nuclear staining of the $Er\beta$ and Ob-R antibodies in adrenal gland suggests possible transcriptional changes during stress. Main aim of the study is to explore the expression of oestrogen receptor beta ($ER\beta$), progesterone receptor (PR) and Obesity – leptin - receptor (Ob-R) in adrenal gland, adipose tissue and CNS after acute and chronic stress using immunohistochemical staining.

Stress and ovarian-derived sex hormones alter cardiomyocyte contractile function in ovariectomized rats

Research Team: University of Debrecen, University J. J. Strossmayer Osijek, University of Szeged

Attila Borbely, University of Debrecen

Gender differences and ovarian-derived sex hormones (in particular progesterone) could modify the effects of stress as was proven in a single acute stress study to investigate the mechanical and biochemical properties of left ventricular cardiomyocytes in the rat. Ca^{2+} -dependent active force (Factive), Ca^{2+} -independent passive force (Fpassive) and Ca^{2+} -sensitivity of force production (pCa50) were determined in single, mechanically isolated, permeabilized cardiomyocytes. During RECOOP CMD the on-going research will continue to investigate the impact of acute and chronic stress and the role of female hormones in altering Ca^{2+} -dependent cardiomyocyte contractile force production, which may have pathophysiological importance during stress conditions affecting postmenopausal women.

Effects of high fat diet, ovariectomy and physical activity on leptin receptor expression in rat brain and white fat tissue

Research Team: Slovak Medical University, University J. J. Strossmayer Osijek, Zora Krivosikova, Slovak Medical University

The aim of the study is to continue the evaluation whether ovariectomy (OVX), high fat diet (HFD) and physical activity (R) compared to sham surgery (SH), standard diet (SD) and sitting (S) conditions affect leptin receptor (Ob-R) distribution in the brain and white fat tissue. Free-floating immunohistochemistry and Western blot methods were carried out to detect Ob-R in the brain and adipose tissue. This is the first report about regulation of Ob-R positive neurons by combined effect of high fat diet, ovariectomy and physical activity to continue the effort to explore correlation between central obesity and CVD. Based on previous study RECOOP scientists believe that these changes are neurological bases for long-term changes in complex

behaviours linked to obesity phenotype and suppose that changes in quantity of Ob-R are present in all parts of the brain that are related to feeding. Based on the established protocol scientists will continue to study the effect of food intake in more detail.

Impact of ovariectomy, high fat diet and lifestyle modifications on oxidative/antioxidative status in rat liver

Research Team: University J. J. Strossmayer Osijek, University of Szeged, Slovak Medical University

Elizabetha Has-Schon, Marija Heffer and Radivoje Radić, University J. J.

Strossmayer Osijek, University of Szeged

Zora Krivosikova, Slovak Medical University

The aim of this study is to estimate the impact of high fat diet (HFD) and estrogen deficiency on the oxidative and antioxidative status in liver of the ovariectomized (OVX) rats. Scientists investigate possible ameliorating effect of lifestyle modifications, such as physical activity or consumption of functional food containing bioactive compounds with antioxidative properties, on oxidative damage in rat liver. Bioactive compounds of selenized onion biscuits (SOB) showed potential in attenuating adverse impact of HFD on antioxidative status. As an indicator of liver oxidative damage in the previous studies lipid peroxidation (LPO)-expressed and were determined in terms of thiobarbituric acid reactive substances (TBARS). While liver antioxidative status was characterized by catalase (CAT), glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR) activities and glutathione (GSH) content. The study results showed that feeding rats with HFD was accompanied by decreased antioxidative enzyme activities and increased LPO, in both OVX and SH rats. Decreased antioxidant defense suggests lowered oxidative stress resistance, which could be reflected in oxidative damage of rat liver in metabolic disorders. RECOOP scientist will continue the study in order to further investigate oxidative damage induced by the HFD and OVX, and determine the possible the GD related to obesity and stress.

Nanoparticles for targeted drug delivery

Research Team: Institute of Cell Biology Lviv, Institute of Physics, Wrocław University of Technology, Institute of Macromolecular Chemistry Prague, Palladin Institute of Biochemistry Kiev, Danylo Halatsky Lviv National Medical University, Slovak Medical University, University of Zagreb School of Medicine, Cedars – Sinai Medical Center, University of Copenhagen, Faculty of Pharmacy Rostyslav Stoika, Rostyslav Bilyy, Institute of Cell Biology Lviv Daniel Horak, Institute of Macromolecular Chemistry Prague Artur Podhorodecki, Institute of Physics, Wrocław University of Technology Tatiana Borisova, , Palladin Institute of Biochemistry Kiev Roman Lesyk, Danylo Halatsky Lviv National Medical University Jana Tulinska, Slovak Medical University Srecko Gajovic, University of Zagreb School of Medicine

An important application of nanobiotechnologies in medicine involves employing nanoparticles and other nanosized materials to deliver drugs or other agents to specific types of cells (E.g. brain cells). For instance, magnetic nanomaterials, including iron oxides (magnetite Fe₃O₄ and maghemite γ -Fe₂O₃), are used as contrast agents for magnetic resonance imaging (MRI) by the Czech partner. RECOOP partner from Poland is synthesizing nanocrystals with the emission/absorbance in near infrared spectral range (NaGdF₄:Yb,Er, NIR-NCs) or PbS-based core shell nanocrystals. In addition, these NIR-NCs can be also excited with the X-ray beam and detected with IVIS Lumina X-ray and FL combine system and can also improve the MRI contrast due to presence of Gd³⁺ ions in the NIR-NCs shell. RECOOP scientists from Ukraine are using luminescent carbon nanomaterials are advantageous compared to fluorescent dye molecules, fluorescent proteins, owing to their stable photoluminescence (PL), excellent biocompatibility and low cytotoxicity. The listed nanoparticles have shown potential applications in bioimaging, diagnosis and drug delivery for NDGD like Alzheimer's disease. In - vivo preclinical tests with animal research (rat) for the evaluation of immunotoxic effect of nanoparticles were already performed. The next step is to investigate the Blood Brain Barrier (BBB) and the targeted drug delivery for Alzheimer's disease using rat model, and Small Animal Imaging Systems (CT) in Zagreb and at CSMC in Los Angeles (MRI).

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

Research Team: Comenius University, Semmelweis University, University J. J. Strossmayer Osijek, Katarina Sebekova, Comenius University

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. 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. The highest concentrations of insulin and insulin receptors within the CNS are found in the olfactory bulb, which also exhibits the highest insulin transport rate across BBB, and the highest rate of insulin degradation. 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. Insulin resistant states are characterized by persistently elevated levels of circulating anorectic peptides, insulin and leptin. However, it remains unclear whether and how insulin resistance affects the expression of insulin and leptin receptors, and thus the modulation of anorexigenic/orexigenic signaling in CNS. Post-mortem free-floating immunohistochemistry of ER β , PR, leptin (Ob-R) and insulin receptors and Western blot methods will be used to detect the density and expression of the above-mentioned receptors. Mapping 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. The cadaver cohorts have insulin resistant vs. insulin sensitive controls deceased, these cohorts would be matched for gender, age, degree of obesity, treatment, and well biochemically characterized.

Change in cardiomyocyte contractile function during age and gender

Research Team: University of Debrecen, University of Pecs
Zoltan Papp, Attila Borbely, University of Debrecen
Tibor Ertl, University of Pecs

In this study, the participating scientists clarify the contribution of myofilament protein alterations to myocardial systolic and diastolic dysfunctions in association with major CVD syndromes (acute or chronic alterations in systolic heart failure or in diastolic heart failure). Wide range of age groups (i.e. from newborns to elderly, also taking sex and gender into considerations with obesity and diabetes) will be investigated. Myofilament protein alterations (changes in expression and posttranslational modifications) have the potential to modulate/limit cardiac performance and hence to contribute to the development of heart failure. Better understanding of the molecular nature of these alterations and of signalling pathways leading to these modifications will lead to better diagnosis and therapy for cardiac dysfunction in various CVD conditions. Tissue samples obtained from humans following transplantation or valve replacement surgeries. Cardiomyocyte function will be studied in permeabilized cardiomyocytes allowing the quantitative characterization of Ca-regulated active and Ca-independent passive properties being informative for systolic and diastolic functions, respectively. Expression and post-translational modifications (e.g. phosphorylation, oxidation) of myofilament proteins will be assayed, in parallel and contrasted to the observed cardiomyocyte mechanical characteristics.

Assessing the contribution of systemic to brain insulin resistance across neurodegenerative diseases

Research Team: Cedars – Sinai Medical Center, Semmelweis University, University of Osijek, Comenius University, University of Zagreb, School of Medicine, University of Copenhagen, Faculty of Pharmacy
Konrad Talbot, Sandor G. Vari, Cedars-Sinai Medical Center

RECOOP scientists would test the hypothesis that systemic insulin resistance and obesity promote pathogenesis of multiple NDGD by inducing brain insulin resistance. Investigators will test the association of systemic insulin resistance with cognition and brain levels of proinflammatory cytokines, adiponectin and leptin in cognitively normal cases compared to those at early clinical stages of the most common NDGD: AD, Parkinson's disease (PD), and frontotemporal lobar degeneration (FTLD). Therefore age- and sex-matched cases would be selected with informed consent from those diagnosed as cognitively normal, probable AD, PD,

or probable FTLD. Patients would be assessed on a battery of cognitive tests appropriate for NDGD conditions. Samples of their adipose tissue, blood, and/or CSF would be measured for (a) fasting glucose and fasting insulin in blood, (b) proinflammatory cytokines (IL-1 β , IL-6, and TNF α), adiponectin, and leptin, in all three types of samples, and (c) A β and phosphorylated tau in CSF. The glucose and insulin data would be used to calculate HOMA-IR, a systemic insulin resistance index. The association of HOMA-IR and cognitive status in the different diagnostic groups would be calculated, as would the association of HOMA-IR with CSF levels of cytokines, adiponectin, leptin, A β , phosphorylated tau, synuclein, and TDP-43 in the different diagnostic groups. Finally, the association of adipocyte and blood levels of cytokines, adiponectin, and leptin with cognitive status would be calculated. These analyses would provide data on the relationship of systemic insulin resistance and related factors to brain pathogenesis and to cognition across NDGD. At CSMC, one of these methods in development to detect IRS-1 pS and SOCS3 in blood-borne exosomes derived from cortical neurons. The other method will be used is in the earliest stage of development, seeks to image IRS-1 pS with an antibody conjugated to an iron nanoparticle. If validated, these methods would allow minimally invasive detection and quantification of brain insulin resistance.

Bioseparation of glycans, lipids and fatty acids to understand the common mechanisms of diseases

*Research Team: University of Debrecen, Institute of Cell Biology Lviv, Semmelweis University, University J. J. Strossmayer Osijek
Andras Guttman, University of Debrecen
Martina Mihalj, University J. J. Strossmayer Osijek
Tamas Tabi, , Semmelweis University*

Change in protein glycation is supposed to serve as marker of various pathological processes. Glycosylation, especially on IgGs, plays a critical role in the bioactivity of this group of important proteins, e.g. in Rheumatoid Arthritis (RA) patients a decrease in the terminal galactose content of the N-linked glycans at the conserved Fc region (Asn297) of IgG occurs. Methods were established at the Horváth Laboratory of Bioseparation Sciences to perform these kinds of studies. The team will analyses N-glycosylation on human immunoglobulins in plasma samples from patients participating in the RECOOP clinical research projects. Micellar Electrokinetic Chromatography (MEKC) bioseparation method will be used to study the fatty acid composition in SAT and VAT in several clinical research projects (CKD, obese pregnant women and NDGD). The snap frozen adipose tissue and blood samples will be used to analyze fatty acid composition and IgG glycation. The results will be correlated to insulin resistance and adipose tissue inflammation.

Study pathogenesis and role of transcription factors in adipose tissue inflammation, in chronic kidney diseases

Research Team: Semmelweis University, University of Szeged, University J. J. Strossmayer Osijek, University of Pecs, Institute of Cell Biology Lviv, University of Debrecen,

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Marianna Pap, University of Pecs

RECOOP scientists will investigate the adipose tissue inflammation in advanced atherosclerotic state of chronic kidney disease in patients subject to organ transplantation compared to healthy individuals of living kidney donors. Investigators will study the role of the gender and obesity. They will study the correlation of SSAO/VAP-1 activity and expression in the adipose tissue and its inflammation and insulin resistance and investigate the role of the regulatory pathways of PPAR γ , CREB and Wnt- β -catenin signalling in the adipogenesis and adipose tissue inflammation. Obesity evaluation will be by WHR and waist circumference. In addition, the alteration of the fatty acid composition during atherosclerotic process, obesity, insulin resistance and adipose tissue inflammation will be studied. CVD risk of the patients would be evaluated on the basis of clinical examination and laboratory test. VAT and SAT samples would be taken and frozen immediately to -80 °C and for laboratory tests, plasma sample would be taken. Investigators will use standard clinical laboratory tests to assay plasma levels of cholesterol, LDL, HDL, triglycerol, glucose, urate, homocysteine, insulin, CRP. RECOOP scientists use immunological tests (ELISA/Western blot) to assay cytokine and adipokine levels in the plasma and adipose tissue. Histopathology will be the research tool to evaluate adipose tissue inflammation and pathology. During evaluation measurement of adipose tissue level of inflammatory mediators (TNF α , IL-1 β and IL-6) by ELISA/Western blot and detection of leukocyte specific markers (e.g. CD11b, CD45) by RT-PCR will take place. Scientists also assay the expression and activity of SSAO/VAP-1, PPAR γ , CREB, Wnt1/3/3a/4 and β -catenin on mRNA and/or protein and phosphorylation level and will correlate them to the CVD risk of the patients. Lipidperoxidation products, as oxidative stress markers in the plasma and adipose tissue would be determined. The activation of main pathways of ER stress would be analyzed using Western blotting and immunohistochemistry. Induction of the chaperon Binding immunoglobulin (BiP) also known as 78 kDa glucose-regulated (BiP/GRP78) protein, the hallmark of unfolded protein response (UPR) activation would be determined. Several proteins involved in the regulation of ER stress mediated apoptosis, like glycogen synthase kinase-3, pro- and anti-apoptotic members of the Bcl-2 family-are also to be determined. MEKC would be used for the analysis of fatty acid composition. The level of gangliosides would be also analyzed in the adipose tissue samples by immunological methods.

Risks of obesity during pregnancy

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Obesity increases preterm birth risk and maternal dyslipidemia may result from exaggerated adipocyte lipolysis. In the study, healthy pregnancy will be compared to preterm birth. During cesarean section (in the Europe around 35 - 40% of the delivery), investigators will VAT and SAT biopsies, waist circumference and WHR will be determined, diameter of adipocytes will be measured from VAT and SAT. The investigators will use standard clinical laboratory tests and the assays of the adipose tissue are the same like in chronic kidney diseases study (see above).

Lifestyle Intervention

Testing Perimenopause as a Sensitive Period for Atherosclerosis Prevention

*Research Team: Institute for Clinical and Experimental Medicine, Prague
Jan Pitha, IKEM*

Based on our results from previous work we proposed the hypothesis, that menopausal transition could be critical period for the progression of atherosclerosis especially in the presence of smoking and that women in menopausal transition could be more vulnerable to environmental insults. Healthy non-smoking women and otherwise healthy smoking women in the transition to menopause will comprise the study group. In women, reproductive status will be defined by STRAW criteria. All control groups will be matched for age, in the case of women also waist circumference; (WHR), in the case of smokers, comparable load of smoking. All participants will undergo following interventions: eight week standardized and supervised physical activity five times a week with at least one week training management. Furthermore, the diet low in saturated fat and salt would be implemented. Before and after intervention the risk factors will be analyzed as follows: waist circumference, WHR, blood pressure, pulse wave velocity, endothelial dysfunction, of plasma lipids, C-reactive protein, the number of endothelial progenitor cells, endothelial microparticles, circulating microRNA 143/145 and methylation of DNA (FTO gene) using already established laboratory methods.

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

Research Team: Department of Family Medicine, University of Split School of Medicine, Institute for Clinical and Experimental Medicine, Prague

Ivancica Pavlicevic, Mario Malički, Department of Family Medicine, University of Split School of Medicine
Jan Pitha, *Institute for Clinical and Experimental Medicine, Prague*

Cross talk between skeletal muscle, adipose tissue and inflammation in the cohort study of assessment health condition among professionals of informational technology (IT) software industry

Research Team: Danylo Halytsky Lviv National Medical University
Oksana Zayachkivska, Department of Physiology, Lviv National Medical University

Under physiological condition skeletal muscle is plastic and secretory organ with broad spectrum of cytokines with multifunctional roles. Physical exercise is inductor of changes in muscle, including secretion of myokines, which important of signaling in glucose metabolism, AMPK-mediated oxidation, brown-fat development, anti-inflammatory reactions, inhibition of cancer cell growth and pancreatic function. Insufficient physical activity is a major contributor of increased risks of obesity, cardio-vascular disease, diabetes type 2, osteoporosis, and depression. In this study, the participating scientists clarify the contribution of cross talk between skeletal muscle, adipose tissue and inflammation to health condition of professionals involved in software industry, having a high occupational stress with more emotional component and sedentary work style, shift work, long term brain-computer interaction and other IT devices, a interesting human population group in approach to understand how limited muscle activities affect human physiology, lifestyle, well being, stress perceived scoring and cognitive functions and what initiate health problem. Cohort study of different age and gender IT software professionals with age and sex-matched healthy control with assessment different aspect of stress level, circadian rhythm and sleeping pattern and the estimation of crosstalk of between skeletal muscle, adipose tissue and inflammation by expression myokines: pro-inflammatory IL-6 and antiinflammarty IL-10, PGC-1alpha-dependent myokine irisin, adipokines: adiponectin and leptin by ELISA will give information about health condition and well being in IT software professionals and may provide targeted recommendations for the treatment and create system of preventive measures which can effectively improve health and quality of life.

New projects

Screening for congenital CMV infection and identification of biomarkers predictive of long-term outcome in RECOOP

Research Team: University of Alabama- Birmingham, Cedars – Sinai Medical Center, Slovak Medical University, University of Pecs, Carol Davila"University of Medicine and Pharmacy, Bucharest, Romania, Charles University in Prague, Faculty of Medicine Hradec Kralove, University Hospital Hradec Kralove William J. Britt, University of Alabama- Birmingham, Department of Pediatrics

Specific Aims:

- 1) Establish screening program for congenital CMV infection
- 2) Establish mechanisms for follow-up of congenitally infected infants
- 3) Identify biomarkers that could identify infants at risk for long term neurologic sequelae

Background

Congenital human cytomegalovirus (CMV) infection represents the most common viral infection acquired by the developing fetus in humans with rates between 0.5-1% in live births. Similar rates of congenital CMV infection have been defined in large natural history studies in the US, Sweden, UK, and in Brazil. In addition, studies in India, China, and sub-Saharan Africa have suggested rates of congenital CMV between 1-3%. Rates of congenital CMV infection in central European countries are not well defined. About 10% of congenitally infected infants develop long-term sequelae secondary to this intrauterine infection. Sequelae can range from severe developmental delays, seizures, and most frequently, hearing loss. Recent studies have demonstrated that congenital CMV is responsible for 25% of hearing loss in infants and children and represents the most common non-familial cause of hearing loss in infants and children. Because over 90% of congenitally infected infants present without recognizable clinical findings associated with intrauterine CMV infection, identification of infected infants has required universal screening of newborn populations for CMV infection. Although current technologies will permit deployment of inexpensive screening programs, identification of the 10% of infants at risk for long-term neurodevelopmental sequelae remains a major hurdle for full implementation of cost effective screening. Without such stratification of infected infants at risk for long-term sequelae, all infants identified by newborn screening will require long term follow-up. Identification of biomarkers predictive of infants at risk for long-term sequelae would offer such a solution and likely speed implementation of screening programs in most newborn populations.

Research plan:

Aim 1: Establish screening program for congenital CMV infection. We will implement a PCR based screening assay for CMV excretion in the saliva of newborn infants that has been validated in a newborn screening program of nearly 100,000 infants in the US. This assay is relatively inexpensive and can be done of samples collected and stored for several weeks, thus allowing batching of specimens. Confirmation of newborn infection can be accomplished within the 1st 3 weeks of life using PCR analysis of CMV in urine. We will establish screening programs in participating hospitals that will enable us to screen a minimum of 85% of all infants born in participating hospitals. Infected infants will be enrolled in a follow-up program to document long-term sequelae from this congenital infection.

Aim 2: Establish mechanisms for follow-up of congenitally infected infants. If screening

programs are implemented, it will be mandatory to also provide follow-up for infected infants. Follow-up will be accomplished through routine pediatric care or alternatively, through a specialty clinic such as Infectious Diseases. Hearing testing during 1st 6 months will include at least one testing by auditory brainstem evoked responses. Normal developmental measures will be recorded and all data included in a standardized case-report form that can be entered into a web based system such as Flexi-Form.

Aim 3: Identify biomarkers that could identify infants at risk for long-term neurologic sequelae. To date, parameters of viral load in the newborn infant and immunologic responses of the newborn infant have not provided information that has allowed stratification of infants at risk for long-term neurologic sequelae. Because long-term sequelae arise secondary to damage to the CNS, only relatively invasive measures such as analysis of CSF or imaging of the CNS have provided predictive information. In this aim we will investigate the possibility that biomarker(s) can be used to identify infants at risk for long-term sequelae. Based on studies in animal model systems and in tissues from human abortuses, it could be argued that activated inflammatory mononuclear cells that infiltrate the CNS contribute to the neurological disease observed in infected infants. Although we cannot directly access the CNS, RNA from peripheral blood mononuclear cells obtained from congenitally infected infants will be used to define the spectrum of miRNAs in these cells. Similarly, we will carry out cell surface phenotyping of mononuclear cells to identify activated, circulating monocytes in these infants. Findings from these studies will be linked to the long term neurologic outcomes of these infants.

Relevant publications supporting proposed project:

1) Spectrum of disease and outcome in children with symptomatic congenital cytomegalovirus infection.

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J Pediatr. 2014 Apr;164(4):855-9. doi: 10.1016/j.jpeds.2013.12.007. Epub 2014 Jan 14.

PMID: 24433826

2) Congenital cytomegalovirus infection as a cause of sensorineural hearing loss in a highly immune population. Yamamoto AY, Mussi-Pinhata MM, Isaac Mde L, Amaral FR, Carvalho CG, Aragon DC, Manfredi AK, Boppana SB, Britt WJ. Pediatr Infect Dis J. 2011

Dec;30(12):1043-6. doi: 10.1097/INF.0b013e31822d9640. PMID: 21814153

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Boppana SB, Ross SA, Shimamura M, Palmer AL, Ahmed A, Michaels MG, Sánchez PJ, Bernstein DI, Tolan RW Jr, Novak Z, Chowdhury N, Britt WJ, Fowler KB; National Institute on Deafness and Other Communication Disorders CHIMES Study. N Engl J Med. 2011 Jun 2;364(22):2111-8. doi: 10.1056/NEJMoa1006561.

PMID: 21631323

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Regulation of inflammatory responses of the digestive system

Research Team: Danylo Halytsky Lviv National Medical University

Oksana Zayachkivska, Department of Physiology, Lviv National Medical University

Aims: the present study will design to examine the effects of molecular inflammatory mediators on mucosal integrity, their possible contribution of esophagitis, key sign of non-erosive reflux disease (NERD) and gastro-esophageal reflux disease (GERD) and evaluate cellular sensors driving chronic inflammation. In addition, the second aim will dedicate how regulation immune homeostasis can be harnessed to develop new diagnostic approach and therapeutics to treat NERD and GERD.

Background: The incidence of esophageal adenocarcinoma (EA) has increased by 500% over the past 30 years. Improved understanding of the mechanisms of esophageal ulcerogenesis and neoplastic progression provides an opportunity to reverse this trend. We previously reported about several important advances in understanding the early biochemical and molecular mechanisms, including biomarkers of glycosalation of ulceration and healing in esophageal mucosa [1, 2, 3]. Our several studies suggested the impairment includes esophageal ischemia and hypoxia secondary to microvascular changes and peroxynitrite-mediated endothelial and enteric neuron damage [4, 5]. Moreover, our recent works have shown that metabolic disorders and diet-related chronic diseases appear to play key roles in the pathogenesis of comorbidities in digestive system [6, 7]. The diagnostic and therapeutic approaches to NERD are limited, in part because of the difficulties of investigating the pathogenesis of this condition in humans. Thus, new study would assist in delineating the early events in NERD pathogenesis, which would hopefully lead to improved early diagnosis and therapies in GERD, premalignant condition of EA.

Research plan: to gain new insights why esophageal lesions in most patients don't heal and lead to precancerous dysplasia and find their novel diagnostic biomarkers of inflammation dysregulation.

1. Zayachkivska O. S., et al. (2007). Protective influence of melatonin against acute esophageal lesions involves prostaglandins, nitric oxide and sensory nerves. *J Physiol Pharmacol.* 58, 371-387.
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Research Management

Monitoring Plan for Clinical Research

Linn Defensor, Office of Research Compliance & Quality Improvement (ORCQI), Cedars-Sinai Medical Center

Aims: The purpose of the RECOOP HST CRMN clinical monitoring plan is to guarantee adequate protection of the rights of human subjects, the safety of all subjects, and to uphold the quality and integrity of the data collected in clinical research conducted by Association members.

Objectives:

1. To ensure that the study is being conducted in accordance with the approved protocol, applicable regulatory requirements and the RECOOP HST Association's standard operating procedure (SOP).
2. To ensure that the reported data are accurate, complete and verifiable from source documents.

Background: The International Conference on Harmonization (ICH) Guidelines for Good Clinical Practice, (GCP 5.18.3) states that the sponsor-investigator should ensure that their trial is adequately monitored. The sponsor-investigator should determine the appropriate extent and nature of monitoring based on considerations such as objective, purpose, design complexity, blinding, size and endpoints of the research study.

The European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) on August 2011 issued guidance documents on the oversight of clinical investigations promoting risk-based approach to monitoring. EMA and the FDA encourage the use of technology to facilitate centralized or remote monitoring.

Method:

Monitoring Team: The monitors will be composed of Sandor Vari, MD, Director, RECOOP HST Association and Linn Defensor, RN, MSHS, CCRP, Global Clinical Trial Leader, CTMN, RECOOP HST Association.

All monitoring visits will be conducted remotely through study specific checklists at predetermined timelines. The RECOOP HST Monitoring Plan will have three scheduled timelines for completion of the checklists:

1. Site initiation Checklist will be completed prior to the first patient enrolled.
2. Interim Monitoring Checklists are completed while the study is ongoing. The frequency of the completion of the checklist will be dependent on the study site and the number of patients enrolled.
3. Closeout Checklist will be completed after all patients are enrolled, all data is collected and all queries resolved.

Predicting Statistical Power and its relation to sample size in Research Studies

Meenu Sandhu, MS Senior Biostatistician Cedars Sinai Medical Center

Aims: Understanding the key components of sample size determination as it relates to statistical power for research studies. A broad perspective highlighting key measurement variables, their variability and the impact on statistical significance.

Background: Sample size determination is often a key step in planning a research study and it can be a challenging one. Among the hurdles to overcome, one must define the key outcome variables, get an estimate of the effect size of importance, obtain an estimate of the error variances associated with the effect size, design of the study, key analyses and practical, clinical, ethical and economic issues associated with study implementation.

Research Methods: Sample Size calculations are based on the interdependence of sample size, study design, degree of departure from the hypothesis, the power of the study and the significance level sought. The design of the study takes into account the number of groups, the number of key variables to be tested, the type of key variables such as categorical or continuous, and the clinical setting. Sample size software such as Nquery⁴, SAS⁶, and many others are often used to determine how many subjects are needed to answer the research question or the null hypothesis.

Conclusion: After taking into account key factors such as study design, measurement variable difference sought, and error variation of difference; the optimal sample size is determined to assure an adequate power to detect statistical significance for the research study.

Publication of RECOOP retrospective studies

Recommendation for modification of the W&CVD RN Retrospective study

Dražen Mlinarević, School of Medicine, Josip Juraj Strossmayer University of Osijek

Working title - Gender-related differences in coronary artery disease risk factors in Croatia, Czech Republic and Hungary

Reiterate the most interesting findings from the retrospective study:

- 1) Patients oldest in ST, mostly due to difference in age of males
- 2) BMI lowest in ST, differences between other 3 centers not significant (NS)
- 3) HT most common in ST, again mostly due to male percentage, the biggest male/female difference in HT incidence is in ST, other centers have similar M/F HT percentages
- 4) Large differences in DM between males and females in ST, DB; 4-way analysis for males is NS, for females $p=0.004$
- 5) Cholesterol highest in ST, OS, PR with DB significantly lower than others, similar male/female patterns; cholesterol difference arises with patients >60 yrs old
- 6) HDL highest in ST, again due to male values, female 4-way comparison is NS
- 7) LDL highest in ST, difference arises with patients >70 yrs old
- 8) TG highest in ST, difference arises with patients >70 yrs old
- 9) Gender-related differences between pooled patients from all centers: females are older ($p<0.001$), have a lower BMI ($p=0.004$), have HT and DM more often ($p=0.002$ and $p=0.025$, respectively) and have a higher HDL ($p<0.001$).

Overall, we can conclude that we found some differences which can be attributed to the benefits of Mediterranean diet in patients from ST – highest age and HDL. On the other hand, ST patients having HT most often and having the highest cholesterol, LDL and TG were surprising results, but can be partially explained by the advanced age of the patients in ST (mean age 68.82 years).

Gender-related differences were also a focus in the retrospective study and we found significant differences within centers and also between pooled male and female samples. Observing the pattern of statistical analyses we can conclude that most of the statistically significant differences between centers were usually the product of differences between male patients, while the females had more similar results in practically all variables. In other words, female data from all centers are more similar than male data.

We also collected demographic data for relevant variables from all three countries, but unfortunately these data are not subject to statistical analysis and can be used observationally.

The sources themselves (statistical yearbooks, WHO database) plead caution with data interpretation (especially nutrition, smoking, alcohol consumption) due to differences in methodology and reliability.

The first two articles^{1,2} represent analyses of subcutaneous and visceral adipose tissue in NASH and in the Framingham study. These articles could present a future direction for the work within

RECOOP, I would like to point out that the group of Prof Radivoje Radic have published in this field and their expertise would be valuable if RECOOP were to proceed in this direction.

The article by Streblow et al.³ concerns the acceleration of atherosclerosis due to infection with microorganisms (CMV, Chlamydia pneumoniae) and similarly to the previous subject this topic can also represent a future direction within RECOOP, since there are microbiologists and virologists in the organisation as well.

The next three articles^{4,5,6} address the prevention and treatment of dyslipidaemia. In our study we did collect data about the therapy of patients with ischemic heart disease and we should include it in the manuscript. I propose adding the data for every drug group if available, most importantly statins, beta blockers, ACE/ARB, MRA, diuretics. If the data was entered then it should not be a problem to retrieve it from Flexiform.

The article about global mortality and morbidity by Wang et al.⁷ in Lancet outlines the need to reduce mortality in low and middle-income countries, as well as improving healthcare registries. Since most countries in RECOOP could be considered developing or middle-income countries and the most common cause of death in these countries are cardiovascular diseases the importance of research in this field can not be exaggerated. Researching CVD in women⁸ is especially important, since the conventional preventative, diagnostic and therapeutic measures tend to be underused, mostly due to a perceived lower risk of CVD in comparison to men. In our sample women had HT and DM, objective risk factors for CVD, more often than men, which indicates that there is need for exploring gender-related differences in CVD.

The EURObservational HF pilot survey⁹ is focused on heart failure which is an entirely different topic to ischemic heart disease, but they can be etiologically related since IHD is the most common cause of HF. More importantly, the current retrospective and prospective study design are quite similar to the ESC-HF pilot and we could try to restructure our manuscript(s) in a similar fashion, which could lead to easier publication. Also, this pilot can be considered a great framework to conduct future studies within RECOOP from a methodological standpoint.

In conclusion, we can publish the retrospective data after restructuring and rewriting.

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Preterm Birth, the risk factors, disparities and discrepancies in data from six Central and Eastern European Centers

Chander P. Arora and Sandor G. Vari

**RECOOP Abstracts for the Criatian Medical Journal
RECOOP Issue in April 2015**

Croatia

School of Medicine University Josip Juraj Strossmayer Osijek

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

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Running title: Hyperbaric oxygenation and ACh-induced relaxation in diabetic rats

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

Please follow the abstract guide: **Abstract shall be structure: Introduction, Methods, Results, Discussion and Conclusion and no more than 300 words**

Introduction:

Methods: The effects of hyperbaric oxygenation (HBO₂) on acetylcholine-induced vasorelaxation (AChIR) were evaluated in fifty-five male Sprague-Dawley rats randomized into 4 groups: healthy controls (Ctr), diabetic rats (DM), and control and diabetic rats that underwent hyperbaric oxygenation (Ctr+HBO₂ and DM+HBO₂). AChIR was measured in aortic rings with/without indomethacin, L-NAME or clotrimazole. CYP450-4A1,2,3 protein isoforms expression was determined by Western blot. (EC Approval No UP/I-322-01/11-01/142 issued by the Croatian Ministry of Agriculture from February 8, 2012)

Results: Plasma antioxidative capacity and systemic oxidative stress were determined with FRAP and TBARS assays, respectively. Data were analyzed with Two-way ANOVA RM and Bonferroni posttest. $p < 0.05$ was considered significant. AChIR was preserved in all groups of rats, but mediated with different mechanisms. In the presence of L-NAME, AChIR in Ctr+HBO₂ and DM+HBO₂ rats was partially preserved in contrast to control and DM groups. Clotrimazole partially blocked vasorelaxation in all groups, but more in both DM groups.

Discussion: The oxidative stress was significantly higher in both DM and DM+HBO₂ groups compared to respective controls. Both HBO₂ groups had higher protein expression of all CYP450-4A isoforms compared to control and both DM groups.

Conclusion: In conclusion, functional studies and expression of CYP450 in both HBO₂ groups suggest improved endothelial function by HBO₂, mediated by CYP450-4A metabolites of arachidonic acid.

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A New Model of Stroke in Type-1 Diabetic Rats

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Keywords: model, cerebral ischemia, transient, stroke, middle cerebral artery, diabetes, rats, female (7)

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

Materials and Methods: Type-1 diabetic Sprague–Dawley female rats (N=25), 12 weeks old, with high chronic hyperglycemia, were divided in 5 groups (N=5 in each group) and subjected to different duration of t-MCAO (20 min., 30 min., 45 min., 60 min. and 90 min.) and reperfusion, under LDF monitoring. 24 hours after reperfusion infarction volumes were evaluated by TTC staining and infarct volume analysis. (EC Approval No UP/I-322-01/11-01/137 issued by the Croatian Ministry of Agriculture from February 6, 2012)

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

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

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Gender difference in expression of estrogen receptor β and leptin receptor in adrenal gland after chronic and acute stress

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Key words: stress, adrenal gland, steroid hormones, leptin receptor

INTRUDUCTION: Plasma glucose, cholesterol and urates level combined with the weight monitoring and glucose tolerance test (GTT) are good markers for metabolic syndrome caused by stress. Leptin has the main role in body weight maintenance but it also lowers the hypothalamus-pituitary axis stress response by inhibition of steroid-hormone secretion from the adrenal cortex. Main aim of the study is to explore the expression of estrogen receptor β (Er β) and leptin receptor (Ob-R) in adrenal gland after acute and chronic stress.

METHODS: Study included 72 four-months-old Sprague-Dawley rats, 24 males and 48 females. Animals were divided into male, NON-OVX and OVX group. Groups were further divided into – control, acute and chronic stress. Chronic stress was performed through 3 repeats of 10-day stress session and acute stress was performed at the day of sacrifice at the age of 28 weeks. Weight, GTT, plasma concentration of glucose, urates and cholesterol were measured. Adrenal glands were stained by immunohistochemistry with ER β , and Ob-R antibodies.

RESULTS: Concentration of analyzed biochemical parameters decreased after acute and chronic stress. Acute and chronic stress changed GTT profile in males and NON-OVX, but not in OVX group. Male chronic stress group showed overall loss of weight, NON-OVX group retained the same body weight and OVX group gained weight at the end of the study. ER β expression in *zona glomerulosa* was significantly higher in OVX control than NON-OVX control ($p = 0.009$) and significantly lower in NON-OVX than OVX chronic stress group ($p = 0.004$). ER β expression in *medulla* was significantly higher in OVX than NON-OVX group ($p = 0.03$) after chronic stress. Ob-R expression in *zona reticularis* is significantly higher in NON-OVX than OVX after chronic stress ($p = 0.03$).

DISCUSSION: This study suggests a significant role of ER β and Ob-R in regulation of adrenal gland stress response and tempting for further confirmation in transcription profile.

CONCLUSION: Stress model was proven by the decrease of biochemical markers, decrease of weight in male and increase of weight in OVX group after acute and chronic stress. Appearance of strong nuclear staining of the Er β and Ob-R antibodies in adrenal gland suggests possible transcriptional changes during stress.

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
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Does leptin receptor expression changes in the brain upon acute and chronic stress?

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Key words: stress, brain, leptin receptor, rats

Introduction: In 1930s Selye observed different physiological changes in experimental animals upon a wide variety of acute and chronic stressors. Stress disturbs homeostasis by challenging the hypothalamus-pituitary axis and in the end leads to pathology. Obesity is one example of the pathology connected to stress – it has been noted that males are losing while females are gaining weight under chronic stress. Hormone that controls energy intake and expenditure is leptin and it acts through its receptor (Ob-R). Ob-R is primarily expressed in hypothalamic neurons – arcuate, ventromedial and dorsomedial nuclei. Association between the expression of Ob-R in brain upon stress is still unclear. Therefore, the aim of the current study was to evaluate whether the acute and chronic stress affect Ob-R distribution in the brain of male, female (NON-OVX) and ovariectomized (OVX) Sprague Dawley rats.

Methods: Study included 24 female and 48 male Sprague Dawley rats divided in acute, chronic and control group. Brains were collected at the age of 28 weeks and free-floating immunohistochemical staining was performed using Ob-R antibody.

Results: Ob-R positive neurons in hypothalamus were analysed in the arcuate nucleus and lateral hypothalamic nuclei. This nuclei show positive Ob-R expression in both female and male rats under the acute stress. Immunostaining in nonhypothalamic areas was analysed in barrel cortex and piriform cortex. Both regions also show Ob-R positive neurons both in female and male rats under the acute stress.

Conclusion: In this preliminary study we observed leptin receptor in the brain of rats in response to acute stress. Further research will show whether there are differences in expression of Ob-R between male, female (NON-OVX) and ovariectomized (OVX) rats upon the acute and chronic stress. We will also investigate whether other regions of the brain are Ob-R positive upon acute and chronic stress.

Source(s) of research support: Internal research grant from Josip Juraj Strossmayer University of Osijek

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

Czech Republic

IKEM

The impact of adipose tissue inflammation in the pathogenesis of atherosclerosis

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Key words: atherosclerosis – adipose tissue – inflammation – macrophages

Introduction: Atherosclerosis and its clinical complications are still the most common cause of death in developed countries. In addition to hypercholesterolemia, atherosclerosis is driven by inflammatory cells, mainly by macrophages. In our project we studied phenotypes of macrophages connected with proinflammatory status in adipose tissue of healthy living kidney donors.

Methods: Anthropometric characteristics were collected from all subjects. Samples of 2,5 grams of subcutaneous, perirenal and perivascular adipose tissue of kidney donors were

exposed to collagenase and repeatedly filtered and stroma vascular fraction (SVF) was eluted from the sample. SVF was then labelled with monoclonal antibodies conjugated with fluorochromes (CD14, CD16, CD36, CD163, CD68 and calprotectin) and subsequently analyzed by flow cytometry. The same surface markers were determined in blood samples of the subjects.

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

Result: Blood monocytes did not express calprotectin and only a minor population expressed CD16 whereas high expression of calprotectin and CD16 in adipose tissue macrophages of living kidney donors was found. Only in the subcutaneous adipose tissue we observed a positive correlation ($p < 0.05$) of number of CD14+ monocytes and CD14+calprotectin+monocytes with body mass index. Two groups of female kidney donors were distinguished: the premenopausal and postmenopausal group. The postmenopausal women had a higher number of CD14+CD16+ macrophages than the premenopausal group in perirenal ($p < 0.04$) and perivascular ($p < 0.05$) adipose tissue. Similarly the postmenopausal women had higher number of CD14+CD16+ macrophages than men in perirenal ($p < 0.05$) and perivascular ($p < 0.04$) adipose tissue.

Conclusion: We demonstrated a positive correlation of BMI with number of CD14+ monocytes and CD14+calprotectin+ monocytes in subcutaneous adipose tissue of kidney donors. We observed significant differences in numbers of CD14+CD16+ macrophages in premenopausal and postmenopausal women and men. The higher number of CD14+CD16+ macrophages may be related to proatherogenic role of this subpopulation of macrophages in postmenopausal women.

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Institute of Macromolecular Chemistry (IMMC)

Maghemite/polyaniline core-shell nanoparticles for biological applications

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Keywords: maghemite, nanoparticles, core-shell nanoparticle, polyaniline

Introduction: Superparamagnetic iron oxide nanoparticles (NPs) received a great deal of attention in a wide range of applications in medicine and biotechnology due to their interesting magnetic properties. Polyaniline is an important conducting polymer. Purpose of this research was to synthesize new polyaniline-coated magnetic NPs and to investigate their combined electrical and magnetic properties for biological experiments.

Methods: Maghemite ($\gamma\text{-Fe}_2\text{O}_3$) NPs were prepared by the coprecipitation of Fe^{2+} and Fe^{3+} salts with ammonium hydroxide which was followed by controlled oxidation with NaOCl. The polyaniline (PANI) shell was obtained by polymerization of aniline hydrochloride with ammonium peroxydisulfate in aqueous solution of poly(*N*-vinylpyrrolidone) in the presence of $\gamma\text{-Fe}_2\text{O}_3$ NPs. The resulting PANI@ $\gamma\text{-Fe}_2\text{O}_3$ NPs were characterized by scanning and transmission electron microscopies, dynamic light scattering, FTIR spectroscopy and elemental analysis.

Results and Discussion: The number-average diameter of the starting $\gamma\text{-Fe}_2\text{O}_3$ NPs was ≈ 10 nm. After the modification with PANI, the size of the PANI@ $\gamma\text{-Fe}_2\text{O}_3$ increased to ≈ 200 nm; particle size distribution was moderately broad. The NPs formed a stable aqueous colloid. The results of thorough characterization of the particles will be presented on the conference.

Conclusions: New magnetic and conducting PANI@ $\gamma\text{-Fe}_2\text{O}_3$ core-shell nanoparticles have been synthesized. They can be useful for biomedical applications, such as in biosensors.

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Hungary

University of Semmelweis

Effects of resveratrol on caspase activation in primary mouse fibroblasts

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Keywords: resveratrol, caspase-3 activation, fibroblast, serum deprivation

Introduction: Resveratrol is a flavonoid that is isolated from red grape. It has been suggested to exert protective effects in age-related disorders, including cardiovascular, renal or neurodegenerative diseases in line with the known beneficial effect of moderate red wine consumption. Besides, on transformed cells it showed antiproliferative, antitumor activity. Reflecting these findings, contradictory data on its effect on apoptosis have been reported, as both its pro- and antiapoptotic activity have been described. The cell type used can be one of the major differences between the various models and the compound might differentially affect tumorigenic and non-transformed normal cells.

In this work we aimed to study the effect of resveratrol on the apoptosis of non-transformed cells to explore its probable cytoprotective effect.

Methods: To study the effect of resveratrol on the apoptosis of non-transformed cell primary mouse embryonic fibroblasts were used. Apoptosis was induced by serum deprivation. Caspase activation, lactate dehydrogenase release and cell viability were assayed by fluorescent methods. The involvement of upstream pathways was also examined.

Results: Serum deprivation of primary fibroblasts induced significant activation of caspase 3 within 3 hours and reduced cell viability after 24 hours. Resveratrol dose dependently prevented caspase activation and improved cell viability with IC50 value of about 100 microM. Among the major upstream pathways p38 kinase was found to be critical in the protective effect of resveratrol.

Discussion: Our results suggest that resveratrol exerts protective rather than proapoptotic effect on non-transformed primary fibroblasts. It prevents caspase activation via p38 kinase dependent pathway suggesting the role of mild stress in its effect.

Conclusion: Resveratrol shows dose-dependent protective effect on primary fibroblasts against serum deprivation induced apoptosis supporting that it is a promising cytoprotective agent which should be explored in the prevention and treatment of various age-related degenerative disorders of the cardiovascular and nervous system.

Institutional Animal Care and Use Committee Approval: 22.1/606/001/2010, February 5, 2010

Acknowledgements: The research was supported by the RECOOP HST Association

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

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

Introduction: There are no uniformly accepted methods to predict which HER2 positive breast cancer patients are more likely to achieve pathological complete remission (pCR) after primary systemic therapy (PST). Our aim was to evaluate the benefit of trastuzumab containing PST in HER2 overexpressing breast carcinomas (with or without hormone receptor (HR) positivity) and to analyze the predictive power of applicable clinical-imaging modalities with regard to tumor response.

Methods: Altogether 188 patients were reviewed who received PST, with a final 43 HER2 positive breast cancer patients being enrolled in the present study. A total of 26 patients received mostly taxane-based PST without trastuzumab (Group 1) and 17 patients received trastuzumab containing PST (Group 2). Presence of pCR after PST was evaluated histologically on surgical samples. Efficacies of tumor response evaluation by means of ¹⁸F-fluoro-deoxy-glucose positron emission tomography and computerized tomography (FDG-PET/CT) as well as by breast-ultrasound (US) were compared. PET/CT was evaluated either regularly (tumor remission measured semiquantitatively by changes in FDG-uptake) or based on metabolic and morphological remission criteria (i.e. Response Evaluation Criteria in Solid Tumors).

Results: Ten patients (38.5%) achieved pCR in Group1, while 8 (47%) in Group2. In both groups significant differences were detected between pCR and non-pCR patients by biological subtypes: pCR was manifest more frequently in the HER2+/HR- than in the HER2+/HR+ subtype. When separating pCR vs. residual disease, PET/CT evaluated by metabolism and morphology based response performed better in both patient groups than PET/CT evaluated regularly or by response measured with US.

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

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Conflict of interest: The authors declare that they have no conflict of interest

Funding: None was involved.

Ethical Committee Approval: Ethical approval for the study was given by the Semmelweis University Institutional Review Board. Date and number of the ethical approval: 12-June-2013; TUKEB No.120/2013. Written informed consent was waived.

University of Szeged

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

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Introduction: Generation of reactive oxygen species and the associated oxidative stress can produce abnormal contractility in the pregnant uterus and can lead to premature labor and stillbirth, therefore antioxidants could be used for prophylactic treatment of premature labor. The antioxidant limonene is a major compound of citrus essential oils. Its applications in food,

cosmetics and household products have multiplied significantly, but its effects on the pregnant myometrium have not been investigated.

Methods: Our aim was to study the effects of limonene on pregnant Sprague-Dowley rat myometrium on the last day of pregnancy *in vitro*, and how that effect can be modified by other agents. Uteri were removed from rats on day 22 of pregnancy. Muscle rings were sliced from the uterine horns and mounted vertically in an organ bath.

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

Discussion: Our findings suggest that, besides the antioxidant action of L-limonene, D-and L-limonene cause myometrial smooth muscle contraction through activation of the A_{2A} receptor and opening of the voltage-gated Ca²⁺ channel.

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

Keywords: limonene, pregnancy, nifedipine, paxilline, antioxidant

The involvement of K_{ATP} channels in the uterus-relaxant effects of β_2 -AR agonists depends on the SUR1 subunit expression in the rat uterus

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Introduction: The β_2 -adrenergic receptors (β_2 -ARs) play an important role in the regulation of the contractility of the pregnant uterus. There have been several reports of the involvement of ATP-sensitive potassium channels (K_{ATP} channels) in the β -AR agonist-induced smooth muscle relaxation. Our aims were to investigate the role of the K_{ATP} channels in β_2 -AR agonist-induced myometrial relaxation in the presence of glibenclamide and pinacidil in early-pregnant (day 6) and late-pregnant (day 22) rat uterus, and to examine the potential correlation between the K_{ATP} channel sulphonylurea-binding regulatory subunit (SUR1) expression and the pharmacological reactivity of β_2 -AR agonists (salmeterol and ritodrine).

Methods: The uterus-relaxant effects of ritodrine and salmeterol (10^{-10} - 10^{-5} M) were investigated on spontaneous contractions cumulatively, alone or in the presence of the K_{ATP} channel blocker glibenclamide (10^{-6} M) and the K_{ATP} channel opener pinacidil (10^{-9} - 10^{-7} M) in vitro.

Results: In the early stage of pregnancy (day 6), when the SUR1 level was markedly elevated, the β_2 -AR agonist-induced myometrial relaxation was inhibited by glibenclamide and potentiated by pinacidil. At the end of gestation (day 22), when the SUR1 level had decreased, neither glibenclamide nor pinacidil influenced the tocolytic effects of the β_2 -AR agonists.

Discussion: The results of this study clearly suggest the functional presence of the K_{ATP} channel in the rat myometrium in the early stage of pregnancy. These findings indicate that no benefit is obtained from the therapeutic application of a combination of a β_2 -AR agonist and a specific K_{ATP} channel opener as a promising tocolytic agent.

Conclusion: The combination of a betamimetic and a K_{ATP} channel opener will not have therapeutic relevance in the treatment of preterm delivery. However, this combination may be of value as a uterus relaxant in the early stage of gestation.

Key words: β_2 -AR agonists, K_{ATP} channels, sulphonylurea receptor, K_{ATP} channel opener and antagonist, rat uterus

Slovakia

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Effect of combined prenatal and postnatal dietary intervention on metabolic status of female Wistar rats

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

Introduction: Western diet is considered an important risk factor in development of cardiometabolic diseases. Being rich in Maillard reaction products (MRPs), it may contribute to development of obesity, insulin resistance or metabolic syndrome. A present epidemics of such diseases has been linked to the rising consumption of glucose/fructose sweetened soft drinks. The aim of our study was to asses the impact of prenatal exposure to MRPs-rich diet on metabolic status of female offsprings, and its further modification by Coca-cola consumption.

Methods: At the first day of pregnancy, female Wistar rats were randomized into 2 groups, fed *ad libitum* either by standard rat chow (CTRL), or MRPs-rich diet – standard rat chow+bread crusts (BC). Offsprings from each group of mothers were divided into 2 groups (n=10-15), and provided either water or Coca-cola for drinking *ad libitum* for 18 days. Oral glucose tolerance test was preformed, circulating markers of inflammation, oxidative stress, glucose and lipid metabolism were assessed.

Results: Higher weight gain was observed in BC groups, significant only in rats drinking water. Both prenatal and postnatal intervention increased carboxymethyllysine levels and semicarbazide-sensitive amine oxidase activity, significance reached between CTRL+water and BC+Cola. Total antioxidant capacity was lower in BC groups, the effect significant in Coca-cola groups. Rats drinking Coca-cola had higher insulin, HOMA-IR, heart rate, advanced oxidation of protein products, triacylglycerols and oxidative stress markers measured as TBARS, with no visible effect of MRPs-rich diet.

Discussion: Metabolic status of offsprings was affected both by prenatal and postnatal dietary intervention. Combination of both challenges was reflected by trends towards worse outcome. Protective effects of missing dietary intervention in mothers during lactation, or a short-term consumption of coca-cola could underlie.

Conclusion: Our results suggest that combined effect of prenatal and postnatal dietary pattern may play a role in development of metabolic disorders in later life.

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The institutional ethical committee approval number: 025/2013/UPF, 6.6.2013

Alterations of peritubular capillaries in experimental renal fibrosis

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Key words: angiogenesis, endothelium, ultrastructure

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

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

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

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

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

Ukraine

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

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

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

Methods. In this work we studied glycosylation of IgG molecules of RA patients at different stages of disease progression and cycles of treatment, and of patients with DM1, using aged-matched groups of NHD. To analyze glycan exposure in IgG molecule we used previously developed and described [Magorivska, Jeremic, Herman, Munoz, Bilyy, Herrmann M. *Annals of the Rheumatic Diseases* 2014; 73:A25] lectin-based ELISA method, which provides information about accessibility of glycans on IgG molecules, besides representative samples of each groups were also analysed with capillary electrophoresis with laser induced fluorescent detection (CE-LIF) to detect the complete IgG glycoforms for specific patients.

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

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

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

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

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Application of novel radiopaque ZrO₂-Gd₂O₃ nanocomposite functionalized with hyaluronic acid for repair of bone defects in experimental animals

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Introduction: Creation of new materials for replacement of bone defects of various aetiology, the ways of improvement of their biocompatibility and osteogenous potential, as well as providing of osteoinduction, osteoconduction and radiopacity, are important tasks in surgical practice.

The aim of this work was to use novel polymer-mineral composites based on nanoparticles of ZrO₂-Gd₂O₃ and functionalized with the hyaluronic acid for regeneration of bone defects in experiment and conducting radiological and histological study of the reparative osteogenesis.

Materials and methods: Animal study was carried out on 24 female rats aged 8-9 months with 300-350 g body weight. Artificial bone defects were formed in rat's caudal vertebra. The animals were divided into 4 groups each composing of 6 rats whose bone defects were treated with: 1) novel radiopaque ZrO₂-Gd₂O₃ nanocomposite with hyaluronic acid in its coating; 2) commercial synthetic material «Easy-Graft™» (DS Dental, Switzerland) prepared on the basis of beta-tri-calcium phosphate; 3) bone regeneration material «Stimulus-Oss» based on animal collagen with addition of 2% chlorohexidine bigluconate and hydroxyapatite; 4) control in which bone defect was sutured under the blood clot. Bone (vertebrae with the regenerate) material was collected for study in 8 and 15 days after surgical intervention. Vertebra macropreparations were investigated morphologically, roentgenologically, histologically, and histochemically.

Results: Application of new experimental model (regeneration of bone defects artificially formed in rat's caudal vertebra) proposed by the authors has demonstrated its advantages in the

reparative osteogenesis. Among 3 different osteoplastic materials used in this study, novel radiopaque ZrO₂-Gd₂O₃ nanocomposite with hyaluronic acid in its coating showed the highest effectiveness in regeneration of the osseous tissue. This was confirmed by the morphological, as well as by histological and histochemical study of wound repair. New material has demonstrated bio-tolerance and high integration with the osseous tissue. Due to its radiopaque core, the developed ZrO₂-Gd₂O₃ nanocomposite can be used for monitoring of treatment course, since it can be easily detected by X-ray method. The obtained results are the basis for new studies with perspectives of their implementation in clinical periodontal practice, maxillofacial surgery, and implantology.

Conclusion: Novel polymer-mineral nanocomposite with ZrO₂-Gd₂O₃ core functionalized with hyaluronic acid is effective in regeneration of bone defects, demonstrates high integration in osseous tissue and bio-tolerance. Its application provides a possibility for monitoring the process of treatment of bone damages since novel nanocomposite can be easily detected at X-ray study.

Keywords: regeneration of bone defects, rats, novel osteoplastic material, polymer-mineral nanocomposite, ZrO₂-Gd₂O₃ core, functionalization with hyaluronic acid.

Ethical approval was received under No.7/2012-09-24 from BioEthics Committee of Danylo Halytskyi Lviv National Medical University (Lviv, Ukraine).

Lectin (GNA, PFA, PNA, WGA) receptors as biomarkers in chorionic villi of human embryos at spontaneous and recurrent miscarriage

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Key words: pregnancy miscarriage, chorionic villi, lectin histochemistry, glycomarkers

Introduction. Pregnancy miscarriage (PM) is the most common complication in human reproduction. It can be classified in spontaneous (SM) (loss of less than 2 pregnancies) and recurrent (RM) (loss of 3 or more pregnancies). 45% of early pregnancy losses are accompanied by the maternal immune intolerance. Since glycoconjugates of cellular surface are involved in cell signaling, they can play a key role in recognizing fetal cells by the maternal immune system.

Methods. 9 samples (6 - SM) and (3 - RM) of the chorionic villi of human embryos miscarried in the 1st trimester of pregnancy at 4-13 weeks of fetal development were studied under the inspection of BioEthics committee of medical university. Control group consisted of 5 tissue samples of chorionic villi obtained after artificial abortion conducted on women's request at the

1st trimester of pregnancy. Carbohydrate determinants of the chorionic villi were investigated by lectin-peroxidase technique using selected lectins - GNA (D-Man), PNA (D-Gal), PFA (L-Fuc) and WGA (NAc-D-Gluc, NeuNA specificity).

Results and Discussion. The highest intensity of exposure of WGA and GNA receptors on structural components of the chorionic villi, namely in syncytiotrophoblast, was observed in the RM group. While in the SM group high level of exposure of WGA receptors, an average exposure of GNA and PNA receptors, and minor exposure of PFA receptors were found. In the control group, the level of binding of all lectins under study was significantly lower than in the experimental groups.

Conclusions. Specific modification of carbohydrate component in the glycoconjugate complexes on cell surface of the chorionic villi at spontaneous and recurrent miscarriage was detected. It is suggested that it leads to the incorrect recognition of these cells by the maternal immune system causing a maternal immune attack and premature fetus death.

Ethical approval was received from Ethics Committees of Danylo Halytsky Lviv National Medical University, Lviv, Ukraine, DH LNMU No.6/2013-06-24.

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Palladin Institute of Biochemistry, Kyiv

**IMPORTANCE OF DOSAGE AND IMMUNIZATION SCHEDULE ON THE
ADJUVANICITY OF POLYLACTIDE-CO-GLYCOLIDE PARTICLES AS ANTIGEN
CARRIERS FOR IMMUNIZATION *PER OS***

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

Diphtheria toxin (DT) is the main pathogenic factor of *Corynebacterium diphtheria* produced by bacteria cells at their colonization sites in respiratory tract mucous membranes. Strong induction of antitoxic immunity in mucosal tissues can protect the body against the infection.

The aim of this work was to evaluate the dosage-dependent immunogenic properties of polylactide-co-glycolide (PLGA) adjuvant particles of two types of size (PLGA-Nano – 100nm and PLGA-Micro – 1µm) conjugated with a recombinant truncated DT.

Research was carried out on BALB/c female mice of the same age and weight, which were treated *per os* with antigen three times with intervals of two weeks.

Institutional Animal and Care Committee of Palladin Institute of Biochemistry of NAS of Ukraine?

Experimental animals were divided into several groups treated with different doses of antigen immobilized on the particles (2,5 ug, 25 ug, 250 ug and 2500 ug of antigen per 1 kg of body weight). Sera were collected one week after each immunization. Immune response was determined by concentration changes of IgA and IgG against DT in peripheral blood of immunized mice in ELISA.

Most significant increase of concentration of blood antitoxic IgA and IgG was detected in groups of mice after the first immunization with antigen in dosage of 250 ug per 1 kg of body weight for both types of PLGA particles. Second and third treatment had not significant effect on the further immune response or even reduced it. IgA and IgG level decrease after second and third treatment could be explained by immune tolerance induction by antigens delivered *per os*.

Obtained results suggest that correct dosage of PLGA Nano and PLGA Micro particles with absorbed antigen and correct peroral immunization schedule could significantly increase of humoral immune response against introduced antigen able to stimulate immune response in mucosal tissues to the adsorbed antigen and vice versa for high doses of oral vaccines and inaccurate immunization plan. That could lead to immune tolerance to introduced antigen. Thus, after optimization of peroral vaccine dosage and immunization schedule PLGA particles can be considered as very potent components for oral vaccines.

Calix[4]arene C-145, prototype of a multiaction antithrombotic agent: *in vivo* study

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Introduction. Sodium salt of calix[4]arene methylene bisphosphonic acid (C-145) was shown to be an efficient inhibitor of fibrin polymerization *in vitro*. In current work was carried out the analysis of the effects of calix[4]arene C-145 on haemostasis *in vivo*.

Methods. C-145 was injected into rabbit's bloodstream in the dose of 7.5 mg/kg. All animal protocols were approved by the institutional animal and care committee of Palladin Institute of biochemistry of NAS of Ukraine (from 4th of February 2014, protocol number 1). After 2, 4 and 24 hours blood samples were collected. Then we measured parameters of coagulation (APTT, PT, TT, fibrinogen level, prothrombin level), anticoagulant system (protein C level), fibrinolysis (spontaneous euglobulin lysis, PAI-1, half-time of clot lysis). Platelets were characterised by flow-cytometry and aggregometry. Effect on endothelium was estimated by the level of secreted tPA.

Results. After injection of C-145 the TT and APTT were prolonged in 2 and 1.5 times respectively. Such prolongation was observed after 24 hours also. However the total fibrinogen and prothrombin level, parameters of fibrinolytic and anticoagulant systems remained constant. C-145 also strongly affected platelet aggregation induced by ADP and provoked thrombocytopenia. Changes of tPA level in blood plasma were not detected.

Discussion. Thus we assumed that the effects of calix[4]arene C-145 on haemostasis based on selectively inhibition of fibrin polymerisation and formation of three-dimensional fibrin network that is the core of thrombus. C-145 did not activate endothelium but affect platelet aggregation that can be an additional mechanism of its antithrombotic action.

Conclusions. Calix[4]arene is a selective inhibitor of fibrin polymerisation that can be successfully used *in vivo* during pathologies of haemostasis as an antithrombotic agent.

Keywords. calix[4]arene, haemostasis, antithrombotic drugs, fibrin polymerization.

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Ethical Committee Approval: date and number?

Platelets as regulators of plasminogen activation system

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Key words: platelet, plasminogen activation, plasminogen activator inhibitor-1, thrombolysis

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

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

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

Conclusion: Platelets could be considered as stimulators for plasminogen activation at physiological concentration of proenzyme and tPA. However, platelets have active PAI-1 supressing plasminogen activation. Platelets can have sites of plasminogen activation and as a result small amount of plasmin is generated. New formed plasmin which is defended from plasma inhibitors thanks its binding to platelet membrane proteins plays an important role at regulation of platelet functioning and thrombi degradation. The obtained data can be used at the development of pharmacological strategy of local thrombolysis.

Ethical Committee or Institutional Animal Care and Use Committee Approval: date and number?

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

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

Introduction: Perinatal hypoxia, accompanied by seizures, lead to life-long cognitive disability, behavioral abnormalities and even epilepsy that are suggested to be due to neurodevelopmental disorders in network construction and maturation. Considering a special role of GABA for an immature brain, our study was focused on the transporter-mediated uptake of GABA in the developing rat brain.

Methods: A model of hypoxia-induced seizures in rat pups at the age of 10-12 postnatal days (pd 10-12) was used for studying an effect of early-life seizures on transporter-mediated uptake of [^3H]GABA. The experiments with **rat brain cortical**, hippocampal and thalamic nerve terminals (synaptosomes) were performed at pd 17-19, pd 24-26, pd 38-40 and pd 66-73 in the control and after hypoxic stress.

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

Discussion and Conclusion: These results are in agreement with our previous findings on the alterations in the ratio of active GAT1/GAT3 expressed in the plasma membrane of nerve terminals after perinatal hypoxia (Pozdnyakova N. et al, Croat Med J., 2014). Such a response of the hippocampus to hypoxia indicates a larger vulnerability of the hippocampus compared to the cortex and thalamus to the action of hypoxia.

Plasma membrane Ca²⁺-pump new inhibitor and supressor of myometrium spontaneous relaxation

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Key words: myometrium, calix[4]arene, contractility, cell calcium

Plasma membrane Mg²⁺,ATP-dependent Ca²⁺-pump (PMCA) is involved in many diseases connected with smooth muscle contraction. Nonetheless, prior to our research there were no low-molecular and selective inhibitors for it. Previously it was shown that calix[4]arene C-90 (5,11,17,23-tetra(trifluoro)methyl(phenyl-sulfonylimino)-methylamino-25,26,27,28-tetrapropoxycalix[4]arene) efficiently and selectively inhibits the ATPase activity of PMCA in the myometrium.

Calix[4]arene C-90 was characterized with infrared spectroscopy and nuclear magnetic resonance methods. PMCA activity was determined on swine myometrium by measurements of P_i. Cell calcium ([Ca²⁺]) was measured with fluorescent microscopy (LSM 510 META) with fluo-4 AM. Laser correlation spectroscopy (“ZetaSizer-3”) was used to determine effective hydrodynamic diameter (EHD) of smooth muscle cells (SMC).

The inhibition coefficient I_{0,5} was equal to 20.2±0.5, and calix[4]arene C-90 (100 μM) decreased PMCA activity velocity maximum by 75%, selectively comparing to other ATPases located in plasma membrane (Na⁺,K⁺-ATPase, Mg²⁺-ATPase). The inhibitory effect of C-90 on PMCA activity associated with cooperative action of four sulfonylamidine groups. Calix[4]arene C-90 increased [Ca²⁺] in myometrium quiescent SMC by 45±9 % comparing to control,

however, during 1.5-2 minutes $[Ca^{2+}]$ was returning to initial level. Additionally, C-90 (50 μ M) caused SMC EHD decrease by 26% like as oxytocin (100 nM), and in concentration 10 μ M decreased uterine smooth muscle relaxation velocity maximum normalized on contraction amplitude by 20% (measured in spontaneous muscle activity).

Since calix[4]arene C-90 effectively and selectively suppresses PMCA activity, increased $[Ca^{2+}]$ in SMC is connected with low PMCA activity, i.e. PMCA takes control of basal $[Ca^{2+}]$ in SMC. Returning to initial level means that other calcium-control systems become involved in Ca^{2+} extrusion, if PMCA is inhibited. EHD and relaxation velocity decrease shows that C-90 may be applied as effective contraction agent in smooth muscles.

These results allowed us to propose calix[4]arene C-90 as a new inhibitor of PMCA (on the plasma membrane level).

We appreciate prof. S.O. Kosterin and V.I. Kalchenko for scientific cooperation.

Danylo Halytsky Lviv National Medical University

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

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

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

Methods. Fifty patients with IHD (32 men and 18 women) aged 45-75 years (average age 56.8 ± 1.7 years) were enrolled in this research. All patients gave written informed consent to participate in research (Protocol of Ethical Committee Approval No 8 from October 22, 2012). The patients were divided into two groups with respect to their ages: group A - middle-aged patients (45-60 years), group B - elderly patients (61-75 years) (WHO, 1963). The patients of both groups matched for sex, disease duration, number of pain attacks. Patients with IHD who have not received treatment by nitro medication, but occasionally used nitroglycerin for angina pectoris were included in the research.

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

disturbance of endothelial function in patients with IHD is characterized by reduced endothelial NO synthesis by eNOS and increased systemic NO synthesis due to increased iNOS activity.

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

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

Assessment of endothelial dysfunction in patients with generalized periodontitis

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

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

Methods: The clinical, paraclinical and immunological studies were carried out in 96 patients from 25 to 50 years old, including 18 patients with the intact periodontium (comparison group). Level of endothelium dysfunction marker – ET-1 was defined in oral liquid by means of the ELISA method and a broad set of reagents Endothelin-1 (“Biomedica” Austria). The electron microscopic studies of marginal periodontium were made (Ethical Committee Approval: 22.04.2013 protocol №4).

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

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

Any financial supports or grants were used for this research.

Efficiency of complex treatment of combined lesions of marginal and apical periodontium with thiotriazoline and chloramphenicol ointment

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Key words: endo-perio lesions, treatment, thiotriazolini, chloramphenicol

Clinical experience shows that patients with marginal periodontitis often have apical chronic inflammation of the teeth as well. Intercommunication of diseases of endodontium and periodontium exists due to close anatomic and functional connection between these tissues.

The status of oral cavity of 65 patients with combined apical and marginal periodontitis has been studied and measured by periodontal indices before, during the treatment and dynamic supervision. Ethical Committee Approval: 29.10.2007, protocol number 8. Investigation included X-ray examination, probing the depth of periodontal pockets, indices OHI-S (Greene and Vermillion), PMA, PI (Russel), PBI (Muhlemann and Son). The endodontic treatment had special value and was conducted as initial procedure for all examined patients. The complex treatment has been worked out and the ointment with thiotriazoline and chloramphenicol was introduced in the scheme of periodontal treatment.

Results of applied treatment indicated to the acceleration of healing process, reduction of exudation period and decrease of exacerbations frequency. Clinical experience also demonstrated positive dynamic in changes of periodontal indices after the conducted treatment.

According to obtained data we suppose that including of the thiotriazoline and chloramphenicol ointment in complex scheme of treatment of endo-perio lesions is expedient and pathogenetically grounded. Proposed remedy has antimicrobial and antioxidative effects, improves microcirculation and stimulates tissues regeneration.

Complex treatment of patients with combined apical and marginal periodontitis including thiotriazoline and chloramphenicol ointment was proved to be effective. Obtained data allows to recommend proposal scheme of such complex treatment of endo-perio lesions into the clinical use.

The work is carried on according to the scientific research of the Department of Therapeutic Dentistry of DHLNMU on budget funds of the university.

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Characteristics teeth anomalies in operated patients with congenital cleft lip and palate

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Keywords: congenital cleft lip and palate deformity and sizes dentition.

Introduction. One of the most common types of congenital anomalies are defects of maxillofacial area, which make up 30-45% of all defects, including congenital cleft upper lip and palate which rank second and is one of the pressing health and social problems, their frequency in the population is an important characteristic of health status. This article explores clinical and morphological characteristics identified strains dentition in patients with congenital cleft upper lip and palate.

Methods. Total primary screening were subjected to 41 patients, including unilateral cleft – 28 with bilateral – 12 patients, 1 – incomplete partial nonunion palate. Ethical Committee Approval date and number: November 21, 2011, №9

Results. . Established that sagittal length of the upper jaw dentition in children operated on for simplex and duplex cleft palate, which in early childhood was made of plastic upper lip, is less than the length of the sagittal mandibular dentition. The most pronounced discrepancy between sagittal lengths dentition of the upper and lower jaws characteristic of patients with bilateral cleft palate ($p < 0,05$). Sagittal underdevelopment of the upper jaw in children with both unilateral and with bilateral cleft palate is most pronounced in the anterior maxilla.

Discussion. The observed ratio of sagittal dimensions of upper and lower jaws in patients with cleft palate is the basis for the development progenic value of the jaws, which is classified as "false progeny." Pronounced sagittal anomalies in patients with cleft anomalies are accompanied by concomitant provision of individual teeth and dentition abnormalities, the most common is a narrowing of the upper jaw dentition and its asymmetry in unilateral cleft.

Conclusion. The features value dentition of the upper and lower jaws and detected anomalies defining features provide orthopedic care to such patients to implement appropriate aesthetic and functional standards.

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Influence of opioid on the condition of the uvea in the course of the experiment

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Key words: eyeball, uvea, opioid, experiment.

Introduction: The scientific article is aimed at determining the structural changes of the vascular layer of the eyeball in rats influenced by opioid.

Methods: The research material includes histological specimen of uvea in white rats. Within the framework of histological examination microscopic sections of the eyeball are stained with hematoxylin and eosin. The medication is studied and photographed at microscope magnification: ob. x 40, ey. x 15. All experiments were approved by the University Animal Care and Use Bioethical Committee (Protocol # 2 / 2012).

Results: The first symptoms of microstructure disorder in all parts of uvea are noticed in two weeks after nalbuphine injection. For the next 4 weeks the abnormal changes are increasing and are being manifested as swelling and polymorphonuclear infiltration of iris, ciliary body and the choroid. The profound destructive changes of eyeball hemomicrocirculatory bloodstream are also disclosed. The six week histological research of uvea in white rats influenced by nalbuphine displayed that the thin-wall extended venules are predominant. Sclerosing caused some thickening of the arteriola walls. The vascular capillary plate is destroyed. The number of capillary tubes is reduced. The wall of capillaries is damaged in some parts and extravascular bloodstroke is displayed (microbleeding). The explicit paravasal swelling is definitely revealed.

Discussion: According to the research results the examination of vessel structure, as well as the histological structure of its walls in a norm and affected by different agencies made it possible to study the importance of vessel factor in the morphofunctional inefficiency of internals. The tissue edema of all parts of uvea discovered during the experiment in 4 and 6 weeks of nalbuphine injection complied with the data presented in the specialized literature about the effect of addictive substances.

Conclusions: The research proved the negative effect of opioid on uvea microstructural organization in the course of the experiment. The opioid influence induced angiopathy which is the releaser for the eyeball destructive changes.

Source of research support: The scientific article is a fragment of the planned scientific work of the department of Normal Anatomy, Danylo Halytskyi Lviv National Medical University.

Disturbance of regulatory mechanisms of spermatozoa in patients with infertility

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

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

Methods. Spermatozoa of patients with male infertility and healthy donors were used. All patients and donors gave written informed consent to participate in research (Ethical Committee Approval Protocol No 8 from October 22, 2012). The activities of ion-transporting systems were determined on the saponin-permeabilized spermatozoa. ATP-hydrolase activity was determined spectrophotometrically, registering process of ATP hydrolysis by the accumulation of inorganic phosphate. Determination of NO_2^- level was carried out using the Griess reaction.

Results and discussion. It has been shown that values of Ca^{2+} -ATPase activity of endoplasmic reticulum increased significantly in patients with infertility. Contrary to this, the activity of Ca^{2+} -independent ATPase systems (Na^+ , K^+ -ATPase, “basal” Mg^{2+} -ATPase and H^+ -ATPase) decreases. Changes in ATPases activities indicate disturbance of ionic homeostasis in spermatozoa. It has been found that with increasing degree of oligozoospermia the calcium level increases in semen, spermatozoa and sperm plasma.

Numerous functions of spermatozoa are mediated by NO and its stable metabolites, acting as modulators of spermatozoa mobility, resistance to changes in environmental conditions, etc. In patients with oligozoospermia the NO_2^- level is not changed in comparison with control group (healthy donors). However, in patient with asthenospermia the NO_2^- level is significantly increased.

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

Possibilities of sonography as intraoperative control method during removal salivary stones from the major salivary glands and their excretory ducts

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Key words: ultrasonography, intraoperative control, salivary glands, sialolithiasis

Introduction: Sialolithiasis is the most common disease of major salivary glands (MSG) non-tumor genesis. The most frequently affected (about 90.0% of all cases) are submandibular salivary glands (SSG). Moreover, in the vast majority of cases (about 75,0%) salivary stone (SS) is single and is located in excretory duct of the gland. However, nowadays the question of choice of the method of intraoperative radiation control isn't fully clear.

Methods: The study included 25 patients with sialolithiasis of SSG who were treated at the department of maxillofacial surgery of the Lviv regional clinical hospital. Ethical Committee Approval: 20.09.2014, № 15

Results: The application of traditional radiography as a method of intraoperative control during removal of SS from SSG is not possible through considerable weight, size, cost of equipment, as well as the presence of radiation load, and a number of contraindications. High accuracy of ultrasonography and acceptable characteristics of the equipment allowed to use this method during the operation – removal of the SS by the following algorithm: assessment of the status and position of the concrement was conducted directly before removing it and immediately after it, in order to assess the condition of the gland and its excretory duct, to identify possible remains of a stone or a previously undiagnosed concrements. All patients' radiographic and sonographic examination results coincided completely. Additionally, in 7 patients ultrasonography diagnosed additional stones, not visualized radiographically.

Discussion: intraoperative ultrasonography in treatment of sialolithiasis is diagnostic method for operative treatment salivary stones, which can provide noninvasive control results of surgery.

Conclusion: It can be argued that intraoperative ultrasonography is a high-precision, non-invasive and easy to use method that can be applied throughout the surgery. The application of this method greatly improves the precision of the surgical intervention. Intraoperative ultrasonography SSG can be recommended for wide application in practice of oral and maxillofacial surgery.

This study was conducted by researchers' funds without attracting additional funds or grants.

Acknowledgements: the researchers express their gratitude to the leadership of the Lviv Natoinal Medical University, head chief of Radiology department Faculty of postgraduate education and head chief Craniomaxillofacial department Dentistry faculty department and leadership of Lviv regional clinical hospital for their opportunities for research and comprehensive care.

Enteral insufficiency syndrome (EIS) in acute pancreatitis: morphologic fundamentals and principles of drug correction

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Key words: acute pancreatitis; enteral insufficiency; pathomorphology.

Introduction: EIS is one of the threatening and unfavorable complications of acute pancreatitis.

Methods: One hundred four-patients with acute pancreatitis (AP) have been examined for EIS signs, depending on the degree of pancreatic damage and pathomorphologic changes in proximal portion of the small intestine. Ethical Committee Approval: № 24 - 23.10.2006

Results: The character of the above-mentioned changes in EIS, its degree of severity has been determined. Clinical evaluation of EIS has been based on analysis of pre- and suboperative signs according to clinically elaborated criteria. They have been compared to the results of pathomorphologic investigation of biopsy specimens of mucosa. Methods of EIS correction have been elaborated, which promote recovery of entero- and colonocytes and level its clinical manifestations.

Discussion and conclusion: Pathomorphologic investigation of biopsy specimens of the small intestine mucosa (obtained in fibrogastroduodenoscopy or suboperative formation of suspension jejunostoma) is an objective and informative criterion of presence of enteral insufficiency syndrome in acute pancreatitis. Morphometric data (correlation of microcilia length to crypts depth) allow detecting degree of severity of EIS. Thus, correlation factor from 1.9 to 2.0 indicates severe degree, from 2.1 to 2.25 – moderate degree, from 2.3 to 2.4 – mild degree of enteral insufficiency. Elaborated drug correction method of EIS on the basis of early enteral nutrition with adapted dairy mixtures in combination with prokinetics and probiotic Enterol (*Saccharomyces boulardii*) constitutes an important and effective component of the complex conservative and surgical treatment of the patients with acute pancreatitis.

Source(s) of research support in the form of financial support, grants: financing from Danylo Halytsky Lviv National Medical University budget.

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Hypohydrosis as a factor of purulent surgical infection development in patients after burn

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Key words: “burn disease”, “hypohydrosis”, “microorganisms”, “purulent surgical infection”

Introduction: Burns have a long history, but the methods, instruments, tactics of treatment and prophylaxis of patients with such injuries are at present one of the most leading problems of contemporary combustiology and plastic surgery. The most common causes of post-operative purulent-septic complications are degree of tissue injury during burn, resulting in dysfunction of sweat glands, as well as reduced level of natural resistance and immunological reactivity of the patient’s body.

Methods: 127 publications, selected in medical search systems by key words: “burn disease”, “hypohydrosis”, “microorganisms”, “purulent surgical infection”, have been analyzed.

Results: Partial or complete damage to sweat and sebaceous glands occurs in burns of the IIIA, IIIB, and IV degrees, causing development of functional impairment of activity of the sweat glands with appearance of hypohydrosis and disturbance of temperature regulation, leading to dryness and skin peeling, decrease in its elasticity and turgor, appearance of macerations and cracks on the skin. The majority of resident flora, vegetating in superficial skin layers and sweat glands, becomes pathogenic microflora when intactness is lost. Acceleration of the process of skin desquamation is caused by impairment of regeneration function, leading, in its turn, to trauma to the superficial skin layers. Thus, they don’t regenerate completely, which may cause maceration of some skin areas and appearance of cracks. As a result all these factors provoke the development of purulent surgical infection in this category of patients.

Discussion: Analyzing the results we revealed that partial or total destruction of the sweat and sebaceous glands caused by burn IIIA, IIIB and IV degree leads to the development of purulent surgical infection in the end.

Conclusion: Vegetative microflora of the sweat glands, accelerated process of desquamation of the epithelium and appearance of hypohydrosis due to deep burns lead to development of purulent surgical skin infection.

Protocol Ethical Committee: №8 from 22.10.2012

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

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Key words: mandibular condyle fractures, endoscopy, osteosynthesis.

Ethical Committee Approval: Lviv National medical University, №4 from 10.05.2014.

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

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

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

Conclusions:

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

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Stress resistance of adipose tissue and liver in offspring of rats fed high-fat, high-sugar or their combination diet

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Keywords: liver, adipose tissue, inflammation, diet, stress

Introduction: Among rapid risen lifestyle diseases is nonalcoholic fatty liver disease (NAFLD), which showed 3-fold increase in its prevalence over the last 30 years. Despite of intensive study of adipose tissue (AT) and liver cross-talking, their changes during developmental programming are still unclear. Thus, we analyzed the effects of maternal stress and overnutrition on AT and hepatocellular stress-resistance in offsprings.

Methods: The experiments were conducted in nonlinear female rats (n=20) and their male offspring (n=72) with Ethical Committee Approval: № 4 from 11.04.2011. Pregnant rats were exposed to stress using Pratt's model (1989), and nutritional insults: high-sugar diet (Kozar, 2009), high-fat diet (Lintermans, 2009) or their combination – high-sugar and high-fat diet (HSFD). The effects of maternal stress and nutrition on severity of AT and liver morpho-functional changes during stress (Takagi, 1964 and administration of indomethacin) were examined in offspring, along with serum levels of IL-1 β , GRO/CINC-1, leptin and adiponectin.

Results: Maternal exposure to stress in combination with HSFD resulted in the most prominent histological changes in offspring: AT inflammation and hepatocellular reorganization. Also in this group were revealed the significant changes in cytokines after stress induction: increased IL-1 β (217%), GRO/CINC-1 (99%), leptin (79%) and decrease of adiponectin (41%) vs control; induction of stress in offspring caused increased IL-1 β (178%), GRO/CINC-1 (57%), leptin (34%) and decrease of adiponectin (53%) vs control; combined stress and indomethacin injury increased IL-1 β (213%), GRO/CINC-1 (27%), leptin (30%) and decrease of adiponectin (64%) vs control.

Conclusion: This rat model used in this study provides a novel insight into stress resistance of AT and liver and their role in NAFLD. Expressed cytokines may indicate early changes in liver and AT functioning. Healthy nutrition and stress management during pregnancy may serve as a valid strategy for preventing liver and AT inflammation and improve their stress resistance in adulthood.

Acknowledgements: We thanks Prof. I. Gout (University College London, UK) for providing ELISA sets for adipokines and interleukins

Features of the reparative processes under influence of opioid in experiment

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Keywords: skin, animal model, reparative process, opioid.

Introduction: To examine the influence of prolonged administration of opioid on the course of the reparative process of multiple post-injection wound of white rat's skin using light microscopy.

Methods: Studies has been carried out on 24 mature white male rats, aged 4.5-7.5 months, with weight of 130-150 g. For histological examination the skin sections were stained with hematoxylin and eosin. The preparations were studied and photographed at the magnification: ob.x8, ey.x15 and ob.x40, ey.x10. The computer system «Aver Media» was used for photography of micropreparations. The University Animal Care and Use Bioethical Committee approved all experiments and the approval # is 2 / 2012.

Results: Studying at the level of light microscopy shows that the administration of opioid for 2 weeks does not affect the reparative process of multiple post-injection wound. In most wound canals the formation of a complete regenerate occurs. After 4 weeks of nalbufin administration the processes of wound healing slow and are complicated by forming of microabscesses. After opioid administration to rats during 6 weeks, destructive changes in the skin have been detected, which in turn leads to the inability of complete regenerate formation of multiple post-injection wound.

Discussion: Regeneration processes of the skin, according to many researchers, are the most expressive in perivascular area of the capillary, and in remote areas of the capillary biosynthetic activity of cells decreases, their degenerative changes increase. Our results confirm that the healing of the skin accompanied by phase changes in the structure of its structural elements - the epidermis, dermis and hypodermis, which is affected by endogenous and exogenous factors.

Conclusions: The experimental results show the negative effect of prolonged administration of opioid on the reparative processes in the skin.

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Biochemical research of osteotropic action of various osteoconductive preparations by experiment

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Key words: experiment, bone fabric, osteotropic preparations

Introduction: The feature of endodontic treatment of complicated caries of permanent teeth with uncompleted apexogenesis consists in providing apexification, optimization of reparative osteogenesis, mineralization of fabrics of tooth. Low efficiency of endodontic treatment caused a search of new osteoconductive preparations of osteotropic action.

Methods: Research on rats was conducted. Defect of bone was created and was filled by osteotropic materials: by compositions based on hydroxyapatite of calcium (HAC) and beta tricalcium phosphate (β -TCP), mineral trioxide aggregate (MTA), Nano Gen. Experiment lasted 14, 30 and 90 days. Efficiency of preparations was estimated on content of calcium (Ca) i phosphorus (P), activity of alkaline (AP) and sour (SP) phosphatase, katalase (KAT), soluble albumin, malondialdehyde (MDA) in bone fabric. Animal Care and Use or Ethical Committee Approval: 23.09.2013; № 7

Results: The most substantial changes were observed on 90 day, results are given in the table.

Groups	Ca ²⁺ /P	AP/SP	MDA, nmol / g	KAT, mmol H ₂ O ₂ /mg albumin per 1 min. $\times 10^{-5}$
HAC	2,6	155	5,05 \pm 0,326	0,89 \pm 0,12
β -TCP	1,9	147	5,31 \pm 0,337	0,79 \pm 0,13
MTA	1,9	127	7,47 \pm 0,445	0,49 \pm 0,07
Nano Gen	1,3	106	7,85 \pm 0,541	0,45 \pm 0,08
Control	1,6	104	3,48 \pm 0,174	1,54 \pm 0,12

Discussion: The most expressed osteoconductive effect among compositions has the composition based on HAC due to increase of Ca²⁺/P and AP/SP balance, decrease of MDA and increase of katalase. MTA in comparison with Nano Gen shows better osteotropic activity.

Conclusion: Highest osteotropic effect of composition based on HAC, that gives opportunity to recommend it for treatment of complicated caries of permanent teeth with uncompleted apexogenesis.

Estimation of the volume of the polynucleated hepatocytes of the rat liver under the opioid intoxication.

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Keywords: liver, rat, opioid

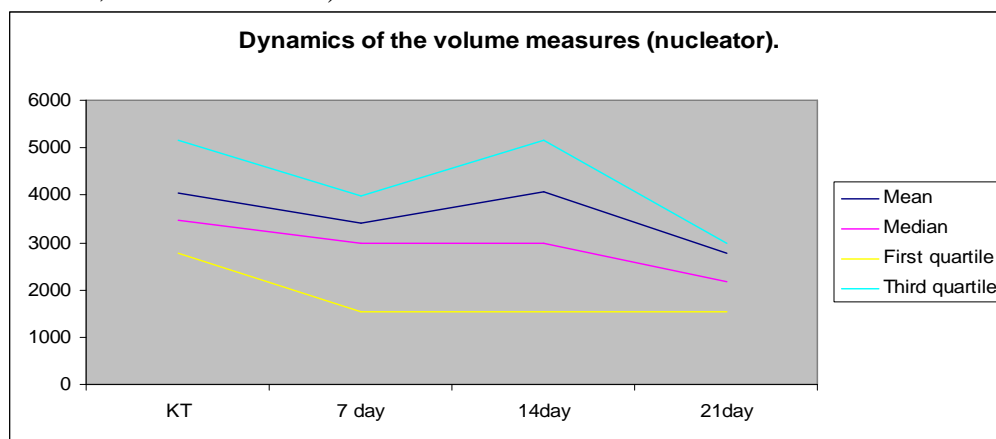
Introduction: The points sampled intercept (PSI) and nucleator (NC) methods were used for researching of the volume of the polynucleated hepatocytes, as a part of larger stereological researching of the rat liver under the influence of the opioid.

Method: We used adult male rat 190-220 g. weight. Experiment was approved by bioethical committee (protocol #2 from 20.02.2012). The rats in experimental group were injected with the opioid "Nalbuphine" every day. Every 7-th day the samples of the liver were taken. Measurements were made with IMAGEJ program.

Results: We have presented results in a table and graph.

Group	Method	n	Mean	SD	Median	25%	75%	CVn(V)
KT	Nuc	68	4028	2387	3475	2780	5152	0,65
	PSI	70	5742	7077	3978	1766	7528	
7 day	Nuc	154	3419	2642	2981	1527	3968	0,48
	PSI	67	4237	4101	2516	1638	5963	
14day	Nuc	239	4085	3561	2981	1527	5152	0,35
	PSI	139	4611	4627	3053	1463	5963	
21day	Nuc	232	2769	1775	2174	1527	2981	0,61
	PSI	136	3817	4751	2695	1288	5113	

Table №1. Descriptive statistics (n - sample size, CVn (V) - coefficient variation for particles volume, volume in μm^3).



Graph №1. Dynamics of the statistic.

Discussion and conclusion: There were decreasing of the mean on the 21th day in both - PSI and NC. On the 7th and 14th days there were increases of the relative number of the small forms hepatocytes, as well as an increase of the relative number of the large forms hepatocytes on 14th day. Based on histology, we can make the conclusion that a decrease in the relative number of

the large hepatocytes on 21th day were caused by their necrosis. About the differences between two methods on practice - NC is close to absolute data in mean as well as in quartiles, thus we can research the dynamics of the volume variation too.

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

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

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

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

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

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

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

Angiographic and clinical presentation of intraoperative vasospasm during endovascular ruptured cerebral aneurysms' treatment.

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Keywords: Aneurysm surgery, subarachnoid hemorrhage, vasospasm

Introduction: The changes of angiometric parameters of cerebral arteries at vasospasm are appearances of complications of endovascular procedures and seem to have high postoperative prognostic value.

Methods: It were inspected 34 patients with subarachnoid hemorrhage (SAH), Ethical Committee Approval: № 5, 2013, May, 20th. Aneurysms of middle cerebral artery (MCA) were verified as a cause of stroke in all cases. Diameter and area of transversal cut of M₁-segment of MCA were measured as on the side of aneurysm before and after operation and on an opposite side before operation with the use of the program «Quantitative vascular analysis», installed on angiograph Siemens Axiom artis.

Results: There was noted a diminishing of minimal diameter of M₁-segment on the average to 1,41±0,16 mm (p=0,02) by comparison to it at the beginning of operation (on the average 2,03±0,14 mm). At patients, who were operated with an favorable result, a narrowing of diameter of bearing artery was insignificant (p>0,5). Determination of minimal area of transversal cut of M₁-segment was also conducted.

Discussion: It is set that diminishing during the operation of minimal diameter of M₁-segment on the side of aneurysm more than 30% from initial likewise of minimal area of M₁-segment transversal cut on 50 % and more testify the progression of vasospasm and predict an unfavorable result of treatment.

Conclusion: It is proposed the classification of intraoperative vasospasm depending on its severity, namely: < 10% -mild intraoperative vasospasm; 10-30 % - moderate intraoperative vasospasm; >30% - severe intraoperative vasospasm, in accordance with the noted correlation of this parameter by the clinical results of surgical treatment of patients with SAH.

Conflict of Interests: The author reports no conflict of interests concerning the materials or methods used in this study or the findings specified in this paper.

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Laparoscopic fluorescence imaging system

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Keywords: laparoscopy, complications, visualization, fluorescence

Introduction: Laparoscopic surgery is a recognized "gold standard" of treatment of a number of surgical conditions. However, despite 25 years of experience in the extensive use of this technology, intraoperative complications are still considered to be its threat attribute. Often such complications occur due to erroneous interpretation of anatomy in result of impaired visualization. Therefore, today an ongoing search takes place for available measures to improve intraoperative visualization.

Methods: Patients completed laparoscopic cholecystectomy with the usage of laparoscopic fluorescence imaging system. During surgery administered intravenous and targeted injection of fluorescents was applied. Ethical Committee Approval: date: 26.05.2014 and number: 5

Result: Indocyanine Green and Fluorescein was used as a fluorescein in the research. Fluorescents were intravenously injected during surgery directly into anatomic structures. Principal advantages of this method include: noninvasiveness and interactivity – securing a sharp imaging in real time, low-toxicity and radiation level. Such method lies in the usage of infrared and ultraviolet illumination as a result of fluorescents emission. This technology provides for definitive vascular and biliary tracts opacification, and improves their visualization. Application of the laparoscopic fluorescence imaging system resulted in the reduction of intraoperative complications during laparoscopic intervention. These facts speak for substantial improvements of visualization in real time.

Discussion and Conclusion: Improvement of imaging methods is one of the priority ways for prevention of intraoperative complications during laparoscopic interventions. Fluorescents and hyperspectral scopy usage is one of the priority directions in prevention of damage to anatomical structures. Maximum security of patient's organs and systems is one of the tasks of intraoperative period. Application of laparoscopic fluorescence imaging system opens up new perspectives for a possible reduction in the incidence of their intraoperative complications.

Confocal microscopy and immunohistochemical study of white rat heart after the effect of opioid intoxication

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Keywords: rat myocardium, opioids, confocal microscopy, immunohistochemistry

Introduction: Drug use prevalence is stable around the world, according to the 2014 World Drug Report of the United Nations Office on Drugs and Crime (UNODC).

Study was thus to investigate the rat atrial cardiac wall on the 7 and 14 day of the experimental opioid intoxication. The male white rats were used in these experiments and divided for 3 exercise groups: the 1 group – with every day injections of “Nalbufin”, the 2 control group with injections of 0,9% NaCl, and the 3rd group – intact animals. All experiments were approved by the University Animal Care and Use Bioethical Committee, the approval # is 2/20Feb2012).

Method: For immunohistochemistry we used the antibodies to μ -opioid receptors, the antibodies to cleaved Caspase-3 and Phospho-Histone H3. For confocal microscopy we used the same 3 antibodies.

Results: Apoptosis start to go in epicardium and perivascular spaces of myocardium on the 7 day of the 1 group, and a lot of cardiomyocytes undergoing apoptosis from the 14 day. The proliferative processes in the rat atrium start in vascular walls, perivascular spaces of the epicardium on the 7 and 14 days of the experiment. The actinum fibers of atrial myocardium become thinner on 7 day of experiment. On 14th day of the interactin spaces become larger then in control and intact groups.

Discussion: Nalbufin are from opioid group affects apoptosis and proliferative processes. These two processes are combined with disorders of actinum fibers. Result – the contractility of heart is decreased.

Conclusion: The apoptosis of atrial myocardium begin to accelerate from 7th day of the experiment. The proliferative processes in atrium start in vascular walls, perivascular spaces of the epicardium on the 7th day and are continued on the 14th day. The actinum fibers become thinner on the 7th day of experimental and start to disorder on the 14th day.

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Experimental substantiation of the application of different plastic stimulators in gingival recession

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Keywords: oral mucosa, plastic stimulators, histomorphology

Introduction: Recession of gums in primary lesions of periodontium tissues demands the minimal – invasive contour plasty with the application of plastic stimulators different in origin and functioning.

Methods: The investigation was carried out on white rats: 1) intact animals; 2) application of natural preparations; 3) introduction of synthetic polymers; 4) application of natural and synthetic compounds in consecutive order; 5) composition of these preparations. In rats, 0.1 ml of the experimental preparation was introduced into the frontal teeth of the mandible intramucously (Animal Care and Use or Ethical Committee Approval: 23.09.2013; № 7). The experiment lasted for 30 days and was followed by the histomorphometric investigations.

Result: Owing to the application of different groups of preparations, the gingival mucosa differed in thickness of epithelial and connective layers. The natural preparations promoted the fibroblasts formation along the microvessels. The connective layer was equal to 826 ± 68 mkm, that is 286 mkm exceeding the level that in intact rats. The synthetic polymers stimulated the formation of elastic fibres, in addition to that the connective layer was equal to 825 ± 93 mkm. On the sections of preparations along with succession of introduction of synthetic and natural compounds there was an insignificant number of fibroblasts with the chaotic arrangement, while the thickness was equal to 825 mkm. After the introduction of composition of preparations no dramatic differences were revealed. Morphometric index was 659 ± 32 mkm.

Discussion: The introduction of preparations with different composition led to the enlargement of number of collagen and elastic fibers, consequently improving the elasticity and resilience of tissues.

Conclusion: All this provoked the changes in morphologic structure of intercellular space and facilitated the plastic stimulation and mesolifting.

**Recommendation for RECOOP Reviewers Assisting in the Review of manuscripts for the
Croatian Medical Journal RECOOP Issue in April 2015**

Czech Republic

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