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Contents

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2011–2014–	Biopolymers & Cell (Abstracts)
2016 –	The Ukrainian Biochemical Journal (Abstracts and publications)
2014 –	Croatian Medical Journal – The RECOOP Annual Scientific Review (Peer reviewed publications)

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Abstracts
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Novel concept on the roles of adipokines in uterine contractility

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Key words: preterm delivery, prolonged pregnancy, uterine contractility, obesity, adipokines, kisspeptin

Obesity is a global health problem even among pregnant women. The most destructive consequence of obesity is the development of long-term low-grade metabolic inflammation, which can lead to the development of several pathologic conditions. Obesity may alter the pregnant uterine contractility and hence makes the gestation period shorter or longer, leading to preterm delivery or prolonged pregnancy, but clear evidence is not available.

Several physiological factors play a role in the altered gestational period and serve as drug targets to treat the consequences. The most important are female sexual hormones, calcium channels, adrenergic system, oxytocin and prostaglandins. However, we have limited information about the impact of obesity on the pregnant uterine contractility and gestation time. Adipose tissue, which is the largest endocrine and paracrine organ, especially in obesity, is responsible for the production of adipokines and various cytokines and chemokines. Recent data suggest that the dysregulation of leptin, adiponectin and kisspeptin during pregnancy contributes to gestational diabetes mellitus and pre-eclampsia.

A preclinical method for obese pregnancy should be worked out to clarify the action of adipokines and assess their impact in obesity. The deeper understanding of the adipokine-induced processes in obese pregnancy may take us closer to the prevention and therapy of preterm delivery or prolonged pregnancy.

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Metabolically healthy central obesity in women associates with low levels of soluble receptor for advanced glycation end-products but not with semicarbazide sensitive amine oxidase/soluble vascular adhesion protein-1

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Key words: metabolically healthy central obesity, women, soluble receptor for advanced glycation end-products, adipokines, semicarbazide sensitive amine oxidase, soluble vascular adhesion protein-1

Introduction: Obesity represents a key risk factor for development of metabolic syndrome, type 2 diabetes, and cardiovascular diseases. In non-diabetic individuals low level of circulating soluble receptor for advanced glycation end products (sRAGE) is considered as a biomarker of risk of development of metabolic syndrome and cardiovascular diseases. Data on association of sRAGE with markers of adiposity are unequivocal. We investigated whether sRAGE levels differ in metabolically healthy (COH) vs. metabolically unhealthy (COU) centrally obese women.

Methods: 47 lean healthy, 17 COH (presenting waist-to-height ratio ≥0.5 but elevated blood pressure, atherogenic lipid profile, and insulin resistance) and 50 COU (centrally obese presenting ≥2 risk factors) women aged 40-45 years were included. Anthropometric characteristics, blood chemistry and hematology data, adipokines, markers of inflammation, sRAGE and soluble vascular adhesion protein-1, and the activity of semicarbazide sensitive amine oxidase were determined.

Results: Central obesity, regardless of presence/absence of cardiometabolic risk factors, associated with low sRAGE levels and elevated markers of inflammation. Both groups of centrally obese women presented hyperleptinemia, while only COH women maintained high adiponectin levels. Soluble vascular adhesion protein-1 concentrations and the activity of semicarbazide sensitive amine oxidase were similar in all 3 groups.

Discussion and Conclusions: Centrally obese women classified as healthy according to standard cardiovascular risk criteria present numerous abnormalities in non-standard markers of cardiometabolic risk, supporting the view that there is no healthy pattern of obesity. The clinical impact of our findings for future prognosis of metabolically healthy obese subjects remains to be elucidated in longitudinal studies.

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This cross-sectional study was conducted in accordance to the principles of the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Slovak Medical University in Bratislava. All subjects signed an informed consent to participate.

Tissue-protective activity of selenomethionine and D-panthetine in B16 melanoma-bearing mice under doxorubicin treatment is not connected with their ros scavenging potential

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Introduction: was to evaluate molecular mechanisms of tissue-protective effects of antioxidants selenomethionine (SeMet) and D-pantethine (D-Pt) applied in combination with doxorubicin (Dx) in B16 melanoma-bearing-mice. Impact of this chemotherapy scheme on a survival of tumor-bearing animal, general nephro- and hepatotoxicity, blood cell profile *in vivo* and ROS content in B16 melanoma cells *in vitro* was compared with the action of Dx applied alone.

Methods: Nephrotoxicity of studied drugs was evaluated by measuring creatinine indicator assay, hepatotoxicity was studied by measuring the activity of ALT/AST enzymes, and myelotoxicity – by light microscopic analysis of blood smears. Content of cellular ROS under Dx, SeMet and D-Pt action *in vitro* were measured by incubating B16 melanoma cells with fluorescent dyes dihydrodichlorofluoresceindiacetate (DCFDA, H_2O_2-specific) and dihydroethidium (DHE, O_2^--specific), and further analysis at FL1 (DCFDA) or FL2 channel (DHE) of FACSCalibur flow cytometer.

Results: Selenomethionine (1200 μg/kg) and D-pantethine (500 mg/kg) in combination with Dx (10 mg/kg) significantly reduced tumor-induced neutrophilia, lymphocytopenia and leukocytosis in comparison to Dx treatment alone. Moreover, SeMet and D-Pt decreased several side effects of Dx, namely an elevated creatinine level in blood and monocytosis, thus, normalizing health conditions of B16 melanoma-bearing animals. However, these tissue-protective effects of SeMet and D-Pt under Dx treatment had no major impact on the survival of melanoma-bearing animas, opposite to their effects towards NK/Ly lymphoma, as observed by us earlier (Panchuk et al, 2016). *In vitro* studies have revealed insignificant impact of both SeMet and D-Pt towards Dx-induced production of hydrogen peroxide and superoxide anions, suggesting that the observed nephro- and myeloprotective properties of SeMet and D-Pt are not dependent on their ROS scavenging activity and are realized by other yet unknown mechanisms.

Discussion and Conclusions: Antioxidants selenomethionine and D-pantethine possess significant nephro- and myeloprotective activity towards Dx action on murine B16 melanoma *in vivo*, but fail to boost a survival of B16 melanoma-bearing animals. The observed cytoprotective effects of studied antioxidants are not directly connected with ROS scavenging.

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Examination of placental vascularization and uterine artery peak systolic velocity in pregnancies complicated by gestational diabetes, pregnancy hypertension and preeclampsia

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Key words: placental vascularization, dimensional power Doppler, pre-gestational BMI, gestational age

Intorduction: The aim of our study was to analyze the correlation between AUtPSV, placental vascularization indices, pre-gestational BMI, gestational age and adverse pregnancy outcome rates in case of pregnancy hypertension, GDM and normal blood pressure pregnancies. We hypothesized that AUtPSV rises when GDM complicates pregnancy hypertension.

Methods: Placental 3-dimensional power Doppler indices, such VI, FI and VFI, and AUtPSV were measured in pregnancies complicated with CHT (N=43), CHT+GDM (N=15), GHT (N=57), GHT+GDM (N=23) and PE (N=17), and compared to pregnancies with NBP (N=109). We analyzed the correlation between the above mentioned indices, AUtPSV results, pregestational BMI and adverse pregnancy outcome rates obtained after delivery.

Results: VI was higher in CHT (p=0.010), while FI was lower in CHT (p=0.009), GHT and PE (p=0.001) compared to NBP. In case of VFI, significant difference was found between CHT and GHT (p=0.002), and NBP and PE (p=0.001). As for adverse pregnancy outcome rates, FI was found prognostic for umbilical pH and birth weight. Pre-gestational BMI was significantly higher in GHT+GDM compared to GHT, as well as in CHT+GDM compared to the CHT group. In case of AUtPSV, significant difference was found between NBP and CHT (p=0.012), NBP and CHT+GDM (p=0.045), NBP and GHT+GDM (p=0.007), NBP and PE (p=0.032), as well as GHT and GHT+GDM (p=0.048) groups.

Discussion and Conclusions: Our study revealed that certain placental vascularization indices and AUtPSV show significant differences due to gestational pathology; thus placental vascularization indices and AUtPSV can be useful in the early detection of pregnancies at risk.

Ethical approval number: 32/2014 Human Investigation Review Board, University of Szeged, Hungary

Identification of myoelectric signals of pregnant rat uterus: new method to detect myometrial contraction

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Key words: contractility, electromyography, uterus, pregnant rat, myometrium

Introduction: The myoelectric processes are crucial for the initiation of myometrial contractions, especially in pregnancy. Although a few clinical studies have been published about the application of electromyography in obstetrics, the comparison and separability of slow wave uterine signals from other smooth muscles waves have not been described yet. Our recent aim was to develop an electromyography method for pregnant rat uterus *in vivo* and to separate them from the GI tract signals.

Methods: Pregnant Sprague-Dawley rats were anaesthetized and their stomach, small intestine and large intestine were removed from the abdomen. A pair of thread electrodes was inserted into the uterus, while a pair of disk electrodes was placed subcutaneously above the myometrium. Additionally, we fixed a strain gauge sensor on the surface of the myometrium and caecum for the parallel detection of mechanical contractions in non-GI resected rats. The filtered electric signals were amplified and recorded by an online computer system and analysed by fast Fourier transformation. The frequency of the electric activity was characterized by cycle per minute (cpm), the magnitude of the activity was described as power spectrum density maximum (PsDmax).

Results: The frequency of the pregnant uterine activity was found at 1-3 cpm, which falls within the same range as that of caecum. Measuring by both electrodes, oxytocin (1 μg/kg) increased and terbutaline (50 μg/kg) decreased the PsDmax by 25-50% and 25-40%, respectively. We found correlation between the alterations of PsDmax values and the strain gauge sensor-detected area under the curve values (AUC). The GI specific compounds (neostigmine, atropine) mainly affected the caecal activity, while myometrium specific drugs (oxytocin, terbutaline) influenced the myometrial signals only.

Discussion and Conclusions: Our method is able to detect the myoelectric activity that reflects the mechanical contraction. The overlapping myometrial and caecal signals are not separable, but they can be distinguished based on the much higher activity and different pharmacological reactivity of the pregnant uterus. Thus, the early signs of contractions can be detected and labour may be predicted in a fast and sensitive way.

Source of research support: This work was supported by project PIAC_13-1-2013-0201, National Research, Development and Innovation Office, Hungarian Government.

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

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

4-Thiazolidinone derivative Les-3833 effectively inhibits viability of human melanoma cells through activating apoptotic mechanisms

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Key words: melanoma, 4-thiazolidinone derivatives, apoptosis, MAPK

Introduction: To evaluate cytotoxic action of 4-thiazolidinone derivative Les-3833 and study the mechanisms of its pro-apoptotic action towards human melanoma cells and human tumor cell lines of other tissue origin.

Methods: The effect of Les-3833 or doxorubicin on the viability of 8 cell lines was studied using MTT assay, while human melanoma cells of WM793 line were additionally examined using light and fluorescent microscopies for evaluating cytomorphological changes, and the Western-blot and flow cytometric analyses were carried out to study signaling pathways of melanoma cell cycling and death.

Results: Les-3833 was the most toxic towards melanoma cells. Its IC50 was 0.22 μg/mL for WM793 cells and 0.3 μg/mL for SK-Mel-28 cells. While IC50 was in between 2.5 to >5.0 μg/mL for human lung A549, breast MCF-7, colon HCT116, ovarian SKOV3 carcinoma cell lines. Les-3833 was relatively not toxic (IC50 > 5 μg/mL) for human embryonic kidney HEK293 cells. Results of Annexin V/PI staining of melanoma cells and activation of caspase 3, PARP, MAPK, and EndoG protein suggest apoptosis in Les-3833-treated cells. Les-3833 also induced ROS production in melanoma cells and their arrest in G0/G1 phase of cell cycle.

Discussion and Conclusion: Novel 4-thiazolidinone derivative Les-3833 is effectively killing human melanoma cells in vitro, and such effect is tumor specific since it is much less pronounced in human carcinoma and leukemia cells. Les-3833 induces apoptosis (morphological changes and increased pro-apoptotic proteins), ROS production, and arrest of melanoma cells in G0/G1 phase of cell cycle.

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Differential pro-apoptotic effects of synthetic 4-thiazolidinone derivative, Doxorubicin and Temozolomide in human glioma U251 cells

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Key words: apoptosis measurement, human glioblastoma U251 cell line, 4-thiazolidinone derivative, Doxorubicin, Temozolomide

Introduction: Compare various pro-apoptotic effects of synthetic 4-thiazolidinone derivative (ID3288), Doxorubicin (Dox) and Temozolomide (TMZ) in the treatment of human glioma U251 cells to improve treatment outcomes of glioblastoma and avoid anticancer drug resistance.

Methods: The cytotoxic effects of used drugs in human glioma U251 cells were measured by cell viability and proliferation (MTT) assay, Trypan blue exclusion test, and Western-blot analysis of the apoptosis-related proteins. In addition, flow cytometry study of reactive oxygen species (ROS) level in glioma cells was carried out. Cytomorphological changes in treated cells were monitored by fluorescent microscopy after cell staining with Hoechst 33342 and Ethydium bromide.

Results: Half-maximal inhibitory concentration (IC50) of Les-3288, Dox and TMZ was calculated for human glioblastoma U251 cells, and rating of the values of this indicator of cellular vitality was assessed. The results of MTT assay proved the superiority of Les-3288: TMZ<Dox< Les-3288, that agree with and also the results of Trypan blue testing: TMZ<Dox≈Les-3288. In general, such ranking corresponded to a scale of pro-apoptotic impairments in the morphology of glioma U251 cells and the results of Western-blot analysis of cleaved Caspase 3. Contrary to Dox, Les-3288 and TMZ did not affect significantly ROS level in treated cells.

Discussion and Conclusion: An increased effect of the synthetic 4-thiazolidinone derivative Les-3288 is realized via apoptosis mechanisms, and it does not involve ROS. It is more effective, comparing with Dox and TMZ, in destroying human glioblastoma U251 cells. Les-3288 compound has a potential as anticancer drug for glioblastoma, nevertheless further preclinical study of the blood-brain barrier is needed.

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Funding: This work was partially supported Lviv National Medical University (LK, RL, BZ), National Academy of Sciences of Ukraine (NF, IG, OK, RP, LL, RS).
Ethical Approval: No animal experiments were conducted.

RECOOP Annual Scientific Review in The Ukrainian Biochemical Journal

Effect of hydrogen sulfide-releasing aspirin on esophageal and gastric mucosa compromised by stress injury

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Key words: H_2S, esophagus, stomach, aspirin, inflammation, cytoprotection, VCAM-1, IL-6

Introduction: The aim was to evaluate the effects of H_2S-releasing aspirin derivative, ATB-340, on esophageal and gastric mucosa compromised by stress injury. Recent data of study H_2S in gastrointestinal tract has proven its potent cytoprotection on mucosal defense. Among acid-related diseases in the gut esophagitis, Barrett esophagus, gastritis are prevalent and characterized by low-grade inflammation, which is a pre-malignant condition. Aspirin is one of the most widely prescribed nonsteroid anti-inflammatory drugs (NSAIDs). Latest data have shown its novel roles in tissue repair, anti-aging and chemoprevention against malignancy. However, aspirin has a well-known ability to induce gastrointestinal damage, and moreover, its combination with proton pump inhibitor therapy causes wide range of side effects. We hypothesized that the novel NSAIDs addition of H_2S-releasing moiety to aspirin may result in defensive activity and decline its pro-ulcer activity.

Methods: Rats were treated with vehicle (control), aspirin (10 mg/kg), ATB-340 (17,5 mg/kg) single or 9 days duration, with or without induction of stress injury. Esophageal mucosa (EM), gastric mucosa (GM) were estimated by histopathological damage scoring. Serological levels of VCAM-1, IL-6 by ELISA.

Results: ATB-340 treatment resulted in protective effect and lower grade of damage score in EM and GM lesions vs effect of aspirin in single or 9 days applications. The serum levels of VCAM, IL-6 in rats who were aspirin-treated and subjected to stress-injury were higher than those in control animals. Treatment with ATB-340 produced an anti-inflammatory effect by decreasing VCAM and IL-6 vs aspirin.

Discussion and Conclusion: Cytoprotective effect of ATB-340 on EM and GM is modulated by inhibiting inflammation and improving endothelial functions.

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The authors are grateful to Antibe Therapeutics Inc (Canada) for providing the ATB-340 and ELISA kits for VCAM and IL-6 (Cedarlane, Canada), and to Prof. Yurij Bisyarin (Bioptat Ltd., Lviv) for helping optimize and perform the histological analysis. Dr. Wallace's research is supported by a grant from the Canadian Institutes of Health Research.

4-Thiazolidinone derivatives rescue osteoblast differentiation from TNAα inhibition in mouse mesenchymal precursor cells

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Key words: osteoblast differentiation, mouse mesenchymal precursor cells, inflammation, bone morphogenetic proteins, 4-thiazolidinone derivatives.

Introduction: Rheumatoid arthritis (RA) is a severe autoimmune inflammatory disorder for which the etiology remains unknown. It has been demonstrated that tumor necrosis factor α (TNFα) plays a crucial role in RA pathophysiology. On another hand, the bone morphogenetic protein (BMP) and Wnt regulatory pathways are key players in signaling mechanisms that induce and support cartilage and bone formation and maintenance. A negative interaction between the pro-inflammatory signals and skeletogenic pathways occurs at the sites of inflammation in RA. In previous studies, we demonstrated that the pro-inflammatory cytokines TNFα and interleukin 1β (IL-1β) mediate such inhibition of the skeletogenic pathway activity.

Methods: We performed *in vitro* evaluation of the functional effect of 4-thiazolidinone-based derivatives (compounds Les-4368, Les-4370, Les-3882, and Les-3288) in different doses (1 μM, 0.3 μM, 0.1 μM and 0.02 μM) on the TNFα-mediated inhibition of the BMP-induced osteoblast differentiation in mouse mesenchymal precursor cells of the C2C12 line. Western-blot analysis was used to elucidate the mechanism of the anti-inflammatory effects.

Results: Treatment of C2C12 cells with TNFα completely inhibited their myoblast differentiation, as well as strongly inhibited their BMP-induced osteoblast differentiation. Treatment of C2C12 cells with two out of four tested 4-thiazolidinone derivatives, Les-4368 and Les-3882, rescued the osteoblast differentiation from the negative control of TNFα, and even converted it from the inhibitor of osteogenesis into its stimulator. The most pronounced effect was shown by the Les-3882 that stimulated osteoblast differentiation at low dose (0.1 μM).

Discussion: Possible modulation of the NF-κB activation by Les-3882 and Les-4368 might be a key mechanism mediating its anti-inflammatory effects. The compounds that were efficient in modulation of the anti-osteogenic effects of TNFα had an opposite effect on the IκBα level. The explanation of such a difference and the more detailed mechanism of such effects requires additional investigations of structural and functional interrelations of novel 4-thiazolodinones.

Conclusion: Les-3882 and Les-4368 rescue osteogenesis from the negative control of inflammation. The best effect was demonstrated by Les-3882 that stimulated osteoblast differentiation at low dose (0.1 μM), presumably, via modulation of the NF-κB signaling pathway.

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Glu- and Lys-forms of plasminogen differentially affect phosphatidylserine exposure on the platelet surface

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Key words: Glu- and Lys-plasminogen, phosphatidylserine exposure, platelets

Introduction: Plasminogen/plasmin system is known for its ability to support hemostatic balance of blood. However, plasminogen may be considered as an adhesive ligand and in this way could affect the functioning of blood cells. We showed that exogenous Lys-plasminogen, but not its Glu-form, inhibited platelet aggregation and suppressed platelet α-granule secretion. The aim of this work was to investigate the influence of Glu- and Lys-form of plasminogen on the formation of platelet procoagulant surface using phosphatidylserine exposure as a marker.

Methods: Human platelets were obtained from human platelet-rich plasma (donors were healthy volunteers, men aged 30-40 years) by gel-filtration on Sepharose 2B. Phosphatidylserine exposure on the platelet surface was evaluated by flow cytometry with FITC-conjugated annexin A5.

Results: Glu- and Lys-plasminogen have different impact on the platelet functioning. Exogenous Lys-plasminogen has no significant effect on phosphatidylserine exposure, while Glu-plasminogen increases phosphatidylserine exposure on the surface of thrombin- and collagen-activated human platelets.

Discussion and Conclusion: Glu-plasminogen can be considered as a co-stimulator of agonist-induced platelet secretion and procoagulant surface formation. Meanwhile effects of Lys-plasminogen are probably directed at platelet-platelet interactions and not related to agonist-stimulated pro-apoptotic changes. The observed different effects of Glu- and Lys-plasminogen on phosphatidylserine exposure can be explained by their structural peculiarities.

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RECOOP Research in Progress

The effect of kisspeptin fragments on the pregnant rat myometrium *in vitro*

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Key words: pregnancy, rat, uterus, kisspeptin

Introduction: Oxytocin (OT) has an important role in the regulation of smooth muscle contractility. Several neuropeptides are known which can activate the OT neurons in the central nervous system, such as kisspeptin. We hypothesize that kisspeptin can modify the myometrial contraction. Our aims were to clarify the myometrial effects of KISS1 58-65 and KISS1 94-121 and to determine their receptors in the pregnant rat uteri throughout gestation.

Methods: Contractions of uterine rings from non-pregnant and 22-day-pregnant rats were measured in an organ bath. The contractions were stimulated with 25 mM KCl and cumulative-dose response curves were elicited in the presence of KISS1 58-65 (10^{-12}–10^{-7} M) and KISS1 94-121 (10^{-12}–10^{-7} M). The contractility studies were also carried out in the presence of the kisspeptin antagonist Kisspeptin-234 trifluoroacetate (10^{-9} M) and after removing the endometrium. The KISS1 receptor expression was determined by RT-PCR and Western blot analysis.

Results: The KISS1 receptors were expressed both in the non-pregnant and pregnant uteri. The highest expression (P=0,017) was found on the 5th day of pregnancy, which refers to the effect of kisspeptin in implantation. The expression of the receptors was higher in the endometrium than in the myometrium. Both kisspeptin fragments caused myometrial relaxation in the non-pregnant and pregnant uteri (50%), however after removing the endometrium the relaxing effect of kisspeptin was ceased, as in the presence of kisspeptin antagonist.

Discussion: The expression of kisspeptin was decreased throughout gestation. Both kisspeptin fragments caused myometrial relaxation, which effect was decreased after removing the endometrium and in the presence of kisspeptin antagonist.

Conclusions: The effect of KISS1 58-65 fragment is endometrium-dependant while the effect of KISS1 94-121 fragment in the endometrium might be independent from the receptor.

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

Omega-3 polyunsaturated fatty acids in functional food may be protective for cardiovascular health

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Key words: polyunsaturated fatty acids, laser Doppler flowmetry, inflammation, microcirculation, hsCRP.

Introduction: Modern Western diet has been characterized by increased intake of saturated fats and omega-6 polyunsaturated fatty acids (PUFA) and low intake of omega-3 PUFA. This change in dietary habits has been accompanied with increased prevalence of cardiometabolic and other diseaseswith inflammatory processes underlying their pathophysiological mechanisms (such as inflammatory bowel disease, autoimmune diseases, obesity). Eicosanoids have an important role in mediating vascular reactivity to various stimuli in health and disease, thus changing tissue blood flow and tissue supply. Omega-3 PUFAs have protective role in cardiovascular system, supposedly due to the changes in eicosanoids production/metabolism. Thus, present study aimed to investigate the effects of omega-3 enriched eggs dietary intake on vascular function and blood inflammatory markers in healthy people.

Methods: Double-blinded prospective studywas performed in 36 volunteers of both sexes, divided in 2 groups-regular eggs (R) and omega-3 eggs (O). Functional vascular study (post-oclussion reactive hyperaemia (PORH) response in skin microcirculation) was performed by Laser Doppler Flowmetry, together with laboratory blood tests (lipidogram, hsCRP). Participants gave written informed consent. The Ethical Committee of Faculty of Medicine approved the study (# R2:19326-7/2016, Date:November 28, 2016).

Results: PORH was improved in O group in subjects of both sexes. hsCRP concentration was decreased, as well as triglycerides (TG) concentration in O group compared to R group, both sexes. TG and hsCRP was significantly improved only in female participants of O group.

Discussion: The results of present study suggest that omega-3 enriched diet has protective effect on cardiovascular function by reducing inflammatory markers and improving vascular function.

Conclusion: Consumption of functional food may be a promising natural venue in reducing low-grade inflammation presented in various cardiometabolic diseases. Hypothetical protective effect is altered metabolism of lipid precursors of proinflammatory or vasoactive metabolites important in vascular reactivity, together with change in activation of immunological system, which require further investigation.

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The Ethical Committee of Faculty of Medicine approved the study (# R2:19326-7/2016, Date:November 28, 2016).
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Insulin resistance in HFHSD induced prediabetic rats. The effect of metformin and liraglutide treatment

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Key words: obesity, diabetes, insulin resistance

Introduction: Recently published papers outlined the gender differences in insulin resistance and suggest a solid association between the metabolic status, insulin signaling and other endocrine mechanism. The oral antidiabetic drug, metformin, apart from improving insulin sensitivity also has beneficial influence on sexual hormone secretion and it is used in some reproductive disorders e.g. polycystic ovary syndrome. Liraglutide is a new antidiabetic drug exerting agonist property on GLP-1 receptor, besides improving glucose homeostasis has considerable effect on appetite and energy expenditure.

Objectives: In our present study, we aimed at examining the mechanism of peripheral insulin resistance in high-fat-high-sugar-diet (HFHSD) induced prediabetic state. The effects of antidiabetic treatments with metformin and liraglutide were also studied.

Method: Animal tissue samples were collected at University of Szeged a Cedars-RECOOP Research Center (CRRC). The insulin signaling assessed in diet induced obesity and prediabetes treated with metformin and liraglutide, and analyzed gender specifically. Plasma insulin level and IRS-1 phosphorylation in tissues were measured by ELISA.

Results: HFHSD led to hyperinsulinemia both in female and male groups; however considerable differences were observed on a case by case basis. The elevated insulin concentration was accompanied with normal fasting plasma glucose levels suggesting compensated insulin resistance and it is also revealed by HOMA-IR index. The antidiabetic treatment (metformin & liraglutide) had no significant effect either on insulin or glucose levels. Only with liraglutide a non-significant elevation of insulin levels was observed in females' plasma indicating further impairment of insulin sensitivity.
At molecular level in males, the HFHSD significantly reduced the phosphorylation of insulin receptor substrate 1 (IRS-1) on tyrosine residues indicating gender specific development of insulin resistance. Metformin and liraglutide treatments on the level of IRS-1 tyrosine phosphorylation improved insulin sensitivity without gender specificity.

Conclusion: HFHSD induced mild, compensated insulin resistance with remarkable interindividual variance. Male animals were found more susceptible for the development of diet induced impaired insulin sensitivity. Antidiabetic treatment showed similar efficacy for improving insulin resistance in both sexes.

Ethical approval: All experiments were approved by the Hungarian Ethical Committee for Animal Research (IV/3796/2015)

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Determination of Fatty Acids in Adipose Tissues by Gas Chromatography

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Key words: fatty acid composition, gas chromatography.

Introduction: Health risks associated with obesity is well recognised. In obesity, an abnormal or excessive fat accumulation in adipose tissue occur. The concentration of free fatty acids is increased in obesity and that this constitutes an important causal factor for the association between obesity and type 2 diabetes. The mechanisms by which free fatty acids (FFA) are involved in obesity is not well known.

Our aim was to investigate fatty acid changes in plasma, visceral and subcutaneous adipose tissue in Type 2 diabetes.

Methods A micro extraction method was developed to extract lipids from different tissues using two solvents, namely n-hexane and diethyl ether, consecutively.
Among separation techniques gas chromatography (GC) is a widely-adopted technique for qualitative and quantitative analysis of complex mixtures of fatty acids. To conduct analysis by GC, fatty acids must first be converted to more volatile non-polar derivatives. For this purpose, Fatty acids were converted to their corresponding fatty acid methyl esters (FAMEs) using alkylation reagent (potassium hydroxide dissolved in methanol). Flame ionization detection (FID) has been used for FAME determination. GC coupled with mass spectrometry (GC/MS) was applied to confirm identification of components.

Results: The saturated fatty acids are detectable in very low concentration in plasma and in fat tissue. Quantitative analysis of monounsaturated and the polyunsaturated fatty acids are feasible in blood and more difficult in fat tissue. Difficult to quantify the essential fatty acids in adipose tissue and in serum contains complex mixtures of fatty acids.

Discussion and Conclusion: Analysing fatty acid content of a biological sample is not easy because of its very low concentration. Further research is needed to optimize analytical method to improve sensitivity of fatty acid determination in adipose tissue samples.

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High fat high sugar diet and liraglutide treatment introduce sex specific changes of adipocytokine receptors in the rat brain while metformin has similar effect on both sexes

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Key words: obesity, diabetes, brain, rat, adipocytokines receptors

Introduction: Chronic overnutrition contributes to chronic conditions such as obesity, diabetes type 2 or neurodegeneration via disruption of hypothalamic pathways in charge of homeostasis maintenance. The aim of this study was to evaluate influence of diet rich in carbohydrates and fat (HFHS) and the influence of selected antidiabetic drugs on the brain of adult Sprague-Dawley rats.

Methods: Sixty four ($N_{♂}$=32, $N_{♀}$=32) Sprague-Dawley rats, 44 weeks old, were separated in 4 groups: 1. Standard diet (SD), 2. HFHS, 3. HFHS + metformin 4. HFHS + liraglutide. Obesity was induced in groups 2, 3, and 4 during first 5 weeks of experiment, followed with 15-week long metformin and liraglutide treatment of groups 3 and 4. The brains were collected and free-floating immunohistochemistry was performed using antibodies for following receptors: insulin (IR-α), leptin (ObR), insulin-like growth factor 1 (IGF-1Rβ) and kisspeptin (Kiss1R). Hippocampus (HIPP) and hypothalamus (HTH) were analysed.

Results: Hypothalamic regulation of satiety under HFHS diet is mostly regulated by upregulation of ObR and IGF in lateral hypothalamus (HTH-LH) of male rats. The same diet introduces more complex change in female hypothalamus: upregulation of Kiss1R in arcuatus (HTH-ARC), downregulation of IR-α and IGF-1Rβ in HTH-LH. Males responded to HFHS diet with upregulation of ObR in all hippocampal regions while female respond was mostly downregulation of all observed receptors. Metformin therapy had almost similar effect in both sexes; general upregulation of ObR and IR-α and no effect on Kiss1R. Liraglutide therapy resulted almost the same in males, with exception of downregulation of Kiss1R in hippocampus. Female response on liraglutide was downregulation of IR-α in satiety centers.

Discussion and Conclusion: HFHS diet itself has sex specific effect on expression of adipocytokine receptors in satiety centres. While metformin affect both sexes similary, liraglutide had more complex effect on female brain what is reflected in over-eating.

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Ethical Committee Approval: Hungarian Ethical Committee for Animal Research: registration number IV/3796/2015.

An up-date in diagnosis Gestational Diabetes Mellitus

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Key words: gestational diabetes mellitus, glycemia, pregnancy, macrosomia, birth.

Introduction: Nowadays, it is clear that identifying of gestational diabetes mellitus (GDM) overpasses the medical discussion of utility, both for the mother and child, both for the short term and long term possible complications.

Methods: Recently, the ACOG, after the recommendations of an independent panel at a National Institutes of Health consensus advocated against diagnosis of gestational diabetes mellitus (GDM) with the criteria recommended in 2008 by the International Association of Diabetes and Pregnancy Study Groups (IADPSG) and adopted by the American Diabetes Association, the World Health Organization, and organizations in other countries. The real reason for this decision is increased costs if GDM prevalence will reach the level of 18% of pregnant women worldwide and 17-25% of pregnant women in United States. The costs derivate from pregnancy care, delivery costs and follow up.

Results: The difference in incidence and costs are done mostly by the number of what is now called "mild GDM cases". The mild GDM was defined in a recent randomized single-blinded trial as a 2-hour, 75-g OGTT value between 140 and 198 mg/dL with a fasting plasma glucose value less than 140 mg/dL (mean, 86 mg/dL) – diagnostic criteria that, are lower than the IADPSG criteria. The results in this study revealed the value of treatment reporting a 66% reduction in "serious complications." In intervention group macrosomia was reduced by 50%, and preeclampsia by 30%. The cesarean rate was unchanged between the 1,000 women in the intervention and routine care groups.

Discussion and Conclusions: After GDM diagnosis, pregnant women need special medical care that includes medical nutritional therapy, counseling about physical activity, self-monitoring of blood glucose, obstetric care. Treating GDM improves outcomes, resulting in less preeclampsia, shoulder dystocia, and macrosomia. A recent systematic review shown reduced risks for perinatal mortality, neonatal intensive care admission and birth trauma in treated women with GDM, but the magnitude of these effects did not reach statistical significance. Medical nutrition therapy is the cornerstone of the medical management of women with GDM and has the following goals: to provide adequate nutrients for the maternal-fetal health and well-being, with appropriate weight gain during pregnancy, to ensure normoglycemia and prevent ketosis. The time of pregnancy represent a good opportunity to deliver nutrition and weight management interventions by authorized health professionals, as pregnant women are clearly more motivated and committed to make changes to improve diet, and in larger way, to improve lifestyle habits.
It is a fact that diagnosis of GDM can be done only in pregnancy and it is a problem of medical ethics to do this and to treat mostly by diet and life style changes.

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Molecular mechanisms of extra-mitochondrial production of H_2O_2 and its role in apoptosis induced by angucycline antibiotic landomycin E in leukemia cells

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Key words: ROS, landomycin E, apoptosis, NADPH-dehydrogenases, caspase-7.

Introduction: Landomycin E (LE) is a novel anticancer antibiotic possessing several unique properties comparing to structurally similar Doxorubicin that has the same quinone core. Among these properties are circumvention of cancer drug resistance and specific early induction of hydrogen peroxide in tumor cells. The aim of this study was to perform in-depth study of molecular mechanisms underlying this properties using modern *in vitro* and *in silico* assays.

Methods: Mitochondrial transmembrane potential ($\Delta\Psi m$) was determined semi-quantitatively by flow cytometry with JC-1 dye, while ROS production was measured by flow cytometry using DCFDA (H_2O_2-specific) and DHE (O_2^--specific) dyes. For identification of cellular sources of LE-induced ROS, various modulators of ROS-generating enzymes and specific scavengers of hydrogen peroxide, superoxide anions and hydroxyl radicals were used. Molecular docking studies of LE interaction with NQO1 and NQO2 enzymes were performed using QXP software and SDOCK+ algorithm. For identification of specific cell death pathways induced by the LE, Western-blot analysis on a panel of 15 proteins involved in apoptosis induction was performed.

Results: LE led to specific 3-fold burst of hydrogen peroxide production as early as 1 h after cell treatment, while only weak (20-30%) depolarization of mitochondria was observed at 6 h time point. There were no signs of superoxide anions under LE treatment, in contrast to Dx which caused late (12-24 h) induction of their production. Using extracellular and cell-permeable catalase revealed that LE-induced H_2O_2 burst occurs in close proximity to plasma membrane. *In silico* search for potential molecular targets of LE revealed its affinity to two NADPH-dehydrogenases NQO1 and NQO2. Specific inhibitors of these enzymes (dicoumarol and quercetin, respectively) revealed that LE is preferably targeting NQO1 in tumor cells. Western-blot analysis has shown that LE leads to early activation of effector caspase-7 at 3 h after drug treatment, while cleavage of initiator procaspase-9 involved in mitochondria-mediated apoptosis was observed much later at 12 h time point.

Discussion: We have shown that specific "curved" tetracyclic quinone core of LE is responsible for NQO1-mediated H_2O_2 burst, while planar tetracyclic core of Dx led to mitochondria-induced superoxide production. Since caspase-7 is associated predominantly with microsomal fraction and membranes, it can be directly activated by LE-induced H_2O_2 produced in close proximity to plasma membrane. Mitochondria seem to play a supplementary role in LE-induced cell death.

Conclusions: Rapid H_2O_2 generation and complex caspase activation contribute to the anti-leukemic effects of LE. Since superoxide generation is considered as the main cardiotoxic mechanism of Dx action, LE might be a better tolerable drug candidate for pre-clinical steps.

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New model of putative duality of presynaptic events for further implementation in artificial neural networks

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Key words: exocytosis; permanent glutamate turnover; non-pathological transporter-mediated glutamate release; brain nerve terminals, artificial neural networks

Introduction: Computational artificial neural network models are a base for development of artificial intelligence. Here, we proposed synaptic-based model for its further development in computational neuroscience and machine learning. Chemical synapse is the main structure in the brain responsible not only for nerve signal transmission, but also for its simultaneous regulation. Presynaptic nerve terminals are of considerable importance providing release of neurotransmitters.

Methods: preparative biochemistry, fluorescence and radiolabel assay.

Results: Analyzing transport of glutamate, the major excitatory neurotransmitter in the mammalian CNS, the authors suggest that there are two main relatively independent mechanisms at the presynaptic level, which can influence the extracellular glutamate concentration, and so signaling, and its regulation. The first one is well-known precisely regulated compound exocytosis of synaptic vesicles containing neurotransmitters stimulated by membrane depolarization, which increases significantly glutamate concentration in the synaptic cleft and initiates glutamate signaling through postsynaptic glutamate receptors. The second one is permanent glutamate turnover across the plasma membrane that occurs without stimulation, and is determined by simultaneous non-pathological transporter-mediated release of glutamate thermodynamically synchronized with uptake. Permanent glutamate turnover is responsible for maintenance of dynamic glutamatein/glutamateout gradient resulting in the establishment of flexible extracellular level of glutamate, which can be unique for each synapse because of dependence on individual presynaptic parameters. These two mechanisms, i.e. exocytosis and transporter-mediated glutamate turnover, are both precisely regulated, but do not directly interfere with each other, because they have different intracellular sources of glutamate in nerve terminals for release purposes, i.e. glutamate pool of synaptic vesicles and the cytoplasm, respectively.

Discussion and Conclusion: This duality can set up a presynaptic base for memory consolidation and storage, maintenance of neural circuits, long-term potentiation, and plasticity. It can be further implemented as a new model for artificial neural networks and intelligence.

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

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Koranyi Frigyes XXII Science Forum English Session

Simulated Ischemia and reperfusion test on cardiomyocytes differentiated from mouse embryonic stem cells and induced pluripotent stem cells

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Key words: Simulated Ischemia, cardiomyocytes, embryonic stem cell, induced pluripotent stem cell.

Introduction: Regenerative therapies have been expected to be a hopeful therapy for cardiovascular diseases by replacing the injured myocardium. In our study we utilized induced pluripotent stem cell (iPSc)-derived cardiomyocte. Oxidative stress was applied to enforce a better differentiation of iPSc into cardiomyocytes. We assessed the hypoxic sensitivity of those cardiomyocyte with ischemia/reperfusion (SI/R) test.

Methods: iPSc line (3.4) and a control mouse embryonic stem cell (mESc) (HM1) line were differentiated into cardiac tissues. Those cells were cultured in embryoid bodies (EBs) for three days and then plated on to a gelatinized surface for further differentiations. Oxidative stress was induced with 1 μM H_2O_2 treatment. We performed SI/R protocol on full EBs. We analysed one set of EBs with fluorescent viability assay, and another set of EBs with flow cytometric analysis to determine cardiac specific marker positive cell survival ratio.

Results: Control mES full EBs were slightly sensitive for SI/R at day-8, which was robust at day-16 (22%, 178% dead vs control level, respectively). The cardioprotective NO-donor could show only a tendency of protection of full EBs. VCAM positive groups were not different significantly. IPSc lines showed similar patterns to the control cell line.

Discussion and Conclusion: The cardiocytoprotective NO donor could not protect full EBs and cardiac marker positive cells against SI/R injury, suggesting that iPS-derived cardiac myocytes at the current development stage are not suitable for testing hypoxic sensitivity and cardiocytoprotective mechanisms.

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Simulated ischemia / reperfusion test of paced engineered heart tissue under chronic hyperglycemic condition

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Key words: Engineered heart tissue, chronic hyperglycemia, paced, diabetes mellitus, simulated ischemia

Introduction: Cardiovascular event is a serious complication of ever increasing incidence of type II diabetes mellitus. 3-dimensional in vitro engineered heart tissue (EHT) is suitable for disease modellings in a dish. We grew an EHT in a hyperglycemic condition and assessed its response to simulated ischemia/reperfusion (SI/R) injury. During the experiment the EHT was continuously paced by external stimulus.

Method: Fibrin-based mini EHTs were prepared from neonatal rat cardiac myocytes and cultured for 20 days. Hyperglycemic (HG) (25 mM glucose) and hyperosmotic (HO) (5 mM glucose + 19.5 mM mannitol) treatment was induced for a week. Then, EHTs were subjected to 120 min SI (93% N2 and 7% CO_2 gas flow + hypoxic solution) followed by 120 min reperfusion (40% O_2) + medium) and control normoxia. Continuous electrical stimulation was kept during SI/R (2p/ps, 4V). Beating rate and force of contraction were monitored during the entire experiment.

Result: Against continuous pacing during SI/R, ischemic EHTs stopped to beat during SI. The HG normoxic EHT showed significantly lower rate force product (beat/min x force) during reperfusion compared to HO ischemic EHTs, which difference was also observed in ischemic groups.

Discussion and Conclusion: The tolerance of hyperglycemic EHTs impaired, which was further worsen with induction of ischemia. The present EHT test system could be a useful tool to test cardioprotection in the presence of comorbidity.

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Engineered heart tissue as a potential comorbidity model: effects of simulated ischemia in the presence of chronic hyperglycemia

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Key words: engineered heart tissue, comorbidity, ischemia, hyperglycemia,

Introduction: Ischemic heart disease is still a major cause of mortality especially in diabetic patients. *In vitro* models of engineered heart tissue (EHT) has a potential to serve as comorbidity model and therapeutic tool for regenerative medicine.
Aim: To test the response of EHT to simulated ischemia/reperfusion (SI/R) injury in the presence of chronic hyperglycemic (HG) conditions.

Method: Neonatal rat cardiac myocytes are used for the preparation of EHTs. EHTs are cultured for 20 days and treated with hyperglycemic (HG) (25 mM glucose) and hyperosmotic (HO) (5 mM glucose + 19.5 mM mannitol) for a week. Then, EHTs were subjected to 120 min SI (93% N2 and 7% CO_2 gas flow + hypoxic solution) followed by 120 min reperfusion (40% O_2) + medium) and time-matched normoxia. Beating rate and force of contraction were monitored during the entire experiment.

Result: SI/R EHTs stopped to beat and, normoxic EHTs showed significantly lower beating activity. The HG normoxic EHT showed significantly lower rate force product (beat/min x force) during reperfusion compared to HO ischemic EHTs, which difference was not observed in ischemic groups.

Discussion and conclusion: Obtained results support that EHT can be used as comorbidity model for diabetic patients. The present *in vitro* system showed an impaired tolerance of EHTs against simulated ischemia/reperfusion in chronic hyperglycemic condition. The present EHT test system may be a useful tool to test cardioprotection in the presence of comorbidity.

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Detailed evaluation of suspected ganglion cell degeneration in the retina of Zucker Diabetic Fatty rats

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Key words: retinal ganglion cells, isodensity maps, type 2 DM, diabetic retinopathy, retina

Introduction: In clinical practice, examination of diabetic patients with Optical Coherence Tomography (OCT) provides one of the earliest potential markers of neuroretinal damage: a detectable reduction of the ganglion cell layer (GCL) thickness. The histological background underlying this is uncertain; in addition to ganglion cell (GC) loss, potential changes in the structure or thickness of the inner plexiform layer (IPL) have also been suspected. Several studies were conducted on animal models to reveal ganglion cell pathology; the results however were contradictory. Our aim was to develop a more sophisticated method and evaluate ganglion cell numbers, distribution patterns and IPL thickness in a model of type 2 diabetes mellitus.

Methods: GCs were labeled in retinal whole mounts of 32 week-old Zucker Diabetic Fatty (ZDF) rats by immunohistochemistry and were counted manually across the retina. Isodensity maps were constructed and analyzed using a custom-built algorithm (MATLAB, Fiji), capable of a regional comparison of the retinas. The number of apoptotic cells and thickness of the IPL were determined on cryosections. Comparison of the control (C) and diabetic (DM) groups was performed by permutation t-test (retinal maps), unpaired t-test (apoptotic cells) and two-way ANOVA test (IPL thickness).

Results: There was no change in staining characteristics of the antibodies and no significant difference in average GC densities was found (C: 1609.78±513.69 vs. DM: 1651±555.23 cells/mm2, mean±SD). The distribution patterns were also comparable in the two groups. Quantitative regional analysis revealed no consequent pattern confined to any region in diabetic specimens. In accord with this, no significant difference was found in IPL thickness at any of the measured locations or in the number of apoptotic cells in the GCL.

Discussion and Conclusion: In contrast to previous reports, our method provides a more detailed quantitative description of GCs and the IPL and excludes any major GC loss even after more than 20 weeks of untreated diabetes in ZDF rats. We recommend adopting this method in other animal models and human pathologic samples and also considering factors other than GC loss (eg. remodeling of dendrites in the IPL) as a potential explanation for GCL thinning on OCT examinations.

Acknowledgement: Grant support: OTKA PD #100245 (to T.R.). All procedures of the present study were approved by the local Ethical Committee for Animal Experimentation of the Semmelweis University and by the Animal Health and Animal Welfare Directorate of the National Food Chain Safety Office of the Hungarian State (number of approval: 22.1/1162/3/2010).

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The neurokinin-1 receptor contributes to the mediation of the fever response to bacterial endotoxin in mice

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Key words: Neurokinin-1 receptor, fever, systemic inflammation, cyclooxygenase-2

Introduction: Neurokinin-1 receptor (NK1R) and its ligand, substance P were shown to be involved in the mediation of systemic inflammation, but the linked mechanism has not been observed yet.

Methods: In adult NK1R knockout (KO) and wild type (WT) mice of both sexes we investigated the deep body temperature (Tb), autonomic thermoeffector and inflammatory biomarker (e.g. cyclooxygenase-2, COX-2, expression) responses to fever-inducing doses of bacterial endotoxin (or saline) infusion.

Results: During the fever response to endotoxin, the increase of Tb and oxygen consumption in NK1R KO mice was significantly attenuated compared to controls (38.1 ± 0.2 vs. 38.5 ± 0.2 °C and 173 ± 9 vs. 189 ± 6 ml/kg/min; $p < 0.05$). The blunted febrile response was accompanied by suppressed expression of peripheral COX-2 of the NK1R KO mice in response to LPS.

Discussion: The absence of the NK1R results in the attenuation of LPS-induced fever. There was a suppression of the febrile response already in the early phase of fever (starting from ~40 min post-LPS infusion). The LPS-induced amplification of the expression of the COX-2 protein was attenuated in the KO mice as compared with their WT littermates.

Conclusion: Our results demonstrate that the fever response of NK1R KO mice to bacterial endotoxin is attenuated, wich is, at least in part, the result of their reduced peripheral COX-2 expression.

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The effects of saccharin on energy balance in rodents

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Key words: saccharin, sweeteners, thermoregulation, oxygen consumption

Introduction: The incidence of obesity has been increasing, consequently artificial sweeteners are more frequently used to substitute the sweet taste of sugar. According to some researches, sweeteners don't play any role in the development of obesity, while others have found a strong correlation between increased food intake, thus obesity, and the use of artificial sweeteners. In our study, we aimed to explore the energetic changes of rats in response to saccharin.

Methods: In our experiments we used male Wistar rats, to exclude the thermoregulatory consequences of the oestrus cycle of the female rats. The animals were administered saccharin (or saline) intaperitoneally (IP) or into the brain and their core and skin temperature (indicator of heat loss) and oxygen consumption (indicator of metabolic rate) were recorded. *In vitro*, we examined the changes of c-Fos expression in nuclei playing a major role in thermoregulation.

Results: We found that IP administration of saccharin caused a significant decrease in the core temperature, which was brought about by suppression of their metabolic rate. *In vitro*, the c-Fos expression significantly decreased in the raphe pallidus and the medial preoptic area.

Discussion: Our findings suggest that saccharin administration results in a lower core temperature and metabolic rate due to suppression of neuronal activation in the raphe pallidus and the medial preoptic area.

Conclusion: Our results support the assumption that administration of saccharin can affect the regulation of energy balance by decreasing core temperature and metabolic rate, which can contribute to the development of obesity.

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Novel cardioprotective strategy against acute myocardial ischemia/reperfusion injury: the development of matrix metalloproteinase inhibitors

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Key words: MMP inhibition; acute myocardial I/R injury

Introduction: It has been demonstrated that acute activation of matrix metalloprotease-2 (MMP-2) during ischemia-reperfusion injury (IRI) contributes to myocardial dysfunction. Therefore, the inhibition of MMP-2 is supposed to be a potent tool to ameliorate cardiac IRI. Despite promising preclinical data, adverse events derived from non-selective and complete MMP inhibition have rendered clinical trials unsuccessful. Therefore, the aim of this study was to identify novel selective MMP-2 inhibitors against acute myocardial I/R injury.

Methods: MMP inhibitor compounds synthetized previously by our collaborators were subjected to a screening cascade. Forty-six selected compounds were used in gelatin zymography experiments to test the inhibitory efficacy on full-length MMP-2. Then, stimulated IRI on neonatal cardiomyocytes was performed to assess the cardioprotective effects of 7 compounds. Finally, based on the data from cell culture experiments, the compound with the highest cytoprotective effect, MMPI-1154, was tested in isolated rat hearts subjected to 30 min global ischemia and 120 min reperfusion to investigate its effect on myocardial infarct size.

Results: Seven compounds examined by gelatin zymography exhibited MMP-2 inhibition at 1 μM and 4 compounds less than 100% MMP-2 inhibition at 100 μM concentration. Six compounds increased cell viability significantly in response to stimulated I/R injury. In isolated hearts subjected to global IRI, MMPI-1154 significantly reduced infarct size at 1 μM as compared to the vehicle-treated hearts.

Discussion and Conclusion: MMPI-1154 can be a promising lead molecule for drug development since it exhibited high selectivity to MMP-2 and significant cardioprotection in preclinical models of myocardial IRI.

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The attitude of specialists and pregnant woman about physical activity during pregnancy

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Key words: pregnancy, exercise, habits, effects, health

Introduction: Studies have shown the beneficial effects of exercise during pregnancy, unfortunately there are many misconceptions about this topic.

Methods*:* Retrospective investigation among mothers-to-be, young mothers and gynaecologists. The participants had to answer to a questionnaire, where they had to choose the answers, according to their opinions. The test was sent to six hospitals in Budapest and one hospital in Miskolc (May 2015 – May 2016), where 255 women and 37 specialist received and filled them out.

Results: According to 94% of the specialists exercise is necessary during pregnancy as it improves the state of the mothers-to-be. Another 33% of those specialists who suggest exercise advise it the ACOG approved way. Placenta praevia is the main contradicting reason (53%), while noting numerous other excluding factors. However few factors were noted which are not excluding, but in those cases exercise is rather advised, like diabetes and obesity. 5% of the respondent said that there are no contradictions to exercise.
52% of the interviewed mothers-to-be said that they worked out before pregnancy; jogging, in most cases. During pregnancy this number decreased to 32% and yoga and special exercises for pregnant women were the dominant forms. 10,98% of the women who exercised did it 3-4 times a week for 15-30 minutes, which is the ACOG approved method. Among the women who exercised regularly while pregnant 51,85% delivered her child naturally and 48,15% had a C-section. The respondents who did no exercise during pregnancy 54,29% had natural delivery, while 45,71% had Caesarean, which contradicts the former researches. 93,48% of the non-sporting and 96,43% of the sporting women were able to get out of bed without help in 12 hours.

Discussion: Unfortunately just the 33% of specialists and 10,98% of women have known the correct way of doing exercise during pregnancy. Less activity can't exert efficiently its benneficial effects, more activity would be dangerous for the foetal developement, because the elevated blood pressure and the anaerobic metabolism may cause foetal hypoxia. In addition specialists haven't assessed the contraindication of exercise well, so they may provide incorrect advices to their patients. In terms of the parameters studied, exercise had no adverse effects during this period.

Conclusion: Although the used factors were undecisive if exercise during pregnancy had positive effects, there are no signs of it to be negative. As any exercise is beneficial for a pregnant woman, it is important that she receives accurate information, to show interests in exercising. Further researches are needed to provide these informations to specialists and women. A scientifically prooven training plan which is beneficial to mothers and their foetus facilitate to performe safely exercise during this period.

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Long-term success of ablation treatment of ventricular tachycardia with ischemic and non-ischemic origin

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Key words: ablation, ventricular tachycardia, arrhythmia, ischemia

Introduction: Catheter ablation (CA) of ventricular tachycardia (VT) is an effective treatment. One of the largest number of such cases in Hungary are performed at our Clinic. Depending on the medical history of coronary disease, ischemic and non-ischemic groups of VT can be distinguished. Our aim was to determine and compare the long-term success of VT ablation in the ischemic and non-ischemic groups.

Methods: In our retrospective study patients with VT (excluding VT with outflow tract morphology) undergoing CA in 2014-2015 were involved. Left ventricular activation and voltage mapping were performed, followed by extensive substrate ablation in the arrhythmogenic zone of VT. Medical history, ablation and follow-up data were collected for analysis. Two groups were distinguished: ischemic and non-ischemic groups. Definition of successful ablation was the lack of recurrence of >30 sec ventricular arrhythmia. Statistical significance level was set at $p<0.05$.

Results: 65 patients (49 men, mean age 69 years [range: 61-86], EF 34% [range: 27-43%]) were included. Median follow-up time was 13 months (range: 5-27 months). Arrhythmia was of ischemic origin in 66% of the patients. Baseline data between the two groups were not significantly different with the exception of age. One-year success rate of all patients, ischemic and non-ischemic patients were 70, 72 and 64%, respectively, with no significant difference between the two defined groups ($p=0.57$). There were 4 complications: 1 death due to late ventricular wall rupture, 2 thromboembolic and 1 punction site complication.

Discussion: One of the definitive treatments for VT – a potentially life-threatening arrhythmia – is CA. Substrate causing VT were usually identifiable with mapping technique during CA in both groups. Success rate and safety of extensive substrate ablation was comparable with literature data according to our results.

Conclusion: Our results suggest that CA is an effective and long-lasting treatment for both ischemic and non-ischemic VT.

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Three-year outcome of catheter ablation for atrial fibrillation in patients with reduced and preserved ejection fraction

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Key words: atrial fibrillation; catheter ablation; heart failure; ejection fraction; outcomes

Introduction: Increasing, however limited data exist regarding the long-term efficacy and impact of catheter ablation (CA) among patients (pts) with atrial fibrillation (AF) and reduced left ventricular (LV) function. We described and compared the three-year success rates of CA performed in our clinic in pts with reduced and preserved LV function.

Methods: We included pts with AF, who underwent CA in 2013-2014. Two groups were distinguished: pts with reduced (EF≤40%) and preserved (EF>40%) LV function. Baseline, procedure and three-year follow-up data were compared of the groups. CA was considered unsuccessful if more than 30 sec AF episode was detected during the follow-up.

Results: 34 (6%) pts had decreased EF, 555 (94%) maintained LV function. Similar age, sex, procedural data were found comparing the two group, while cardiovascular comorbidities, type of AF, CHA2DS2-VASc-score varied significantly. Three-year success rate year by year was 64, 47, 31% in the reduced EF group, and 72, 55, 45% in the preserved EF population respectively. No significant difference was found between those rates (p=0.25). Likewise, no significant difference was detected according to AF types. EF increased significantly in reduced EF group after CA (from median 33 to 41, p=0.004).

Discussion: Regarding the reduced LV function conservative therapy is often considered for AF, although guidelines recommend CA as well (IIB). While multicentre, prospective, randomized studies are still in process, success rates of CA highly varies in the literature. Our study provides an update on success rates of CA among persistent AF and reduced EF population as well, which is similar to other leading international studies. Additionally, we proved a significant increase of LV function after CA.

Conclusion: According to our study, we consider CA as an appropriate choice for symptomatic, antiarrhythmic-resistant pts with AF and reduced LV function.

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Nanomedicine & Drug Development

Next Generation Nanotherapeutics in Postgenomic Era for Combination Cancer Treatment and Imaging

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Key words: Nanoimaging and nanotherapy, metastatic tumor treatment, drug delivery, biobarriers, nanotoxicity.

Introduction: Differential diagnosis of brain magnetic resonance imaging (MRI) enhancement(s) remains difficult without invasive biopsies, especially in the brain. Such MRI enhancement(s) can result from metastasis of primary tumors, radiation necrosis, infections, or a primary brain tumor. Neurological symptoms are often the same on initial presentation. Different biobarriers make it difficult to reach certain tumors by most classical chemotherapeutic drugs rendering them largely inefficient. Brain tumors are a classical example due to the relatively impermeable blood brain barrier (BBB) and blood tumor barrier (BTB). To improve drug delivery and tumor targeting, nanopolymers appear to be highly promising. They can provide molecular combination therapy using one delivery system and thus, personalized therapy designed to treat individual tumors with specific marker expression profiles. Additionally, we developed a new class of nanoconjugates, nanoimaging agents (NIAs) and tumor-specific nanodrugs, based on a poly(β-L-malic acid) biodegradable polymer for precise noninvasive MRI diagnostics.

Experimental methods: We used a natural nanobiopolymer, polymalic acid (PMLA), as a nanoplatform for the family of PolycefinTM drugs to treat primary and metastatic tumors. We synthesized nanoconjugates carrying MRI contrast agent gadolinium-DOTA with novel "3-5-8 star-PEG molecules" and antibodies recognizing tumor-specific markers and crossing (BBB). Nanoconjugates were administrated intravenously in a newly developed double brain tumor xenogeneic mouse model bearing human EGFR/EGFRvIII- and HER2-positive tumors in different brain hemispheres. After treatment tumors were evaluated by immunohistochemistry and western blot analysis. Treatment efficacy was examined in *in vivo* experiments with mouse brain-inoculated human cell lines: EGFR-positive triple negative breast cancer (TNBC) MDA-MB-468, A529 lung cancer, and BT-474 HER2-positive breast cancer to mimic brain metastases. To treat brain tumors from HER2-positive breast cancers and TNBC, PMLA-based nanodrugs had covalently attached antisense oligos (AONs) against HER2 or EGFR; monoclonal antibodies (mAbs) to either EGFR (Cetuximab) or HER2 (Herceptin), and to transferrin receptor (TfR) for delivery through mouse endothelial system including BBB/BTB. Fluorescent dye Alexa Fluor 680 was attached for imaging.

Results: Imaging In the nanoMRI the signal in the targeted tumors was higher and remained longer than with a commercial MRI tracer MultiHance (Fig. 1). Treatment. Animal survival after Polycefin treatment of metastasis from lung, HER2+ breast cancer and TNBC was significantly higher than in untreated (PBS) or therapeutic mAb (Herceptin or Cetuximab) treated animals. Survival increases were as follows: 66% for lung cancer, 75% for HER2-positive breast cancer, and 103% for TNBC. Inhibition of tumor molecular targets in treated tumors was associated with reduced expression of key proliferation and stem cell markers, as well as with increased tumor cell apoptosis.

Discussion: After nano-MRI diagnosis, breast and lung cancer brain metastases were successfully treated with tumor-targeted nanoconjugates carrying AON to EGFR/EGFRvIII or HER2. Inhibitors were delivered to the tumor cells while sparing healthy brain tissue. Inhibition of tumor molecular targets in treated tumors was associated with reduced expression of key proliferation and stem cell markers, as well as with increased tumor cell apoptosis.

Conclusions: We developed versatile biodegradable and non-toxic diagnostic and treatment (theranostic) nanoconjugates based on naturally derived PMLA. A significant advantage of our novel nanosystem is precise delivery of imaging agents and specific mRNA suppressors (e.g., AON) to the tumor cells for efficient tumor MRI diagnosis and treatment.

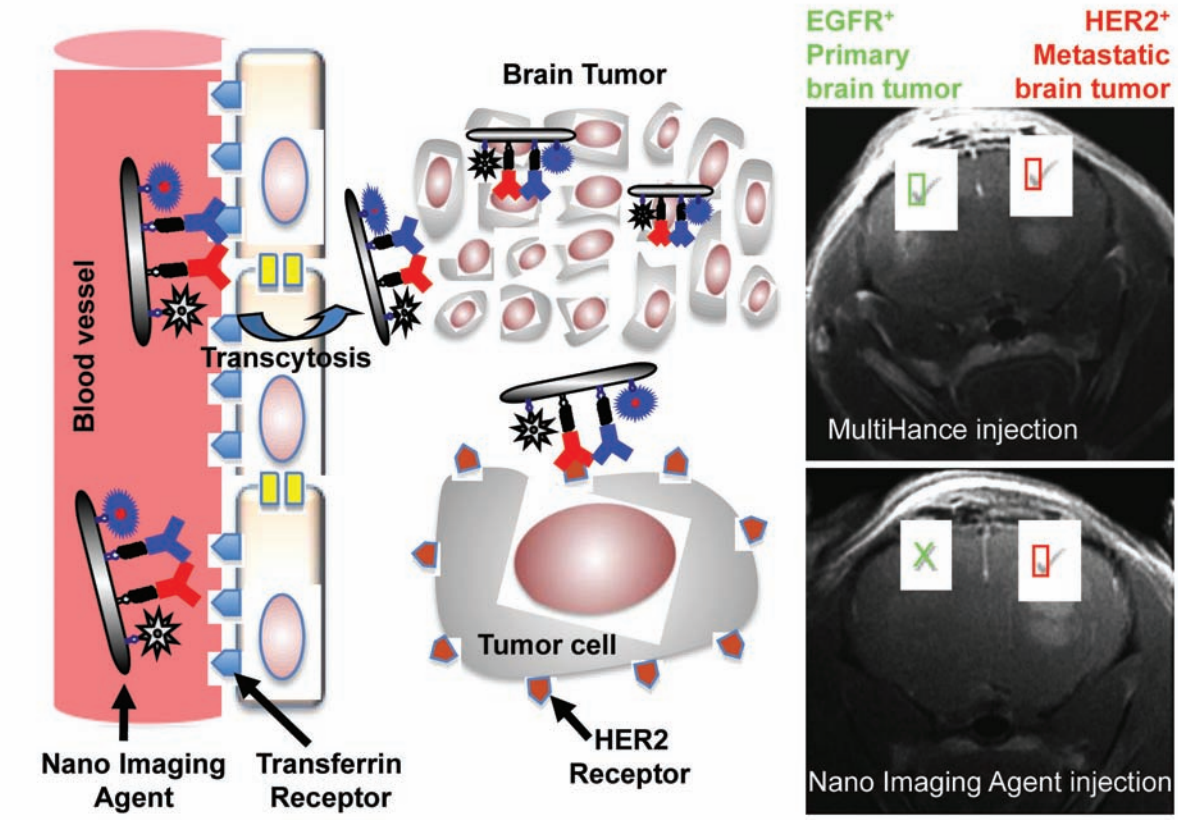

Figure 1. HER2- and EGFR-expressing brain tumors were differentiated by nanoMRI-targeted agents

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Institutional Animal Care and Use Committee approval: All animal experiments for brain and breast cancer treatment were performed according to the guidelines of the Institutional Animal Care and Use Committee (IACUC protocols # 5289 and #4658) at Cedars-Sinai Medical Center, Los Angeles, CA, USA.

Analysis of research activity at the Department of Regulation of Cell Proliferation and Apoptosis (Institute of Cell Biology, NAS of Ukraine): main directions, perspectives, and networking within RECOOP-HST

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Key words: nanomaterials, anticancer drugs, antioxidants, auto-immunity, cell signaling

Introduction: Main directions of research carried out at the Department in **2012-2016** are as following: 1st direction **"Nano-related research: novel materials for drug and gene delivery and for affinity bio-separations"**. Most important publications in 2012-2016 count **28**, total Impact Factor - **101.31**. 2nd direction **"Immunity, autoimmunity, cell signaling, and apoptosis"**. Most important publications in 2012-2016 count **26**, total Impact Factor - **116.73**. 3rd direction **"Cancer, novel drugs and antioxidants"**. Most important publications in 2012-2016 count **9**, total Impact Factor - **15.02**.

Results: Articles published in 2012-2016 in collaboration established with other Cedars RECOOP-HST Research Centers (CRRC):

1. Zasońska BA, Boiko N, Horák D, Klyuchivska O, Macková H, Benes MJ, Babic M, Trchová M, Hromádková J, Stoika R. The use of hydrophilic poly(N,N-dimethylacrylamide) for promoting engulfment of magnetic gamma-Fe2O3 nanoparticles by mammalian cells. *J Biomed Nanotechnol.* 2013; 9(3): 479-491. **IF = 5.256**.
2. Zasońska BA, Boiko N, Klyuchivska O, Trchová M, Petrovský E, Stoika R, Horák D. Silica-coated γ-Fe2O3 nanoparticles: Preparation and engulfment by mammalian macrophages. *J Nanopharmaceutics Drug Delivery.* 2013; 1: 182-192.
3. Grama S, Boiko N, Bilyy R, Klyuchivska O, Antonyuk V, Stoika R, Horak D. Novel fluorescent poly(glycidyl methacrylate) – silica microspheres. *Eur Polym J.* 2014; 56: 92-104. IF = **3.242**.
4. Kit Y, Starykovych M, Vajrychova M, Lenco J, Zastavna D, Stoika R. Detection of novel auto-antigens in patients with recurrent miscarrige: description of an approach and preliminary findings. *Croat Med J.* 2014; 55: 259-264. **IF = 1.4**.
5. Panchuk R, Skorokhyd N, Chumak V, Lehka L, Omelyanchik S, Gurinovich V, Moiseenok A, Heffeter P, Berger W, Stoika R. Specific antioxidant compounds differentially modulate cytotoxic activity of doxorubicin and cisplatin: in vitro and in vivo study. *Croat Med J.* 2014; 55(3): 206-217. **IF = 1.4**.
6. Dumych T, Lutsyk M, Banski M, Yashchenko A, Sojka B, Horbay R, Lutsyk A, Stoika R, Misiewicz J, Podhorodecki A, Bilyy R. Visualization of melanoma tumor with lectin-conjugated rare-earth doped fluoride nanocrystals. *Croat Med J.* 2014; 55(3): 186-194. **IF = 1.4**.
7. Podhorodecki A, Noculak A, Banski M, Sojka B, Zelazo A, Misiewicz J, Cichos J, Karbowiak M, Zasonska B, Horak D, Sikora B, Elbaum D, Dumych T, Bilyy R, Szewczyk M. Lanthanides fluorides doped nanocrystals for biomedical applications. *ECS Transactions.* 2014; 61: 115-125. **IF = 0.54**.
8. Tomin A, Dumych T, Tolstyak Y, Kril I, Mahorivska I, Bila E, Stoika R, Herrmann M, Kit Y, Bilyy R. Desialylation of dying cells with catalytically active antibodies possessing sialidase activity facilitates their clearance by human macrophages. *Clin Exp Immunol.* 2015; 179: 17-23. **IF = 3.278**.
9. Kit Y, Bilyy R, Korniy N, Tomin A, Chop'yak V, Tolstyak Y, Antonyuk V, Stoika R. Two-step chromatography purification of IgGs possessing sialidase activity from human blood serum. Biomed Chromatogr. 2015; 29(3): 328-332. **IF = 1.723**.
10. Horak D, Plichta Z, Starykovych M, Myronovskij S, Kit Y, Chopyak V, Stoika R. Calf thymus histone-conjugated magnetic poly(2-oxoethyl methacrylate) microspheres for affinity isolation of anti-histone IgGs from the blood serum of patients with systemic lupus erythematosus. *RCS Advances.* 2015; 5: 63050-63055. **IF = 3.84**.

11. Antonyuk V, Grama S, Plichta Z, Magorivska I, Horak D, Stoika R. Use of specific polysaccharide-immobilized monodisperse poly(glycidyl methacrylate) core-silica shell microspheres for affinity purification of lectins. *Biomed Chromatography.* 2015; 29(5): 783-787. **IF = 1.662**.
12. Bilyy R, Podhorodecki A. Can we use rare-earth nanocrystals to target glycans for the visualization of melanoma? *Nanomedicine (Lond).* 2015; 10(13): 1997-2000. **IF = 5.413**.
13. Senkiv J, Finiuk N, Kaminskyy D, Havrylyuk D, Wojtyra M, Kril I, Gzella A, Stoika R, Lesyk R. 5-Ene-4-thiazolidinones induce apoptosis in mammalian leukemia cells. *Eur J Med Chem.* 2016; 117: 33-46. **IF = 3.902**.
14. Myronovkij S, Negrych N, Nehrych T, Tkachenko V, Souchelnytskyi S, Stoika R, Kit Yu. Identification of Ser-Pro-Cys peptide in blood serum of multiple sclerosis patients. *Protein Pept Lett.* 2016; 23(9): 808-811. **IF = 1.069**.
15. Panchuk RR, Skorokhyd NR, Kozak YuS, Lehka LV, Chumak VV, Omelyanchik SN, Gurinovich VA, Moiseenok AG, Stoika RS. Antioxidants selenomethionine and D-pantethine decrease negative side effects of doxorubicin in NK/Ly lymphoma-bearing mice. *Croat Med J.* 2016; 57(2): 180-192. **IF = 1.483**.
16. Kobylinska LI, Boiko NM, Panchuk RR, Grytsyna II, Klyuchivska OYu, Biletska LP, Lesyk RB, Zimenkovsky BZ, Stoika RS. Putative anticancer potential of novel 4-thiazolidone derivatives: cytotoxicity toward rat glioma C6 in vitro and correlation of general toxicity with the balance of free radical oxidation in rats. *Croat Med J.* 2016; 57(2): 151-163. **IF = 1.483**.
17. Tomin A, Dumych T, Kril I, Antonyuk V, Chopyak V, Munoz L, Stoika R, Herrmann M, Bilyy R. Magnetic separation of apoptotic cells with lectin-conjugated microparticles (Magnetische abtrennung apoptotischer zellen mit lektin-konjugierten mikropartikeln. *Materialwiss Werkstofftech.* 2016; 47(2-3): 189-192. **IF = 0.425**.
18. Myronovkij S, Negrych N, Nehrych T, Redowicz MJ, Souchelnytskyi S, Stoika R, Kit Yu. Identification of a 48kDa form of unconventional myosin 1c in blood serum of patients with autoimmune diseases. *Biochem Biophys Reports.* 2016; 5: 175-179.
19. Negrych N, Myronovskij S, Nehrych T, Stoika R, Kit Yu. Identification of the unique properties of IgGs and their heavy chains in blood serum of multiple sclerosis patients. *J Autoimmune Disorders.* 2016; 2(2): 1-5.
20. Chapter in monograph: B.A. Zasonska, V. Patsula, R. Stoika, D. Horák Surface-modified magnetic nanoparticles for cell labeling // "The Chemistry and Physics of Engineering Materials" Editors:A. A. Berlin, R. Joswik, N. I. Vatin, / Modern Analytical Methodologies. Apple Academic Press, NJ, USA. – 2015; V. 1, Chapter 10: 275-288.

Conclusions:

1. Number and Impact Factor of Publications of the co-workers of the Department in the peer-reviewed international journals demonstrate great potentials for collaboration in three main directions of research: 1) Nano-BioTech; 2) Immunity-Autoimmunity; 3) Cancer.
2. During 2012-2016 comparing to former 5 years, there was a shift in research activity at the Department from Cancer research to the NanoBioTech and Immunity-Autoimmunity research.
3. In future, research activity at the Department in the NanoBioTech direction will be somewhat decreased to give more opportunities to basic research. A significant decrease in the Immunity-Autoimmunity research is expected due to leaving of the Department by several principal investigators. An increase in the ratio of Cancer research at the Department is expected.
4. Potentials of the Department in the NanoBioTech research within RECOOP-HST Association were realized mostly via CRRC Institute of Macromolecular Chemistry in Prague and Lviv National Medical University. Beside the growing opportunities for collaboration in cancer research with CRRC Lviv National Medical University, ICB is collaborating with non-RECOOP organizations: Ivan Franko Lviv National University and Lviv National Polytechnic University (both in Ukraine) and the Institute of BioOrganic Chemistry in Belarus.

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

Antitumor effect of 4-thiazolidinone derivatives at treatment of mice with NK/Ly lymphoma

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Key words: 4-thiazolidinone, murine NK/Ly lymphoma treatment

Introduction: Recently, we demonstrated that novel 4-thiazolidinone derivatives (ID3288, ID3833, ID3882) were capable of inducing apoptosis *in vitro* in rat C6 glioma and human glioblastoma U251 cells [1]. Therefore, we continued the investigation of the antitumor effects *in vivo*, in NK/Ly lymphoma-bearing mice treated with the same derivatives.

Methods: Studied compounds were synthesized at LNMU [2]. Healthy BALB/C mice were inoculated intraperitoneally with murine NK/Ly lymphoma cells. Anticancer drugs were injected next day after tumor inoculation and thereafter every 2nd day for 10 days. Doxorubicin (Dox) was used in 1 mg/kg of body weight dose for comparison with ID3833 - 2.5 mg/kg, ID3288 and ID3882 - 5 mg/kg. Lymphoma development was controlled by measuring the volume of ascite (VA). The increased intraperitoneal fluid was produced as the consequence of the injected lymphoma cells. Number of different cells in mice blood, as well as the activity of aminotransferases were monitored at 14th and 21th days of experiment.

Results: Dox and ID3833 were the most effective in treatment of NK/Ly lymphoma-bearing mice, while ID3288 demonstrated slightly weaker effect, and ID3882 did not show any antitumor action. The untreated tumor-bearing mice had the VA 150% comparing to the Dox-treated having VA 100%, the ID3833-treated – VA 105%, the ID3288-treated – VA 115%, and to the ID3882-treated – VA 140%. After 14 treatment days, the activity of aminotransferases in mice blood was increased, but after 21 days it returned to normal level. In the Dox-treated tumor bearing mice, the number of red blood cells was strongly reduced after 21 days, contrary in mice treated with the studied derivatives where the counts did not change significantly. At the same time, the applied treatment normalized the increased number of blood neutrophils. Dox increased the number of lymphocytes, and the studied derivatives did not.

Discussion: The 4-thiazolidinone derivatives extended a lifespan of the mice with NK/Ly lymphoma in the order: ID3882<ID3288<ID3833≈Dox. These results of anticancer activities are like the pattern found in the *in vitro* study of rat and human glioma cells [1]. Investigated compounds also possessed much lower general toxicity in rats comparing to Dox [3].

Conclusions: Mice treated with ID3833 or Dox stayed alive with a reduction of lymphoma for more 60 days after tumor inoculation. Therefore, the studied drugs have the anticancer capacity to treat animals with lymphoma.

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Bio-Ethics Committee Approval: Experimental procedures using laboratory animals were approved by the Ethical Committee of Danylo Halytsky LNMU, Protocol N4, 18.04.2016.

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Glutamate transport in presynaptic rat brain nerve terminals in the presence of *O*-methyl-β-cyclodextrin-modified magnetic nanoparticles

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Key words: glutamate; cholesterol; O-methyl-β-cyclodextrin; maghemite nanoparticles; nerve terminals.

Introduction Unspecific regulation of glutamate transport in the nerve terminals can be achieved by modulation of cholesterol content in their plasma membrane.

Methods chemical synthesis, preparative biochemistry, fluorescence and radiolabel assay.

Results Lowering of the cholesterol concentration in isolated presynaptic rat brain nerve terminals (synaptosomes) using cholesterol-depleting agents decreases the rate of uptake and increases the extracellular level of glutamate. Extraction of cholesterol from the plasma membrane and its further removal from the nerve terminals by an external magnetic field can be achieved by means of magnetic nanoparticles with immobilized cholesterol-depleting agent such as O-methyl-β-cyclodextrin (MCD). A simple approach is developed for preparation of maghemite (γ-Fe_2O_3) nanoparticles containing chemically bonded MCD. The method is based on preparation of a silanization agent containing MCD. MCD-modified γ-Fe_2O_3 nanoparticles reduce the initial rate of synaptosomal uptake and accumulation of L-[^{14}C]glutamate and increase the extracellular L-[^{14}C] glutamate level in the preparations of nerve terminals. The effect of the MCD-γ-Fe_2O_3 particles is the same as that of MCD per se (the ability to influence the uptake and ambient level of L-[^{14}C]glutamate in synaptosome preparations).

Discussion Magnetic manipulation of the MCD-γ-Fe_2O_3 particles enables removal of bonded cholesterol. Because of this unexpected fact, we met difficulties in the interpretation of the results obtained.

Conclusion Neuroactive properties of γ-Fe_2O_3 and MCD-γ-Fe_2O_3 nanoparticles can be used to advantage in neurotheranostics.

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Multilayered magnetic nanoparticles with phenolic antioxidants on the surface

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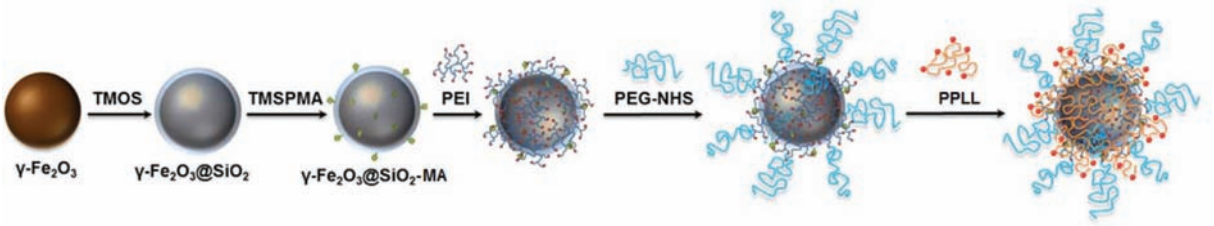
Key words: magnetic nanoparticles; oxidative stress; polyphenols

Introduction: Iron oxide nanoparticles have found applications as cell labeling probes, contrast agents for magnetic resonance imaging, nanoheaters for hyperthermia, carriers in controlled drug delivery systems, etc. The surface of the particles has to be modified (typically with SiO_2 and PEG) to render them with colloidal stability, non-toxicity, and reactive functional groups.



Methods: Maghemite (γ-Fe_2O_3) nanoparticles were prepared by aqueous precipitation of Fe salts and oxidation. They were coated with tetramethyl orthosilicate (TMOS) to create γ-Fe_2O_3@SiO_2 and subsequently reacted with 3-(trimethoxysilyl)propyl methacrylate (TMSPMA) to yield γ-Fe_2O_3@SiO_2-MA nanoparticles. The particles were then grafted with poly(ethyleneimine) (PEI), N-hydroxysuccinimide-terminated poly(ethylene glycol) (PEG-NHS), and finally with phenol-modified poly(L-lysine) (PPLL) (see the Figure). Phenolic compounds included phenol, phloroglucinol, and chlorogenic acid. The resulting products were characterized by elemental analysis, transmission electron microscopy, dynamic light scattering, and FTIR spectroscopy. Radical scavenging activity of the particles was measured by 2,2-diphenyl-1-picrylhydrazyl assay.

Results: The average diameter of the starting γ-Fe_2O_3 particles was 14 nm. After modification with SiO_2 and coating with the polymers, the diameter increased up to 17 nm; particle size distribution was moderately broad. The particles formed a stable aqueous colloid due to their high surface ζ-potential and steric stabilization. PPLL-modified particles proved to be an efficient antioxidant exhibiting promising results as a radical scavenger.

Discussion and Conclusions: Colloidally stable polymer-coated maghemite nanoparticles with phenolic antioxidants on the surface were developed. The particles had a reduction in undesirable side-effects induced by reactive oxygen species produced during anticancer drug treatment. Moreover, the particles could be easily separated using a magnetic field. Finally, attached poly(L-lysine) served as a transfection agent increasing the cellular uptake of the particles.

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Optical and structural properties of nanoparticles

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Key words: nanocrystals, quantum dots, imaging, near infrared

Introduction: In recent years, the study of nanoparticles (NPs) in both fundamental and technological research has attracted considerable attention due to their potential applications in various fields, like electronics, photovoltaics or medicine. Especially nanocrystals doped with lanthanides (RE-NCs) and quantum dots (QDs) are promising candidates for multimodal (optical and magnetic) imaging. However, in order to apply NPs in biomedicine, the synthesis methods have to enable the control of size and shape.

Methods: In order to obtain high-quality NPs, co-precipitation and co-thermolysis methods were applied. As the precursors acetates, trifluoroacetates and oleates were used.

Results: Flower-shaped NaGdF4:Yb,Er RE-NCs were obtained by an adjustment of fluorine-to-lanthanides molar ratio. When the concentration of NH4F solution was reduced more complex structures were obtained. In order to obtain different sized CdS and CdSe NCs various molar ratios of precursors were applied. Also shapes of such NPs were tuned using various ligands. Unfortunately, all of NPs after the synthesis were suspended in non-polar solvents, which prevents their direct use in biomedicine. However, it was possible to transfer them into the water, using simple ligand exchange protocol.

Discussion: Emission of as-obtained lanthanide-doped NCs and QDs can be tune in visible (NaGdF4:Yb,Er (Tm)/Eu, CdS, CdSe) and in NIR range (PbS) and their excitation wavelength is aligned with the optical window of tissues. What is more such RE-NCs are characterized by lack of the bleaching and blinking.

Conclusions: Fluoride $NaGdF_4$ NCs and CdS, CdSe, PbS QDs were synthesized and transfer into the water in order to apply them in the cells. Cytotoxicity tests showed that toxicity of these materials is on an acceptable level. Presented NPs have been already applied in optical imaging (HeLa cells, melanoma tumor) and in magnetic resonance imaging (MRI).

This work was supported by the grants from the National Science Centre, Sonata Bis 3 Project No. UMO-2013/10/E/ST5/00651.

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Optical imaging in near infrared spectral range with use of colloidal quantum dots

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Key words: microscopy, quantum dots, imaging, near infrared

Introduction: One of the key areas merging physics and biology is optical imaging. This includes designing new optical probes as well new imaging systems. Good candidates for such markers are colloidal quantum dots, which enable tuning of the emission spectra with the size of the quantum dot. For the biological cells imaging, the best emission range should be within the biological optical window (around 800-1300 nm), within which the absorption rates of water, melanin, hemoglobin and epidermis are the lowest.

Methods: In our work, we designed the hydrophilic PbS/CdS core-shell colloidal quantum dots with narrow emission band (FWHM < 100 nm) which peak position can be tuned in the spectral range of 850-1600 nm. These quantum dots were incubated with the mouse endothelium and macrophage cells for about 2.5 h.

Results: To fulfill these demands we build wide-field microscope with the 532 nm laser line excitation and emission signal detection from 300 up to 1600 nm. In addition, to verify if the recorded emission corresponds to QDs not to scattered light or to other artifacts, the system was equipped with the monochromator what enables online spectral detection of recorded images.

Discussion: We tried to image the probes in the biological systems in a short time, in a large scale, in 3D, with the highest possible spatial resolution, recording images with the highest frequency (dynamic imaging) and the best in NIR spectral range. The last condition helps significantly to reduce the light scattering, autofluoroscence and to increase the light penetration depth what is the most important for *in vivo* imaging.

Conclusion: In our work we will present the results of optical imaging in NIR spectral range of mouse endothelium and macrophage cells which were incubated with hydrophilic PbS/CdS core-shell colloidal quantum dots for about 2.5 h.

This work was supported by the grants from the National Science Centre, Sonata Bis 3 Project No. UMO-2013/10/E/ST5/00651.

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

Enhancement of cytotoxic activity of doxorubicin conjugated to polymer-coated γ-Fe_2O_3 nanoparticles towards tumor cells of different origin

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Key words: polymer-coated γ-Fe2O3 nanoparticles, doxorubicin, tumor cells.

Introduction: Doxorubicin is the most commonly used anticancer drugs in clinics. However, its main shortcoming are cardio-, hepato- and nephrotoxicity towards organism of cancer patient. Thus, novel approaches should be developed to decrease side effects of Dx and enhance its anticancer action. Thus, the aim of this study was to evaluate cytotoxic activity of novel drug delivery system based on doxorubicin-bound poly[*N*-(2-hydroxypropyl) methacrylamide-*co*-methyl 2-(*N*-methylmethacrylamido)acetate]-coated maghemite nanoparticles (γ-Fe_2O_3@PHPMA-DOX) towards various tumor cell lines *in vitro.*

Methods: Human leukemia cell lines of different lines (Jurkat, HL-60/wt, HL-60/vinc, K562) were treated with γ-Fe_2O_3@PHPMA-DOX nanoparticles and their cytotoxic activity was compared to free doxorubicin. Short-term (24 h) cytotoxic effect of anticancer drug was studied under the Evolution 300 Trino microscope (Delta Optical, Mińsk Mazowiecki, Poland) after cell staining with Trypan blue (0.1%). For measurement of proapoptotic activity of these nanoparticles, fluorescent microscopy (DAPI staining) and flow cytometry (annexin V/PI staining) were used. All experiments were repeated 3 times.

Results: Coating of PHPMA-DOX on γ-Fe_2O_3 nanoparticles led to enhancement of their cytotoxic activity by 15-20% compared to free DOX. This phenomenon was observed both for sensitive and drug-resistant tumor cell lines (overexpressing P-glycoprotein and MRP-1) used in the study. Annexin V/PI staining has shown that such enhancement of cytotoxicity of novel γ-Fe_2O_3@PHPMA-DOX nanoparticlesis explained by increased proapoptotic activities of these particles (by 15% compared to free DOX). These data were also confirmed by cytomorphological studies of chromatine hypercondensation in murine B16 melanoma cells (DAPI staining), showing that complexes of DOX with the nanoparticles led to a more pronounced apoptosis compared to free drug. We suggest that higher proapoptotic activity of these nanoparticles allows them to eliminate both sensitive and drug-resistant tumor cells *in vitro* with the same efficiency.

Discussion and Conclusion: Cytotoxic action of doxorubicin-conjugated PHPMA-coated γ-Fe_2O_3 nanoparticles was found to be 15-20% higher compared to doxorubicin alone. The particles have advantage of magnetic manipulability and targeting. The obtained data indicate necessity of further *in vivo* studies of novel DOX-nanoparticle conjugates on experimental tumor models in mice.

Acknowledgement: Support of the RECOOP HST Association and the Cedars-Sinai Medical Center is acknowledged. This study included only *in vitro* experiments on tumor cell lines, thus was no need in approval from Ethics Committee of ICB.

Novel antitumor anthracyclines from natural products

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Key words: cancer, *anthracycline* antibiotics, cytotoxicity

Introduction: *Anthracycline* antibiotics remain an important class of antitumor agents. Here we report the production of novel compounds by techniques of gene cloning and gene mining and study of their cytotoxic activity against tested cancer cell lines.

Methods: PCR was performed using *Taq* DNA polymerase and primer pairs specific to *snogI* gene. Restriction endonucleases, Klenow fragment, alkaline phosphatase, T4 DNA ligase, *Taq* polymerase were purchased from standard commercial sources (MBI Fermentas) and used according manufacturer's instructions. Identification of strains was based on 16s rDNA sequence analysis. The structures of antibiotics were elucidated based on HR-ESIMS and NMR data. Human HCT-116 colon carcinoma cells, human embryonic kidney HEK293T, and human cervix carcinoma KB-3.1 were treated with rubimycinone A to assess acute cytotoxicity.

Results: Plasmid-mediated recombination approach was developed to conduct gene disruption in *Streptomyces nogalater*. Recombinant strain, NaI1, was constructed by disruption of a putative aminotransferase (*snogI*) gene. Phenotype analysis of the mutant strain showed that novel compound was produced. NaI1 strain possessed strong antibacterial and cytotoxic activity against tested bacteria and cancer cell lines. Chemical analysis of a terrestrial actinomycete (Lv-6-8) isolated from the root zone of *Yucca aloiofolia* yielded a new anthraquinone possessing a 3-furanone ring system, rubimycinone A. Rubimycinone A displayed moderate to good antibacterial and cytotoxic activity against a panel of bacteria and cancer cell lines. The IC50 values against HCT-116, HEK293T, and KB-3.1 were 0.76, 0.16, and 1.14 μg/mL, respectively.

Discussion and Conclusion: Knockouts of *sno*-genes provide the opportunity of a new approach to modified *anthracyclines* by gene manipulations. In summary, novel antibiotics exhibited moderate to good effects on cancer lines. Rubimycinone A represents the first example of a natural product with a 3-furanone ring attached to an anthraquinone ring system.

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Institutional Animal Care and Use Committee Approval – protocol № 6, 20.06.2016.

Impact of izatine-pyrazoline-thiazolidinone compound ID-3833 towards human tumor cells *in vitro*

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Key words: thiazolidinone, tumor, apoptosis

Introduction: 4-tiazolidinones are heterocyclic sulfur- and nitrogen-containing compounds possessing anti-tuberculosis, anti-inflammatory, immunomodulatory, and antitumor activities. The main goal of current study was to elucidate death signaling pathways involved in toxic activity of izatine-pyrazoline-thiazolidinone compound ID-3833 towards tumor cells *in vitro.*

Methods: Cytotoxic activity of ID-3833 was measured by Trypan Blue exclusion assay. Mitochondrial transmembrane potential (ΔΨm) was determined semi-quantitatively by flow cytometry with JC-1 dye (5.5'-tetrachloro-1.1',3.3'-tetraethylbenzimidazolocarbocyanine iodide). Induction of apoptosis (chromatin hyper-condensation) was studied in drug-treated cells after their DAPI staining. Western-blot analysis was used for evaluation of expression of proteins involved in death signaling pathways in Jurkat T-cells

Results: LC_{50} of ID-3833 ranged from 1.1 to 4.8 μM depending on used cell line. DAPI staining of human breast adenocarcinoma cells of MCF-7 line treated with 2 μM of ID-3833 confirmed induction of apoptosis in malignant cells. In 24 h of cells incubation with studied compound the % of depolarized mitochondria increased from 8.34 (control) to 14.64%. Western-blot analysis revealed that ID-3833 caused an activation of the initiator caspase-2, -8, -9 at 12 h after drug treatment and rapid processing of the effector caspase-3,-6,-7, as well as cleavage of their substrate DFF-45.

Discussion: We have shown that ID-3833 possessed a significant anti-neoplastic action towards leukemia and carcinoma cells, and induced apoptosis in these cells. Since cell treatment with ID-3833 did not result in significant increasing of depolarized mitochondria and led to cleavage of both pro-caspase-2 and -9, we suggest that its anti-neoplastic action is implemented by a mixed-type apoptosis.

Conclusion: ID-3833 is a potent anti-tumor drug for tumor cells of different origin. The value of its average LC_{50} is similar or even higher than such value of Doxorubicin ("gold chemotherapy" standard). Further investigations of molecular targets of ID-3833 action in tumor cells are in progress.

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Neurodegenerative Diseases

Dostoyevsky's illnesses: neurological aspects

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Key words: insular cortex, ecstatic seizure, Dostoyevsky's illness

Introduction: Re-evaluation of famous individuals' illnesses in the light of recent neurobiological research data has been a great challenge for medical pathobiographists. The retrospective diagnosis of Fyodor Mikhailovich Dostoyevsky's (1821–1881) neurological and psychiatric disease proves to be particularly interesting. While the Russian writer's retrospective diagnosis of epilepsy is well known, the gambling disorder accompanying him throughout his life is not as widely known.

Methods: Literary and scientific overview (1928–2015) on the subjects of Dostoyevsky's epilepsy and gambling disorder.

Results: Although the retrospective diagnosis regarding the type of the writer's epilepsy has been raising serious arguments among epileptologists since the 1960s, recent neurobiological data suggest a solution to these questions claiming the insular cortex to be the origin of the seizures. Regarding Dostoyevsky's pathological gambling, this hypothesis is consistent with another finding from recent neuroscience, namely that the malfunction of the insula could be an important underlying pathology in gambling disorder.

Discussion and Conclusion: Considering Dostoyevsky's neurological (ecstatic seizures) and psychiatric (pathological gambling) disease and the crossroads, these two disciplines make regarding the underlying pathology, we would like to suggest a speculative theory that these two disorders have a common insular pathomechanism, namely, the malfunctioning of the risk prediction–risk prediction error coding system. Furthermore, based on Dostoyevsky's case, regarding gambling disorder in general, we would like to hypothesize that the three common gambling-related cognitive distortions (near-miss effect, gambler's fallacy, and the illusion of control) can be all attributed to the impairment of the anterior insular risk prediction–risk prediction error coding system.

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Acknowledgements: Due to the nature of our study, no ethical approval was needed.
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Neuromodulatory effects of detonation nanodiamonds

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Key words: detonation nanodiamonds; glutamate; γ-aminobutyric acid; exocytosis; brain nerve terminals

Introduction: Nanodiamonds are one of the most perspective nano-sized particles with superb physical and chemical properties. Nanodiamonds consisted of a specific carbon complex, the core of which is sp^3 hybridization carbon (diamond), and a surface layer composed of sp^2 hybridization carbon (non-diamond carbon) firmly connected with the core. The non-diamond carbon content is substantially reduced due to chemical treatment. The size of nanodiamond particles vary from 10-20 nm to 10 μm. There are carbonyl, hydroxyl and carboxyl functional groups on the surface of the particles. Physical-chemical properties such as a magnetic susceptibility and the amount of incombustible residue in samples of detonation nanodiamond vary depending on the synthesis regime and the method of chemical cleaning of the product and therefore, the neuroactive properties of nanodiamonds from different batches can be different. We recently demonstrated neuromodulatory properties of carbon nanodots with other than nanodiamonds hybridization types, i.e., sp^2 hybridized graphene islands and diamond-like sp^3 hybridized elements.

Methods: preparative biochemistry, fluorescence and radiolabel assay.

Results: Here, neuromodulatory properties of uncoated nanodiamonds from detonation synthesis were analyzed in brain nerve terminals based on the effects on the uptake and ambient level of glutamate and γ-aminobutyric acid (GABA), the major excitatory and inhibitory neurotransmitters, respectively. Nanodiamonds in a dose-dependent manner attenuated the initial velocity of Na^+-dependent transporter-mediated uptake and accumulation of L-[^{14}C]glutamate and [^{3}H]GABA by nerve terminals and increased the ambient level of these neurotransmitters. Also, nanodiamonds caused a weak reduction in acidification of synaptic vesicles and depolarization of the plasma membrane of nerve terminals.

Discussion and Conclusion: Therefore, nanodiamonds and carbon dots, despite different types of hybridization, exhibit very similar effects on transport of glutamate and GABA in nerve terminals. This common features of both nanoparticles are presumably associated with their nanoscale size. Neuromodulatory properties of nanodiamonds can be used for simultaneous labeling of the nerve terminals in neurotheranostics and modulation of key processes of glutamate- and GABAergic neurotransmission. Medical application involving hypo/hyperthermia, external magnetic fields, and radiolabel techniques can be perspective for nanodiamonds.

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Experiments were carried out in accordance with the European Guidelines and International Laws and Policies (Directive 86/609/EEC); the protocols were approved by the Animal Care and Use Committee of the Palladin Institute of Biochemistry (Protocol # 1 from 05/01-2016).

Neurogenic potential of mouse embryonic neural stem cells and adult human oral mucosa stem cells

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Key words: neural stem cells, human oral mucosa stem cells, cell differentiation

Introduction: One of the critical parameters in selecting the appropriate regenerative approach and cell-based treatment of brain diseases is neurogenic potential of investigated stem cells. The aim of the study was to compare isolation, cultivation and differentiation protocols for mouse embryonic neural stem cells and adult human oral mucosa stem cells. The main goal was to evaluate the neurogenic potential of aforementioned cell populations.

Methods: Neural stem cells were isolated from the telencephalic wall of 14.5 days old mouse embryos, obtained from the B6.Cg-Tg(Thy1-YFP)16Jrs/J transgenic mice strain, which expresses yellow fluorescent protein (YFP) in all parts of the neuron. Afterwards, neurospheres were cultivated in Dulbecco's Modified Eagle Medium Nutrient Mixture F-12 (DMEM/F-12) enriched by N2, B27, EGF and FGF2. After dissociation, cells were seeded on poly-D-lysine and laminin coated coverslips and followed up to 14 days in differentiation medium. Oral stem cells were obtained from the punch biopsy performed on healthy adult volunteers. Cultivation was performed in medium consisting of DMEM and 10% foetal bovine serum, EGF and FGF2, followed by the seeding of these cells onto fibronectin coated coverslips and tracking of their growth for 30 days in differentiation medium.

Results: One day after isolation, mouse neural stem cells uniformly expressed nestin, the marker of neurogenic potential, which after three and especially seven days, became replaced by set of neuron markers: approximately 75% of cells expressed NeuN, Map2 and Tuj1. Along with differentiation, these cells started to express markers of synaptogenesis (Nlgn1, SynCam, PSD95 and Syn1). Human oral mucosa stem cells retained expression of nestin and Oct4 for longer period lasting more than ten days. Also, expression of Sox2 and Map2 appeared after 14 days and was detected in approximately 80% of cells. Neurons obtained from both cell populations exhibited same morphology after 14 or 21 days.

Conclusion: Mouse neural stem cells represent an ideal cell population for preclinical testing of procedures based on application of stem cells for brain diseases due to their unmatched uniformity and supreme potential of obtaining neurons in a very short period. However, clinical trials require human stem cells isolated from accessible sources with significant potential for regenerative medicine. Despite the fact that more time is required to obtain neurons from oral mucosa stem cells, our study has shown that their accessibility and embryonic origin, which oral mucosa stem cells share with nervous system, makes them an appropriate candidate for clinical trials.

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All experiments on animals described in this work received approvals of the Internal Review Board of the Ethical Committee of the School of Medicine, University of Zagreb: HR-POK-006 from 2nd July 2015, and 380-59-10106-17-100/27 received on 26.01.2017. All experiments were carried out in accordance with the EU Directive2010/63/EU on the protection of animals used for scientific purposes.

A Novel System for Inducing Chronic Intermittent Hypoxia in Mice as a model of sleep apnea in humans

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Key words: sleep apnea, hypoxic chamber, intermittent hypoxia, apoptosis

Introduction: Sleep apnea is a chronic, widely underdiagnosed condition characterized by disruption of sleep architecture and intermittent hypoxia due to short cessations of breathing. It is associated with myocardial infarctions, congestive heart failure and stroke, and it represents one of the rare modifiable risk factors for Alzheimer's dementia. Reliable animal disease model is needed to understand the link between sleep apnea and the various clinically linked disorders.

Methods: An automated system for inducing hypoxia was developed, in which the major improvement was the possibility to efficiently adjust the length and intensity of hypoxia in two different periods. The mice were kept in their usual cages adapted with the system on the cage lid. As a proof of principle they were exposed to a three week period of intermittent hypoxia for 8 hours a day, with 90 s intervals of 5, 7 % and 21 % oxygen to validate the model. Treated (n=8) and control mice (no hypoxia, n=7) were handled in the same manner and their hippocampal brain regions compared by histology.

Results and Discussion: The chamber provided a fast, reliable and precise intermittent hypoxia, without inducing noticeable side effects to the animals. The validation experiment showed that apoptotic neurons in the hippocampus were more numerous in the mice exposed to intermittent hypoxia than in the control group.

Conclusion: The new design of a hypoxic chamber provides a fast, adjustable and reliable model of obstructive sleep apnea which was validated by apoptosis of hippocampal neurons.

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The experiments were approved by the Ethical Committee of the University of Zagreb School of Medicine (permit number: 380-59-10106-14-55/230).

Stress detection in medical students using physiological approach

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Key words: Lifestyle diseases, physical inactivity, human stress detection

Introduction: The modern medical education is becoming a more intensive and technical overload than in previous times. Over the last decade medical students (MS) use a lot of information from various modern gadgets, interactive learning courses, and digital trails (from social media, emails, etc.) that cause circadian disruption and early manifestations of lifestyle diseases. The aim was to analyze the possibility of diagnostic use of multimodal stress investigation based on a non-invasive physiological-based assessment of MS health and to create a problem- and emotional-based coping system for MS work stress and its outcomes.

Methods: Participants were recruited among medical students of the Lviv National Medical University (n=100). Stress detection was addressed using various approaches including a battery of questionnaires (International Physical Activity Questionnaire; daily time of physical inactivity; Pittsburg Sleep Quality Index; assessment of usage of technological gadgets, and evaluation of functional gastrointestinal disorders (FGID) by Rome III Consensus), heart rate variability (HRV), skin conductance (SC), pupil diameter (PD) based detection, and saliva study, as well as stress self-report.

Results: Approximately 65% of MS have a negative stress self report, despite decreased daily skeletal muscle contractile duration, circadian dysfunction and extended time of using gadgets. Changes in HRV, SC, PD that lead to autonomic nervous system dysbalance (ANSD) were in 70% of MS; among them about 30% have signs of FGID and abnormal saliva microcrystalline pattern. Smoking students have higher risk for prevalence of upper FGID.

Discussion: The obvious changes in saliva microcrystalization in persons with higher physical inactivity and circadian disruption may present external stimuli for induction of ANSD and FGID. A physiologically based program of increased duration of skeletal muscle contractility will be helpful in prevention of ANSD and its health outcomes.

Conclusion: An integrative view of stress detection could be a novel diagnostic tool for detection of early health disorders. Maintaining regular skeletal muscle contractile activity and normal circadian rhythmicity are a promising physiological approach for a problem- and emotional-based coping system for improvement of MS health.

Acknowledgements: The Ethics Committee of Lviv National Medical University (15.02.2016; N2).

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Metabolic Disorders

Effects of high fat and high sugar diet on hippocampal volume and cellular morphology in rat model

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Key words: Diabetes, Obesity, Metabolic diseases, Hippocampus

Introduction: Obesity is a strong risk factor for diabetes, and patients with type 2 diabetes have increased risk of developing Alzheimer's disease. Altered metabolism, inflammation and insulin resistance are pathological features presented in both diseases. In accordance with that, some anti-diabetic agents seem to be capable of improving cognition in Alzheimer's patients.
Aim of our research was to assess changes in hippocampi in obese rats without and on anti-diabetic medications.

Methods: Animals used in experiment were from HFHSD protocol previously described by prof. Robert Gaspar. Brains were fixed in 4% paraformaldehyde, cryoprotected in 30% sucrose. Brains were scanned with MRI (Bruker 7T, voxel size 450μm×450μm×2mm) and total hippocampal volumes (THV) were measured using 3DSlicer. After scanning 35μ slides were cut on cryostat (*Leica CM 3050 S*) and silver impregnated under UVC light. Regions of interest were identified: cornu ammonis 1, 3a and 3b (CA1, CA3a, CA3b) and dentate gyrus (GD). Specimens were photographed on Zeiss Axio MOT2, and digitally analyzed with Cell Profiler. Surface areas of neurons (SAN) were assessed and median surface area was calculated for each region and animal.

Results: HFHSD female rats tend to have larger THV (median 25,41 mm^3) compared to controls (22,36 mm^3). Contrary to that, HFHSD male rats have reduced THV (23,05 mm^3) compared to the controls (25,11 mm^3). Female rats on medications tend to have smaller hippocampi compared to the HFHSD animals without medication (metformin group 23,97 mm^3, liraglutide 22,67 mm^3). In males this effect is absent.
Regarding the SAN, females fed on HFHSD tend to have larger hippocampal neurons compared to the controls, whereas HFHSD males do not differ compared to the control group. Liraglutide and metformin do not influence SAN in hippocampi of male animals, but they seem to slightly reduce the neuronal hypertrophy in females.
Although aforementioned effects were not statistically significant, we demonstrated positive correlation between SAN and THV in females for CA1 ($p=0.00871$) and CA3a (0.03156), but not for CA3b and GD regions. In males, positive correlation between THV and SAN was confirmed for CA1 ($p=0.00232$), CA3a ($p= 0.0117$), CA3b ($p=0.01592$) and GD regions ($p=0.00171$).

Discussion and Conclusion: Hippocampus in rat model seems to react sex-specifically to chronic overeating. In females, a neuronal hypertrophy takes place and THV increases; hypertrophy does not occur in males, but slight hippocampal atrophy emerges, which might suggest a net neuronal loss. Liraglutide and metformin failed to demonstrate a statistically significant effect on hippocampal morphology in obese rats.

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Ethical approval was issued by Hungarian Ethical Committee for Animal Research (IV/3796/2015).

Oxidative and antioxidative status of nuchal muscles in rats exposed to chronic and acute stress

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Key words: acute stress, chronic stress, nuchal muscles, oxidative stress, ovariectomy

Introduction: Acute and chronic stress can induce the production of reactive oxygen species which exert a multitude of biological effects that ranges from physiological regulatory functions to damaging alterations and thus participating in the pathogenesis of a number of diseases.

Methods: In order to estimate the impact of acute and chronic stress on oxidative and antioxidative response in nuchal muscles (*Semispinalis capitis, Splenius capitis, Splenius cervicis*) of male (M), female (NON-OV) and ovariectomized female (OV), Sprague-Dawley rats were exposed to acute and chronic stress protocol. As an indicator of liver oxidative damage, lipid peroxidation levels expressed in terms of thiobarbituric acid reactive substances (TBARS) were determined, while liver antioxidative status was determined by catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST) activities and oxidized/reduced glutathione (GSH/GSSG) ratio.

Results: Both acute and chronic stress significantly increased TBARS content in in nuchal muscles of all groups relative to control groups. Chronic stress decreased antioxidative defence mechanism in nuchal muscles of males and females. Acute stress did not have significant impact on the most of the antioxidative enzyme activities (CAT, GPX, GST, SOD and GR) in males and females, although GSH/GSSG ratio was reduced. Ovariectomy increased LPO and reduced some of antioxidative enzyme activities (CAT, GPX).

Discussion and Conclusion: Oxidative and antioxidative response to acute and chronic stress, in nuchal muscles of males and females, showed similarity in most of the measured parameters, and it differed from the response in ovariectomized females. Unlike acute stress, chronic stress disrupts antioxidative defence significantly more, thus it can cause changes in muscle contraction and muscle fatigue, which may lead to chronic pain in neck and headache.

Ethical approval: This study was performed at the Animal Facility of the Faculty of Medicine Osijek and was approved by the Ethics Committee of the Croatian Ministry of Agriculture, approval number: 2158-61-07-11-51.

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Role of Glycome Imaging in Evaluation of the Gastric Mucosa Damage

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Key words: glycome, lectin histochemistry, gastric lesions, indometacin, oligopeptides.

Introduction: Nonselective cyclooxygenase (COX) blockage is an established cause of stomach mucosa (SM) injury. But interaction between COX and glycans expression in SM remains unknown. Another hot issue in experimental gastroenterology is application of oligopeptides, some of which are reported to exert cytoprotective properties.
Purpose of research was to assess SM glycome in indometacin-induced (II) gastric lesions (GL) in rats, pretreated with a tripeptide T-34 (H-Glu-Asp-Gly-OH).

Methods: Studies were conducted on white male rats, devided into 3 groups (n=6 per group): 1) control; 2) intragastrically (ig) administered indometacin (ind), 35 mg/kg; 3) ig pretreated with T-34 (10μg) 30 min before ind introduction. 24 h later rats were sacrificed. SM glycome was assessed using lectin-peroxidase technique. Lectin panel included fucose- (Laburnum anagyroides agglutinin [LABA]) and galactose-specific (Peanut (PNA), Soybean (SBA), Helix Promatia (HPA) lectins. Study design was approved by the Institutional Animal Care and Use Committee (Approval №2, February 16th, 2015).

Results: COX blockage caused erosive damage of SM (S=12 mm2), attenuated by T-34. In control rats, strong reactivity of SM to LABA- and SBAlectins was revealed, whereas there was faint labeling by HPA and PNA. In II GL, tendency to increased reactivity of epitheliocytes to LABA and decreased staining of chief cells and mucocytes were noted. Cytoprotective effect of T-34 resulted in reduction of fucose-specific receptors of epitheliocytes ($p<0.05$) and increased LFuc content in chief and parietal cells. In GL, PNA and SBA showed increased reactivity to parietal cells, which was decreased by T-34. HPA- and PNA-staining revealed an increased number of mucocytes under the effect of tripeptide. HPA reactivity in chief cells was restricted only to the T-34-pretreated group.

Discussion: COX blockage results in changes of mucosal glycosylation patterns in SM. T-34 caused redistribution of II glycoconjugate alterations, approaching the glycome of control rats.

Conclusions: Glycome examination has diagnostic significance for evaluation of SM injury. T-34 is promising for further studies towards elucidation of its cytoprotective effect, dosage and route of administration.
Source(s) of research support in the form of financial support, grants: The research was funded by the authors themselves.

Acknowledgements: Study design was approved by the Institutional Animal Care and Use Committee (Approval №2, February 16th, 2015).

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Cholesterol content and phospholipid composition in adipocytes of rats with obesity-induced insulin resistance and its changes after N-stearoylethanolamine administration

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Key words: obesity, insulin resistance, adipose tissue, dyslipidemia, N-Stearoylethanolamine

Introduction: Obesity induces molecular changes that promote associated disorders, such as insulin resistance (IR) and type 2 diabetes. Low insulin sensitivity occurs primarily due to defects in the pathway of insulin action in target tissues and there is a hypothesis that IR may originate in adipose tissue and followed by dyslipidemia. Cholesterol is known as the main modulator of phospholipids content in cell membranes. That is why the aim of our study was to investigate the total cholesterol content and phospholipid composition of adipocytes of obesity-induced IR rats and its changes induced by the N-Stearoylethanolamine (NSE) administration.

Methods: The experimental model was induced by the 6-month high-fat diet (58% of fats of the total diet) and confirmed by the peroral glucose tolerance test. NSE was administrated as water suspension *per os* in a dosage 50 mg/kg daily during 2 weeks. Abdominal fat isolated from rats was digested with Type 1 Collagenase solution. The fraction separation of adipocytes lipid extract was carried out by thin-layer chromatography. The cholesterol level was measured by gas–liquid chromatography. The phospholipid composition was determined using 2D thin-layer chromatography and estimated by measuring inorganic phosphorus content.

Results: The total cholesterol content significantly increased in adipocytes of obese IR rats compared to control. The phospholipid composition indicated a reduction of phosphatidylcholine and the total content of phosphatidylinositol with phosphatidylserine, whereas the content of lysophosphatidylcholine, sphingomyelin and phosphatidylethanolamine increased in IR group compared to control. NSE administration caused a statistically significant decrease in total cholesterol level and had a considerable effect on normalization of individual phospholipids content.

Discussion and Conclusions: As far as NSE administration decreased the total cholesterol content and normalized phospholipid composition of adipocytes, we can consider NSE as a prospective agent for the treatment of obesity-induced complications.

Funding: This study was supported by the Program of National Academy of Sciences of Ukraine: “Investigation of N-Stearoylethanolamine effect on mammals with insulin resistance and cognitive disorders” (state registration № 0114U003215).

Ethical approval: All experiments involving animals were carried out with the approval of the Animal Care and Use Committee of the Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine (Protocol №1 from 08/09-2015).

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Different antioxidative enzymes expression in aorta and brain blood vessels in obese pre-diabetic elderly rats of both sexes treated with metformin or liraglutide

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Key words: obesity, oxidative stress, blood vessels, antioxidative enzymes, liraglutide, metformin

Introduction: This study aimed to determine differences in micro and macrovascular antioxidative enzyme gene expression of pre-diabetic obese elderly Sprague-Dawley (SD) rats of both sexes. Additionally, this study aimed to determine whether liraglutide and meformin change the gene expression of antioxidative enzymes in this model.

Methods: Male and female SD rats were divided into: CONTROL group (fed with standard rat food); HSHFD group fed with commercially available carbohydrate and fat rich food from 20-65 weeks of age; HSHFD+metformin treatment group (50 mg/kg/day s.c.) and HSHFD+liraglutide treatment group (0.3 mg/kg/day s.c). During the entire protocol rats had free access to food and tap water.
Animals were anesthetized with isoflurane (Forane® isofluranum). Aortas and brain blood vessels (BBV) were used to determine superoxide dismutase (SOD) izoforms, glutathione peroxidases 1 and 4 (GPx1 and GPx4) and catalase (CAT) gene expression.

Results: No significant enzyme expression differences were observed among male aortas, while in female HSHFD+liraglutide aortas, expressions of Cu/Zn SOD, EC-SOD, GPx1 and CAT were increased compared to the other studied group. MnSOD, EC-SOD and GPx1 gene expressions in female CONTROL BBV group were significantly increased compared to other female groups and EC-SOD and GPx4 in male CONTROL BBV were significantly increased compared to other studied male groups.

Discussion: There are sex and vascular site differences in gene expression of different antioxidative enzymes and the resulting changes are more evident in macrovessels. Drugs may modify oxidative status more in females than males.

Conclusions: Obesity significantly reduces the expression of antioxidant enzymes particularly in macrovasculature of both sexes. Our results showed that more significant changes are observed in the expression of antioxidative enzymes in macrovessels (aortas) than in the microcirculation (BBV) in female and male with/without application of drugs. The liraglutide therapy proved to be more effective in reducing oxidative stress than metformin by increasing expression of antioxidative enzymes more strongly, especially in females.

Ethical Committee Approval: All experimental procedures conformed to the European Guidelines for the Care and Use of Laboratory Animals (Directive 86/609). They were approved by the Hungarian Ethical Committee for Animal Research (IV/3796/2015).

Sources of Funding: The study was supported in part by Bohdan Malaniak CSMC - RECOOP Young Scientists Research and by VIF-MEFOS-15 (Faculty of Medicine Osijek, Croatia).

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Cholesterol-dependent alterations in synaptic vesicle fusion and exocytotic glutamate release in vitamin D deficiency

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Key words: Vitamin D deficiency, exocytosis, cholesterol, membrane fusion

Introduction: Vitamin D deficiency (VDD) impacts a wide range of biochemical pathways and may accompany metabolic disorders. The association exists between obesity and low serum level of 25-OH-D_3, with higher doses of vitamin D recommended for obese individuals. Brain is one of the most critical systems affected by VDD. Prolonged VDD correlates with the predisposition to several neurological disorders. This study aimed to track neurosecretion and stimulated release of excitatory neurotransmitter glutamate in brain nerve terminals in VDD.

Methods: Techniques included gas chromatography, dynamic light scattering, registration of the exocytosis, intracellular pH, membrane fusion and glutamate release.

Results: VDD rats displayed increased cholesterol levels in erythrocytes and plasma membranes (PMs) of cortical nerve terminals. Increased cholesterol level in synaptic PMs of VDD rats (by 27%) was associated with higher cholesterol level in SV membranes. Depolarization-induced exocytotic glutamate release from nerve terminals was suppressed by 16±3% in VDD. Furthermore, intentional reduction/increase of cholesterol levels in PMs and SVs attenuated both homo- and heterotypic membrane fusion.

Discussion: There was correlation between high cholesterol levels in presynaptic PMs and erythrocytes in VDD. High cholesterol level in presynaptic membranes disturbs homo- and heterotypic membrane fusion. The latter may be one of the reasons for impaired depolarization-induced glutamate release in VDD.

Conclusion: Vitamin D is implicated in cholesterol metabolism in the brain and beyond. The level of PM cholesterol in VDD as well as the enrichment/depletion of synaptic PM cholesterol strongly impact the cholesterol level in SVs and substantially modify membrane fusion and exocytotic glutamate release.

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Ethical Approval: Studies were approved by the Institutional Animal Care and Use Committee (protocol N4, 14.09.2015).

Infection and Inflammation

Important role of cyclophilin D in the pathogenesis of LPS induced acute lung injury

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Key words: lipopolysaccharide, acute lung injury, cyclophilin D, reactive oxygen species

Introduction: Lipopolysaccharide (LPS)-induced sepsis is characterized by an intense systemic inflammatory response leading to acute lung injury (ALI). Mitochondrial dysfunction and reactive oxygen species (ROS) play important role in the pathogenesis of sepsis.
In the present study we investigated the role of cyclophilin D (CypD), a regulatory component of mitochondrial permeability transition pore (mPTP), in the development of LPS-induced acute lung injury.

Methods: ALI was induced by intraperitoneal LPS administration. Twenty four hours after LPS challenge histology and immunohistochemistry were carried out and lungs were also analyzed by electronmicroscopy. Cytokine determination by ELISA and Western blot analysis as well as RT-PCR were performed.

Results: Histologic and electronmicroscopic examinations revealed attenuated microscopical and ultrastructural changes in lungs of CypD deficient mice after LPS treatment. Immunohistochemistry showed decreased staining of nitrotyrosine and 4-hydroxy-2-nonenal major products formed by peroxynitrite-mediated nitration of proteins and peroxidation of membrane lipids respectively, in mice lacking CypD after LPS administration. In these animals phosphorylation and thereby activation of MAP kinases, Akt, nuclear factor-κB (NF-κB) and I-κB was also inhibited. Quantitative PCR showed decreased expression of NF-κB-mediated proinflammatory genes, consistent with findings of TNFα- and IL-1ß-determination by ELISA.

Discussion: mPTP opening leads to enhanced production of mitochondrial reactive oxygen species known to contribute to the pathogenesis of ALI. We found that 24 hours after LPS challenge mice lacking CypD, exhibited attenuated histological as well as ROS- and NO-induced cellular damage of the lungs. As a consequence of the lower oxidative toxicity redox sensitive cellular pathways were also downregulated leading to ameliorated activation of major inflammatory transcription factor, NF-κB.

Conclusion: We demonstrated that the lack of CypD significantly reduces the severity of LPS-induced lung injury and has clear impact on the LPS-induced signaling cascade and proinflammatory gene expression involved in the pathogenesis of ALI.

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Viral infection of NIH 3T3 cells

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Key words: coxsackieviruses, NIH 3T3 cells, virus replication, cytopathic effect

Introduction: Type B coxsackieviruses (CVB) belong to species *Enterovirus B* (genus *Enterovirus*) of the family *Picornaviridae*. Enteroviruses, small positive-stranded RNA viruses are commonly found human pathogens associated with both acute and chronic diseases. Coxsackieviruses induce lytic infections resulting in cytopathic effect (CPE) which includes visible changes and destruction of the host cell morphology *in vitro.* Their viral RNA can remain persistent for prolonged times in cells and organs post infection. Our aim was to study the replication of coxsackieviruses in NIH 3T3 cells with resistance to Puromycin alone or along with Blastomycin in combination with truncated variant of the Dicer ribonuclease. NIH 3T3 cells are a mouse embryonic fibroblast cell line, when 3T3 means cell transfer and inoculation protocol. Some studies describe moderate replication of enteroviruses in NIH 3T3 cells, or their presence without apparent CPE.

Methods: Selected two genetically modified clones of NIH 3T3 cells were infected with CVB3 (Nancy strain) passaged in Vero cells (monkey kidney epithelial cells). Cells were infected with a multiplicity of infection of 0.1. The cells were observed for the cytopathic effect (CPE) after infection. Presence of viral RNA was analyzed by reverse transcriptase polymerase chain reaction (PCR) and Nested-PCR.

Results: Morphological changes were absent in the infected and control cells in both clones of NIH3T3 cells. However, **rounding of cells was observed after third and fourth passages of the virus in the same cell line, but this effect did not increase on further passages of the virus. We observed presence of viral RNA irrelevant of the rounding effect.**

Discussion and Conclusion: Cytolytic viruses, involving enteroviruses, indicate persistent types of infections. We suggest a slow virus replication induced by presence of cytokines in the studied cells. Our results show possibility of development of a viral persistence in these cells.

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Comparison of different swabs type usable in the enteroviral molecular diagnostics

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Key words: enterovirus, diagnostics, swabs, PCR

Introduction: In routine laboratory diagnostics, rapid and early diagnosis methods are required based on the molecular detection of the viral genome. To detect the enteroviral RNA in the clinical sample, the tissue excision, blood or swabs are sent to the virological lab. Usually, the swabs are transported in a special medium (VTM) that ensures the stability of the virus. In a pilot study we had made an attempt to standardize the use of dry swabs (without VTM) and realized that these were with cotton-tips. The aim of this study was to examine the impact of different swab types and storage temperature on the enteroviral RNA detection by PCR.

Methods: Different types of swab (polyester tipped with VTM and swabs without VTM [polyester or cotton tipped]) were immersed in serial dilutions of stock virus CVB3 (Nancy) and inserted into a sterile container. Duplicates of each swab type were treated immediately (vortex in 500µl RNAse free water), other swabs were stored (1, at 4 °C for 12 days; 2, at -80 °C for a, 12 days; b, 1 month and c, 2 month) and treated sequentially. Enteroviral RNA was detected in processed suspension after RNA isolation.

Results: Viral nucleic acid was demonstrated in all the tested swabs. Enteroviral RNA was detectable after two months storage at -80 °C, as well as after 12 days storage at 4 °C (with a reduction in virus titer 1-2 $\log_{10}$ based on the swab type). Cotton- tipped swabs showed better results when compared to polyester tips.

Discussion and Conclusion: In this study, we confirmed the suitability of dry swabs (without VTM) in the molecular diagnosis of enteroviral infections for clinical and epidemiological purposes. All tested swabs were suitable. Cotton tipped swabs are readily available, inexpensive and do not affect the detection of enteroviral genome.

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Standardization of immunohistochemical staining for enteroviral capsid protein VP1

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Key words: Coxsackievirus B4, enteroviruses, capsid protein VP1, immunohistochemistry, antibody

Introduction: Immunohistochemistry is a key tool for analyzing target molecules localized within tissues, and is used routinely for almost every aspect of modern biomedical research. Monoclonal and polyclonal antibodies help to localize and visualize corresponding antigens in sections of tissues therefore allowing visualization of histopathological changes. Immunohistochemical techniques have been applied for localization of the enterovirus markers mainly the (capsid) viral protein 1 (VP1). Enteroviruses are one of the most common and important pathogens of humans. They are associated with a wide spectrum of human illnesses ranging from subclinical infections to rapidly fatal diseases and have been implicated in chronic diseases of the heart and pancreas. The aim of the work was to determine the suitable concentration of primary antibody for detection of VP1 in the mouse pancreatic tissue.

Methods: CD-1 outbred mice were infected with coxsackievirus B4-E2 by oral route. Pancreas tissues of mice were fixed in 4% formal saline, embedded in paraffin wax and 4-7μm sections were prepared for immunohistochemical analysis. Detection of VP1 in pancreas samples taken at day 5 post infection was done using labeled streptavidin-biotin (LSAB) method and monoclonal mouse anti-enterovirus antibody. Different dilutions of the primary antibody (1:150, 1:250 and 1:300) were investigated.

Results: The standardization of the procedures showed, that 1:250 was the best and the most suitable dilution for localization of VP1 by this primary antibody.

Discussion and Conclusion: In our study enteroviral VP1 was found in endocrine and excocrine pancreas mainly in the pancreatic acinar cells and also in the peripancreatic fat tissue. The pancreatic tissue showed severe inflammation (pancreatitis) and necrosis of the acinar tissue.

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Pathogenesis and sequence differences between Coxsacievirus B4 viruses of different origin

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Key words: enteroviruses, clinical isolate, environmental isolate, mice

Introduction: Enteroviruses display quasispecies dynamics, characterized by high rates of mutations and recombinations. The aim was to study the influence of intratypic virus strain variability on the course of infection in terms of pathogenesis, tissue and organ tropism, and possible persistent infection in relation to genetic variability of coxsackievirus strains of different origin: environmental and clinical.

Methods: Outbred CD1 mice were orally infected with Coxsackievirus serotype B4 of different origins (i) isolated from the cerebrospinal fluid of patient with meningitis – CVB4 AL, (ii) isolated from the stool of the same patient – CVB4 AS, (iii) environmental isolate – CVB4 COV. Replicating virus and viral RNA were detected. Virus was localized in particular organs using immunohistochemical staining. Stock viruses that had been used for the mice infection were partially sequenced and compared.

Results and Discussion: CVB4 AS isolate was detected in pancreases more than in brains and unlike other viruses it was shedded in faeces up to 45 days. Although closely related, clinical isolates adapted easily to particular organs depending on the origin of the isolate. CVB4 COV was detected in pancreas of mice up to 45 days and this was the only virus that developed acute pancreatitis in mice. The virus was detected in the acinar tissue and in islets of Langerhans, whereas clinical isolates were detected only in islets of Langerhans. Sequencing showed similarity between the clinical isolates, but several differences were found between the CVB4 AL/AS and CVB4 COV at positions 573, 2660, 2711 and 2945.

Conclusion: All three viruses were different from the diabetogenic prototypes CVB4 E2 and CVB4 JVB.

Ethics committee statement: The study was approved by the Ethical Committee of the Slovak Medical University from November 5, 2006 and the State Veterinary and Food Control Authority of the Slovak Republic from March 22, 2007 under number 3035/07-221/3.

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Study of antimicrobial effects of buffered ascorbic acid

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Key words: ascorbic acid, biofilm, eradication

Introduction: Catheter sepsis is a serious medical problem, especially in patients with permanent central venous catheters. Antimicrobial catheter locks are used to prevent development of the sepsis. Aim of this study was to assess the antimicrobial potential of buffered ascorbic acid solution and its usefulness in prevention of catheter sepsis.

Methods: The influence of buffered ascorbic acid on *Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter* sp., and *Candida* sp. was studied *in vitro.* Effects on planktonic microorganisms, biofilm formation and biofilm eradication was studied using culture techniques combined with detection of microbial extracellular polymeric substances (crystal violet staining) and cell viability (alamar blue assay). Finally, the effects were observed on biofilm formation prevention on surface of Hickman catheter *in vitro*, simulating bacterial contamination of the catheter.

Results and Discussion: The results showed significant bactericidal effects of ascorbic acid on tested Gram-negative non-fermenting bacteria (*Pseudomonas aeruginosa* and *Acinetobacter* sp.). The growth curves did not show this effect on other tested bacteria and yeast. Significant decrease of biofilm formation was observed in all tested bacteria, but influence on *Candida* sp. was seen. A significant decrease in cell viability was observed in all tested organisms in the mono-microbial biofilms, when treated with ascorbic acid.

Conclusion: Our results support the rationality of using the ascorbic acid solution as a protective lock to prevent catheter related sepsis.

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Reproductive Health

Correlation between placental vascularization indices and sFlt-1/PlGF ratio in preeclampsia screening: study progress

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Key words: 3-D ultrasound, placenta, sFlt-1/PlGF,vascularization, VOCAL.

Introduction: This is a prospective, cooperative observational ultrasound study fitted with serum and urine analyses. The purpose of the study is to determine the feasibility, utility and efficacy of the measurements of placental vascularization indices, measured by 3-dimensional power Doppler (3-DPD), as a potential tool in preeclampsia (PE) screening in combination with soluble fms-like tyrosine kinase and placental growth factor (sFlt-1/PlGF) ratio.

Methods: Patients with increased risk for PE, such as: previous PE (prevPE), chronic hypertension (CHT), gestational hypertension (GHT), pre-gestational diabetes mellitus (preDM), are recruited for ultrasound examinations and blood tests in Szeged and Osijek every four weeks between 20th and 36th gestational weeks. The study initiated in November, 2016.
During ultrasound examination fetal biometry is evaluated first, then uterine artery peak systolic velocity (aUtPSV) is measured and finally placental 3-DPD measurements are performed and recorded. Volume files are analyzed in Szeged by VOCAL (Virtual Organ Computer aided AnaLyses, version 10.0) software.
After the ultrasound measurements, maternal serum samples are taken for sFlt-1, PlGF, liver enzyme, creatinine, and platelet count measurements, urine sample is taken and blood pressure is also measured.

Results: Thirty patients were recruited and 43 examinations were made between November, 2016 and January, 2017. We lost 2 patients during this 3 month period, 1 patient was already delivered preterm, and we found 2 PE cases. Most of the patients recruited are prevPE or CHT patients. Blood samples for sFlt-1, PlG and the ultrasound volume files will be analysed in March, 2017 before the congress presentation.

Discussion: The study initiated in November 2016 and we already have 30 patients recruited out of 100 originally planned to follow until September 2017.

Conclusion: The study is in good progress. Our optimistic estimation for PE detection rate was 10% and it seems that we can reach that goal.

Source of research: Bohdan Malaniak CSMC RECOOP Young Scientists Research Grant 2016

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Maternal serum C-reactive protein and white blood cell count and intra-amniotic complications in women with preterm prelabor rupture of membranes

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Key words: preterm delivery, intra-amniotic inflammation, microbial invasion of the amniotic cavity, non-invasive marker

Introduction: Spontaneous preterm labor represents the major cause of perinatal morbidity and mortality. Preterm premature rupture of membranes (PPROM), a specific phenotype of spontaneous preterm labor, appears in about 30-40% of all preterm births. PPROM is very often complicated by microbial invasion of the amniotic cavity (MIAC) and intra-amniotic inflammation (IAI). The data regarding associations between maternal serum C-reactive protein (CRP) concentrations and maternal white blood cell (WBC) count and infection-related intra-amniotic complications in PPROM are conflicting. Moreover, there is a lack of information about an association between white blood cell count and the presence of MIAC and/or IAI. The main aim of the study was to evaluate maternal serum CRP concentrations and WBC count in pregnancies complicated by PPROM based on the presence of MIAC and/or IAI.

Methods: Two hundred eighty-seven and four hundred twenty-five women with singleton pregnancies complicated by PPROM were included to the studies to evaluate maternal serum CRP and WBC, respectively. Maternal blood and amniotic fluid were collected at the time of admission, and concentrations of interleukin-6 were measured using a point-of-care test. Maternal serum CRP was measured using a high-sensitivity immunoturbidimetric analysis. MIAC was diagnosed based on a positive PCR result for *Ureaplasma* species, *M. hominis*, and/or *C. trachomatis* and/or by positivity for the 16S rRNA gene. IAI was characterized as an amniotic fluid point-of-care IL-6 concentration ≥ 745 pg/mL.

Result: Women with MIAC and IAI had higher maternal serum CRP concentrations than women without these complications (with MIAC: median 6.9 mg/L vs. without MIAC: median 4.9 mg/L; $p = 0.02$; with IAI: median 8.6 mg/L vs. without IAI: median 4.7 mg/L; $p < 0.0001$). When women were split in the four subgroups based on the presence of MIAC and/or IAI, women with the presence of both MIAC and IAI had higher maternal serum CRP than women with IAI alone, with MIAC alone and women without MIAC and IAI (both MIAC and IAI: median: 13.1 mg/L; IAI alone: 6.0 mg/L; MIAC alone: 3.9 mg/L; and without MIAC and IAI: median 4.8 mg/L; $p < 0.0001$). The maternal serum CRP cutoff value of 17.5 mg/L was found to be the most effective at identification of the presence of both MIA and IAI with sensitivity of 47%, specificity of 96%, positive predictive value of 42%, negative predictive value of 96%, and the positive likelihood ratio of 10.9. Women with MIAC and IAI had higher white blood cell count than women without these complications [with MIAC (n=112): median $12.8 \times 10^3/\mu L$ (IQR 10.8-15.8) vs. without MIAC (n=313): median $12.0 \times 10^3/\mu L$ (IQR 9.9-14.5); $p=0.002$; with IAI (n=88): median $13.8 \times 10^3/\mu L$ (IQR 11.8-16.7) vs. without IAI: median 11.9 (IQR 9.9-14.2); $p<0.0001$]. When women were split in the four subgroups based the presence of MIAC and/or IAI, the difference was found among these subgroups ($p<0.0001$).

Discussion: Maternal serum CRP is among the most commonly used clinical non-invasive markers to predict infectious-related and inflammatory complications in women with PPROM, despite the absence of the strong evidence for its use in relation to these indications The following are the key findings of this study: i) higher maternal serum CRP concentrations and WBC were associated to presence of MIAC; ii) higher maternal serum CRP and WBC concentrations were associated to presence of IAI; iii) the highest maternal serum CRP concentrations were associated to women with both MIAC and IAI; iv) maternal serum CRP concentrations

had better diagnostic indices than WBC to identify PPROM pregnancies complicated by both MIAC and IAI; v) maternal serum CRP cutoff value of 17.5 mg/L was found to be most effective at identifying of women with the presence of both MIAC and IAI; and vi) maternal serum CRP concentrations weakly correlated with amniotic fluid IL-6 concentrations.

Conclusion: The presence of both MIAC and IAI was associated with the highest maternal serum CRP concentrations. Maternal serum CRP had a very good specificity and negative predictive value for an identification of women with both MIAC and IAI. The diagnostic value of WBC to predict these PPROM complications was weak.

The study's protocol was approved by the Institutional Review Board (July, 2014; decision No. 201407 S14P).

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Examination of bioactive factors in human breast milk samples

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Key words: breast milk, bioactive factor, LUMINEX, breastfeeding, development

Introduction: Breast milk contains several bioactive compounds that play important roles in the development of the newborn. Earlier we have shown that PACAP38 and MIF are present in high levels in breast milk, but previous prospective studies have only focused on the water phase of breast milk. In the present experiment we aimed to examine the changes of different bioactive factors in different layers of milk samples during the first 6 months of lactation.

Methods: We collected 5 ml milk every month during the first 6 months of nursing. First we separated the milk samples to lipid phase and water phase by centrifugation. We used ultrasonication to factor the lipid phase. We measured the PACAP and MIF concentrations with ELISA technique from each sample. With Luminex technique we examined the concentration of Fractalkine, MIP-1ß, Eotaxin, RANTES, EGF, MCP-1, GRO, Flt-3L, CD40, and MDC in these samples.

Results: In our experiment we detected the long-term presence of examined bioactive factors in the lipid phase of human milk for the first time. We measured higher MIF concentrations in the water fraction than in the lipid fraction. We were also the first to show an increasing tendency of the MIF concentration in the lipid layer of human milk during a long-term 6-month follow-up period. With Luminex technique we also detected significant changes in the level of other bioactive factors in 3 different milk fractions.

Discussion: We detected the above-mentioned factors in different concentration ranges in the 3 layers of breast milk. Since these factors have several effects on gastrointestinal, immune and nervous systems, they might have an ideal concentration in breast milk, which is essential for ideal development.

Conclusions: Our future aim is to establish the exact influence of the above-mentioned factors with additional clinical and molecular biological experiments.

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Acknowledgement: Ethical Committee Approval: PTE KK 6383 (Valid until:16/08/2018)
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Obesity in pregnancy: the impacts of bone morphogenetic protein, wingless and inhibitor of DNA-binding proteins

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Key words: obesity, pregnancy, adipokines, BMP-Smad signalling, Wnt proteins, Id proteins.

Introduction: Obesity is rapidly emerging as one of the most serious health problems worldwide since its reaching epidemic proportions. It is known that the adipocytes produce different cytokines that are critical for energy homeostasis and fertility. Elevated level of adipokines also can lead to dysregulation in wingless (Wnt) /β-catenin and bone morphogenetic proteins (BMP) and Id (Inhibitor of DNA-binding).
The main goal of our study is the understanding the molecular mechanisms that mediate the negative effects of obesity on myometrical function and uterine contractility though the pregnancy.

Methods: Q-PCR and Western and Northern blot analysis were used to detect the gene expressions at RNA and protein levels. Mesenchymal stem cells differentiation *in vitro* was used to control the BMP and Id effects.

Results: In our preliminary studies we showed the *Id1/2/3* genes expression is induced by the different BMP proteins in mesenchymal C2C12 cells. Moreover: we demonstrated that Id1 is a direct BMP target gene. Id1 efficiently inhibits the myoblast differentiation and can cooperate with key osteoblast regulator Runt homology protein 2 (RUNX2) in driving the osteoblast differentiation. Currently we are investigating the crosstalk between adipokines and Wnt/β-catenin signaling as the potential mediators of the negative effects of obesity on myometrical function and uterine contractility though the pregnancy.

Discussion: It is known that targeted inactivation of the key Wnt proteins and of closely cooperating BMP proteins leads to early embryonic death due to malformations in mesenchymal tissue and blood vessels. In addition, Id proteins affect the adipogenic differentiation (Id2, Id3) and myogenic differentiation (Id1, Id3). Id proteins could influence the uterine myometrial contractions. Id3 affected the adipose tissue vascularization.

Conclusion: Our results suggest Wnt, BMPs and their target genes Id proteins as the crucially important regulators of adipogenic and myogenic differentiation thus mediating the negative effects of obesity on myometrical function and uterine contractility though the pregnancy.

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Experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Institute of Cell Biology NAS of Ukraine.

Evaluation of markers of haemostatic system in pregnancy complicated by pre-eclampsia

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Key words: haemostasis, pre-eclampsia, protein C, soluble fibrin, prothrombin

Introduction: Pre-eclampsia is a pregnancy complication characterized by high blood pressure and signs of damage to another organ system. The connection between pre-eclampsia and thrombosis provides insights for the aim of our work: to investigate the list of haemostasis parameters that need to be monitored during pre-eclampsia.

Methods: Blood samples were collected from 43 pregnant females diagnosed with pre-eclampsia and 224 normal pregnant females. Soluble Fibrin (SF), fibrinogen and D-dimer were quantificated using immunodiagnostic test-systems DiaProph-med©. Protein C, factor X activity and ATIII levels were measured by standard methods. Accumulation of functionally inactive forms of prothrombin (FIFPs) was detected as the difference between the levels of prothrombin activated by ecamulin (activates all forms of prothrombin) and thromboplastin (activates only native prothrombin).

Results: Pregnant females with pre-eclampsia have a high fibrinogen level (4,4±1,02 mg/ml) that could be evidence of the inflammation process. In 50% of those patients protein C level was dramatically decreased (<70%). We also detected FIFPs and SF in blood plasma samples of 50 and 40% of patients, respectively. Distinct differences in factor X and ATIII levels in blood plasma of females diagnosed with pre-eclampsia and normal pregnant females were not shown.

Discussion: Both FIFPs and SF detected in blood plasma of pregnant females with pre-eclampsia are evidence of thrombin generation and as a consequence, the risk of thrombus formation. However, being formed directly under the action of thrombin, FIFPs can be acknowledged as the early marker of activation of the coagulation system. The level of SF may correspond to the level of D-dimer in that high SF and D-dimer levels could demonstrate a balance in the haemostatic system, whereas a low D-dimer level along with high amounts of SF could indicate a higher procoagulant potential that leads to thrombosis.

Conclusions: Determination of protein C, SF and FIFPs levels and individually selected qualifying tests (Fibrinogen, D-dimer levels; factor X and ATIII activity) can enhance the understanding of pathogenesis of pre-eclampsia and support the therapy.

Source: This work was carried-out in the frame of the basic theme of the Palladin Institute of Biochemistry of NAS of Ukraine "Study of regulation mechanisms of blood coagulation and fibrinolysis interplay with vascular and platelet haemostasis".

Ethical approval: Patients signed informed consent prior to blood sampling at the Department of obstetrics and gynaecology №1, according to the Helsinki declaration. This study was approved by the Ethics Committee of O.O. Bohomolets Medical University (09.05.2016, N12).

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Alteration in expressions of RhoA and Rho-kinases during pregnancy in rats: their roles in myometrial contractions and onset of labour

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Key words: RhoA, Rho-kinases, onset of labor, contraction, pregnant myometrium, rat

Introduction: Activation of RhoA and Rho- associated kinases (ROCK I and II) has a pivotal role in the regulation of smooth muscle contraction via phosphorylation of myosin-light chain and myosin phosphatase. There are few data on the RhoA and ROCKs expression levels in rat uteri. Therefore, our aim was to investigate the mRNA and protein level of RhoA and ROCKs during pregnancy, during parturition and post-partum and to evaluate the effects of the ROCK (Y-27632, fasudil and RKI 1441) and RhoA inhibitors (simvastatin) on myometrial contractility.

Methods: The mRNA and protein expressions of RhoA, ROCK I and ROCK II were measured in non-pregnant, pregnant uteri (days 5, 15, 18, 20, and 22 of pregnancy), during parturition and post-partum (1, 3, 5, 7 day after delivery) by Real-time PCR and Western blot analysis. The effects of Y-27632, fasudil, RKI 1441 and simvastatin were investigated on oxytocin induced uterine contractions (non-pregnant, 22 day of pregnancy, during parturition and post-partum day 1).

Results: The mRNA and protein levels of RhoA decreased on the 5th day of pregnancy to day 22, then a sharp increase was detected at term. The mRNA and protein concentration of ROCKs was down-regulated in the early stage of pregnancy, while it sharply increased during parturition. Simvastatin relaxed the myometrial contractions, although its inhibitory effects were not followed by the alteration of RhoA. The strongest inhibitory effect of fasudil was found on non-pregnant myometria, while it elicited milder relaxation on day 22, during parturition and postpartum day 1. The maximum relaxing effects of Y-27632 and RKI 1441 were altered in a proportional way with the target protein expressions.

Discussion and Conclusion: We suppose that the lower expression of ROCKs during pregnancy may contribute to the maintenance of relative quiescence in the pregnant myometrium and the sudden increase of mRNA and protein expression of RhoA and ROCKs during labor suggests that they may contribute to the enhanced contractility and the initiation of delivery. In conclusion, the RhoA/ROCK signaling pathway might be a potential target for the development of new tocolytic agents.

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

Glycome and the miscarriage: a new approach to the problem

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Key words: recurrent miscarriage, spontaneous miscarriage, lectins, pregnancy, glycoconjugates.

Introduction: There are two types of pregnancy miscarriage – the sporadic (SM) and recurrent (RM). The frequency of RM among reproductive age women reaches 5%. There are many reasons of RM and in approximately 50% of all cases they are defined as "immune infertility", the mechanisms of which are not completely known.

The purpose of the study was to investigate morphological changes and glycome features of the structural components of human embryo chorionic villi (CV) that had died as a result of the first trimester miscarriage.

Methods: Histological material included 33 4-13-week-old human embryo CV tissue samples, obtained after SM, RM and 20 control group CV samples. They were stained with hematoxylin and eosin, PAS stain and alcian blue with different pH levels. Lectin peroxidase technique was applied, using 8 lectins (Con-A, HPA, LABA, SBA, SNA, CNFA (CNL), AIA, VAA).

Results: Destructive changes were observed in RM chorionic villi samples. With the help of lectin peroxidase technique, we have identified one most active lectin – LacdiNAc-specific CNFA (CNL).

Discussion: Considering that we recorded significant affinity of LacdiNAc-specific lectin CNFA (CNL) to the structural components of microvilli, we assume that a normal LacdiNAc group can play a protective (camouflage) role against negative effects including the immune system of the mother.

Conclusion: We propose that one of the mechanisms of a regular early pregnancy loss can be a reduced expression of LacdiNAc - carbohydrates in some chorionic villi structural components, and as a result, the unmasking of an embryo against negative external factors, including those of an immune nature.

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Ethical committee approval: Research performed in compliance with the main provisions of GCP (1996), the European Convention on Human Rights and Biomedicine on 04.04.1997 and the Helsinki Declaration of the World Medical Association on ethical principles of scientific medical research involving human subjects (1964-2008) and MOHC of Ukraine order №690 on 23.09.2009, as evidenced by the conclusion of biomedical ethics commission LNMU (protocol number 2 from 15.02.2016).

In-vivo analysis of placental vascularization and perinatal outcome in pregnancies complicated by intrauterine growth restriction

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Key words: intrauterine growth restriction, perinatal outcome, placenta, ultrasound

Introduction: Our goal was to examine placental vascularisation using 3-dimensional power Doppler (3-DPD) technique in the second and third trimester of pregnancies complicated by intrauterine growth restriction (IUGR).

Methods: Vascularisation of placentas was assessed in the second and third trimester of 52 pregnancies complicated by IUGR as well as 171 normal pregnancies using 3-DPD technique. We have evaluated the correlation between specific parameters and gravidity, parity, body-mass index, placental localisation, estimated fetal weight, birth weight, emerging intrauterine complications, umbilical cord arterial pH and Apgar score. We applied the Merce-type sonobiopsy and volumes were analyzed with Virtual Organ Computer-aided Analysis (VOCAL) programme.

Results: 3-DPD vascularisation indices of the placenta showed significant differences between the study group and control group. Placental vascularisation is lower in pregnancies complicated by IUGR than in normal ones. Deterioration of the vascularisation correlates to perinatal outcome.

Discussion: In our prospective study focusing on the determination of placental vascularisation indices (VI, FI and VFI) with the help of Mercé-sonobiopsy, we found that placental vascularisation is lower in cases of IUGR pregnancies compared to normal pregnancies. We did not find significant discrepancy regarding maternal age, as well as gestational age at the time of examination between IUGR and the control group, thus maternal age had no influence on the condition of arteries or on angiogenesis during pregnancy. Placental vascularisation indices are approximately constant during the pregnancy. The frequency of Caesarean section was significantly higher in the case group compared to the control group. Growth-restricted infants respond sensitively to stress during labour and poorly tolerate vaginal delivery, thus Caesarean section is performed more frequently.

Conclusion: The examination of placental vascularisation by 3-DPD technique can be a method to distinguish perinatal complication in IUGR pregnancies.

Source(s) of research support: No funding support.

Approval by the Ethical Committee: No 135/2011

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The contribution of chromosomal abnormalities in the genesis of early reproductive losses in population of Western regions of Ukraine

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Key words: spontaneous abortion, G-banding, cytogenetic, chromosome abnormalities, mFISH.

Introduction: Karyotypes of spontaneously aborted conceptus provides valuable clinical information for couples, as well as for research. Chromosomal abnormalities (mostly aneuploidies) account for ~45% of fetal losses in first 8–15 weeks of gestation (w.o.g.). Most of detectable aberrations are numerical, namely polyploidy, autosome trisomy, or X monosomy.

Methods: For analysis, spontaneous abortion (SA) cells of chorionic villi were separated from the decidual cells, and chromosome samples were prepared. These samples were subjected to cytogenetic study using G-banding technique. For analysis of conception products in which banding analysis was not possible due to absence of metaphases interphase, mFISH was performed by probing a panel for specific chromosomes 13, 14, 15, 16, 17, 18, 21, 22, X, Y.

Results: Cytogenetic and molecular cytogenetic studies were performed on 419 spontaneously aborted fetuses of 4 to 14 w.o.g. G-banding results were obtained in 133 of 419 cases. For the remaining 286 cases, mFISH analysis was performed. Uncultured cells were probed with a panel for chromosomes 13/21, 14/22, 15, 16, 17, 18, X, Y, and in 150 cases (35.8%) an abnormal karyotype was detected. None of these chromosomal abnormalities was influenced by fetus gender (male/female - 205/214). Most of detected aneuploidies were triploidy (27.3%), monosomy X (22.7%), trisomy 16 (18.7%), trisomy 21 (6.7%), trisomy 15 (5.3%), and trisomy 22 (5.3%).

Discussion: Chromosomal aneuploidies in conception products play a key role as the reason for reproductive failure in humans. Combination of GTG-banding analysis with the mFISH approach allowed a detection of low level mosaics of the aneuploidies.

Conclusion: Effectiveness of combining karyotyping and mFISH for increasing the detection rate of spontaneous abortions in human was demonstrated. A pattern of chromosomal aneuploidies in samples of conception products is presented for patients with reproductive failure who live in the Western regions of Ukraine.

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Ethics Committee Approval: Protocol № 51/2017 from 1.02.2017 of the BioEthics Committee of the Institute of Hereditary Pathology, NAMS of Ukraine.

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The effects of the antibiotics on the expression of aquaporin 5 in the pregnant rat uterus

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Key words: aquaporin 5, amoxicillin, doxycycline, fosfomycin, pregnancy

Introduction: The aquaporin (AQP) water channels are small hydrophobic integral membrane proteins. Most of them are expressed in female reproductive tissues and they play important roles during pregnancy. Earlier we proved that AQP 1, 2, 3, 5, 8 and 9 are detectable in the late- pregnancy rat uterus and that AQP5 expression showed a dramatic down-regulation on the last day of pregnancy that is regulated by oxytocin and may play a role in delivery. Since antibiotics are among the drugs used to stop preterm labor, our aim was to study the changes in AQP5 expression and uterine contractility after antibiotic treatment.

Methods: 3 groups of pregnant rats were involved in the study. Amoxicillin (Group 1, 40 mg/kg) or doxycycline (Group 2, 30 mg/kg) was given orally for 1 week from day 16 of gestation. Fosfomycin (Group 3, 40 mg/kg) was given orally on day 21 of gestation. On pregnancy day 22, uterine samples were collected. We used reverse-transcriptase PCR and Western blot techniques for the detection of the changes in AQP5 expression. Uterine contractility was investigated in an isolated organ bath system.

Results: Fosfomycin and amoxicillin pretreatment caused a significant increase of AQP5 mRNA and protein levels on the last day of pregnancy ($p<0.05$). Doxycycline pretreatment caused a significant decrease of AQP5 mRNA and protein levels ($p<0.05$). The fosfomycin and amoxicillin pretreatment significantly reduced the uterine contractions both to potassium chloride and oxytocin treatment ($p<0.05$).

Discussion and Conclusion: We suppose that the AQP5 expression is inversely related to the uterine contractility because the increased AQP5 expression is accompanied with decreased contractions. Fosfomycin and amoxicillin treatment during pregnancy may be favorable in the therapy of preterm labor.

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

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

The antioxidant α-tocopherol modifies the smooth muscle effects of NSAIDs

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Key words: NSAID, antioxidant, myometrium, trachea, rat

Introduction: Nowadays, the use of antioxidant supplements is becoming increasingly popular. However, the effect of these materials on various medications has only been scarcely investigated. It is well known that activity of cyclooxygenase enzymes liberate reactive oxygen species (ROS). Our aim was to investigate how the antioxidant tocopherol influences the effects of NSAIDs in the smooth muscles in rats *in vitro.*

Methods: Contractility of smooth muscle tissues from 22-day-pregnant and non-pregnant Sprague-Dawley rats were measured in isolated organ bath *in vitro.* α-Tocopherol-succinate (10^{-7} M) was applied as antioxidant, while non-selective diclofenac (10^{-9}–10^{-5} M) and COX-2 selective rofecoxib (10^{-10}–10^{-6} M) were used in cumulative doses as NSAIDs. The COX activities of the samples were measured by enzyme-immunoassay.

Results: In the presence of tocopherol, the uterus relaxant effect of the diclofenac and rofecoxib increased significantly on the 22-day-pregnant smooth muscle tissues. On the non-pregnant uteri, however, the tocopherol did not influence the relaxant effect of NSAIDS. It was also found that tocopherol decreased and increased the tracheal tone-reducing effects of rofecoxib and diclofenac, respectively. These differences can partially be explained by the different COX-activities in non-pregnant and pregnant uteri and the tracheal tissue.

Discussion: The antioxidant tocopherol significantly modifies the smooth muscle effect of NSAIDs. Our results suggest that the observed influence depends on the selectivity of NSAIDs and also on the type of smooth muscle and the local COX-activity.

Conclusion: The use of antioxidants may significantly alter the impact of NSAIDs on smooth muscles.

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

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

Alteration of nitric oxide synthase activity in spermatozoa of infertile men with different forms of pathospermia

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Key words: male infertility, spermatozoa, nitrix oxide, NO-synthase

Introduction: Infertility is a widespread complex problem affecting approximately 15% of couples globally. Studies indicate that more than 40% of infertility is related to the male factor. Defective sperm function is the most common cause of male infertility. Nitric oxide has been recognized as a signaling molecule and effector in various biological processes. The aim of the present work was to detect changes in the activity of NO-synthase isoforms of sperm cells in patients with different forms of pathospermia.

Methods: Spermatozoa of infertile men and healthy donors were used. NOS activity was determined by measuring L-citrulline by a highly specific method with antipyrine.

Results and Discussion: The state of NO-synthase system in the sperm cells of fertile men was characterized by the dominance of eNOS activity. iNOS activity was detected at extremely low levels compared to eNOS. Sperm of infertile men, as compared to fertile men, was characterized by decreased eNOS activity. According to obtained results, the eNOS activity was decreased by 1.4 – 1.5-fold ($p < 0.05$) in patients with oligozoo-, astenozoo- and oligoastenozoospermia and by 3.2-fold ($p < 0.001$) in patients with leucocytospermia in comparison with normozoospermic men. Contrary to this, the iNOS activities in sperm cells of infertile men with oligozoo-, astenozoo-, oligoastenozoo- and leucocytospermia were 23-, 30, 31- and 56-fold ($p < 0.001$) higher, respectively, compared with fertile men. The most expressed violations of the NO-synthase pathway of L-arginine were observed in patients with leukocytospermia. This manifestation could be explained by the fact that white blood cells stimulate the formation of reactive oxygen, and the induction and development of oxidative and nitrative stress.

Conclusion: It was found "uncoupling" of NO-synthase in sperm cells in infertile men, and redistribution of activities in the NO-synthase system with their shift toward a Ca^{2+}-independent inducible isoform, which indicates NO hyperproduction.

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Fetal weight to placental volume ratio analysis in pregnancy complicated by diabetes mellitus

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Key words: fetal weight to placental volume ratio, gestational diabetes mellitus, placental volume, type-1 diabetes mellitus, ultrasound

Introduction: Our purpose was to analyze the fetal weight and placental volume ratio in diabetic pregnancies during mid-pregnancy.

Methods: One hundred and forty-nine diabetic pregnancies (75 gestational diabetes mellitus (GDM) and 74 diabetes mellitus type I (T1DM) with good glycemic control) and 232 healthy patients were analyzed by three-dimensional sonographic volumetry of the placenta, while fetal weight was estimated by two-dimensional technique.

Results: The gestational age specific estimated fetal weight (EFW) (EFW_{GDM}: 1840.8±932.82 gram; EFW_{T1DM}: 1475.6±914.7 gram (mean±standard deviation) and placental ratio (PR) was significantly higher ($p<0.05$) in pregnancies complicated by GDM and T1DM (PR_{GDM}: 5.05±1.67 gram/cm3, PR_{T1DM}: 4.13±3.2 gram/cm^3) compared to control group (Q) (EFW_Q: 232.±186.49 gram; PR_Q: 1.84±0.8 gram/cm^3), whereas placental volume (PV) was significantly higher ($p<0.05$) only in GDM (PV_{GDM}: 334.3±111.5 cm^3) compared to control group (PV_Q: 232±78.9 cm3).

Discussion: In contrast to GDM, T1DM with good glycemic control did not predispose to any changes in placental sonographic volumetric differences compared to control values.

Conclusions: Fetal weight related to the placental volume are already elevated in second trimester in pregnancies complicated by gestational diabetes mellitus and type I diabetes mellitus compared to normal pregnancies.

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Expression of GABA signaling pathway key molecules in mouse and human cervical tissue

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Key words: endocervical glands, mucus, GABA, estrogen

Introduction: Cervical mucus is a glycoprotein gel produced by secretory epithelium in the endocervical glands. The role of the mucus is protective; it provides a barrier to the infection. Cervical mucus has an important role in fertility, due to the cyclic changes of its properties which are related to the levels of hormones in the blood. It is indicated that, besides the slow processes of transcription and translation of mucus components, the cycling hormones can regulate rapid process of mucus secretion from the endocervical glands. Since the regulation of mucus production in the bronchial goblet cell is mediated by GABA signaling pathway we hypothesized analogous GABA-mediated regulation of the endocervical glands.

Methods: In order to test the hypothesis, the expression of key GABAergic molecules in mouse and human cervix was tested by reverse transcription polymerase chain reaction (RTPCR) and immunohistochemistry.

Results: GABA signaling pathway key molecules were present in mouse and human cervix. The examined molecules in mouse were β2 subunit of GABA receptor A ($GABA_AR$ β2) and estrogen receptor ERα. The following molecules were shown to be expressed in the human: glutamic acid decarboxylase (GAD), vesicular GABA transporter (VGAT), β2 subunit of GABA receptor A ($GABA_AR$ β2) and estrogen receptor ERα. GABAR, GAD and VGAT, elements of the GABA signaling pathway were present in the mouse and human cervix and their intracellular localization was in accordance to the presented theory.

Discussion: This study is the first step in verifying the novel concept assuming the role of GABA signaling pathway in the regulation of the endocervical mucus secretion. Subsequently the mechanism of the estrogen driven regulation of mucus secretion remains to be elucidated in the context of GABA mediation.

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All experiments received approval of the Internal Review Board of the Ethical Committee of the School of Medicine, University of Zagreb under the number HR-POK-006, issued on July 2nd 2015. All experiments were carried out in accordance with the EU Directive 2010/63/EU and 2004/23/EC.