Poster Sessions

Partner Organizations

Poster Session Schedule

18:00 – 20:00 **Poster Sessions -** ***Conference Rooms # 4 and 5***
Sponsored by CSMC - RECOOP HST Association

Preterm Birth
Cardiovascular Diseases

11:30 – 14:00 **Poster Sessions -** ***Conference Room # 6***
Sponsored by CSMC - RECOOP HST Association

Cancer Research
NanoBioTechnology

11:00 – 12:00 **Poster Sessions –** ***Conference Rooms # 7***
Sponsored by CSMC - RECOOP HST Association

Stress, Obesity and Metabolic Diseases
Neurological Disorders and Brain Research

Empowering the first year students at the faculty of medicine in skopje for scientific writing

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Introduction: From the beginning of the medical studies it is very important to develop skills in the critical reading of published literature and writing. Scientific writing and publishing is essential for spreading information about health issues and problems and possible solutions for changing the practice, and for promoting of the research findings and activities. Medical students should become familiar with the principles of scientific communication because research normally should lead to publication in scientific journals. The aim of the paper is to present the activities and results with the 1st year students at the Faculty of Medicine in Skopje for empowering them for scientific writing.

Methods: After a lecture delivered on the principles of the scientific communication and for preparing seminar paper and manuscript for publishing in a scientific journal (guidelines sent to the students in electronic form), the students in the 1st semester of the academic year 2012/2013 at the Faculty of Medicine in Skopje were asked to prepare (optional) a seminar paper.

Results: Out of total 246 enrolled students in the 1st year of study at the Faculty of Medicine in Skopje, 198 (80.1 %) submitted seminar paper at the end of the 1st semester. Grading of the seminar papers was done upon five criteria, each of them carrying two points or possible total 10 points, which counts 10% in overall possible points for the final grade of the course Introduction to the medicine. The average grade for all seminar papers was 3.98.

Conclusion: Scientific education and research work is an important dimension of the medical curricula to set standards for student analytic and writing performance in accordance with the Bologna process. Usually that is the weak part of many medical programs and too few students reach this goal till graduation. We tend to increase the awareness and motivated the students to make the first steps in direction of scientific writing and publishing.

Key words: scientific communication; biomedicine, publishing ethics; references, Vancouver style, peer-review

Poster Sessions

Preterm Birth

Maternal excess iron intake during pregnancy increases oxidative stress and affects birth outcome.

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Goal: To identify and assess the risk of excess iron supplementation during pregnancy in the CEE countries. Background: Oxidative stress of placenta has been known as a pathology of gestational disease. Pregnancy is associated with a fine equilibrium between enhanced requirement for iron to treat anemia and free radical formation leading to oxidative stress and harm by excess iron usage. Oxidative stress can lead to gestational diabetes, insulin resistance and preterm birth. Method: This study explores potential association of iron use with anemia, gestational diabetes and preterm birth in Central and Eastern Europe (CEE). Partners of Mother and Child Health Research (M&CH) Network of the Regional Cooperation in Health, Sciences and Technology (RECOOP HST) Association, made an undertaking to identify this association from five sites in CEE; Czech Republic, Hungary, Romania, Slovakia and Ukraine. Iron use data from total of 30,406 term (26,902) and preterm births (3,504) in a retrospective study, from 2007 to 2009 in five university hospital centers of CEE. Term and preterm data was analyzed using SAS to investigate iron use frequencies for each core. Discussion: The rates of iron use and rates of anemia vary from 2.2 % (Ukraine) to 39 % (Slovakia). There was no relationship in iron use and diabetes except for Hungary and Slovakia where iron use was higher in subjects who developed diabetes. Logistic models indicate that anemia is a very strong predictor in all the participating CEE countries except Ukraine and smoking is an additional predictor in all these countries except Hungary. In Slovakia, current (pregnancy induced or existing) diabetes and BMI are also significant predictors of Ironuse. Conclusion: The results of the Iron use in the retrospective study are crucial to make adjustments in the ongoing prospective study and able to design iron supplementation schemes for the safe prevention and correction of iron deficiency or excess. Biomarkers index through the course of pregnancy could be more sensitive to changes in maternal iron status rather than the overall outcome measures.

Coxsackievirus infections during pregnancy

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Background: Coxsackie B viruses (CVB), of which 6 serotypes are known (CVB1-6), belong to the Enterovirus B species. Infections by CVB are highly prevalent, but often sub-clinical or cause a mild flu-like illness. Neonatal infections and chronic diseases (where autoimmunity and/or viral persistence may be involved) such as type 1 diabetes, chronic myocarditis and idiopathic heart failure, are associated with CVB. Sero-epidemiological surveys have associated enterovirus infections during pregnancy with increased risk for offspring to become diabetic, even years after birth. Some case histories and experimental data in mice indicate that these viruses may incidentally be transmitted vertically or by (fecal) contamination during, and shortly after birth. Few case reports suggest that infection during pregnancy leads to preterm delivery, fetal growth retardation, or even embryopathy. These observations, suggesting vertical transmission, have still to be confirmed.

Project Objectives: Systematic data/sample collection for determining 1. incidence of CVB infections during pregnancy in different countries 2. effect of CVB infections on the course of pregnancy 3. transmission of the virus to fetus/neonate and resulting complications due to infections 4. follow-up of children of mothers infected during pregnancy 5. factors (socio-economic, nutrition, smoking) influencing the course of pregnancy and children of the infected mothers.

Countries involved in sample/data collection and testing: Croatia, Czech Republic, Slovak Republic

Expected Results: A conclusive systematic data on the CVB infections during pregnancy, consequences of these infections and mode of transmission.

Influence of single and multiple mutations on the pathogenesis of CVB2 strain in mouse model.

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Coxsackieviruses B (CVB) are human enteroviruses which cause a wild spectrum of diseases. Coxsackievirus B2 (CVB2O) the prototype strain is capable of persistent infection in rhabdomyosarcoma (RD) cells. This strain was adapted to RD cells provoking cytolysis (CVB2ORD). Two amino acid changes were identified in the capsid and one in the 2C region of the adapted virus.

Objective: The aim of this study was to compare the pathogenesis of mutated CVB2 in VP1 and 2C region and the prototype in an experimental mouse model.

Methods: Male A/J mice were intraperitonally infected with the CVB2O, CVB2ORDII VP1 (cytolytic in RD cells), CVB2OVP1 I118F, CVB2OVP1 Q164K (cytolytic in RD cells), and CVB2O2C K185R. Controls were mock infected. Mice were sacrificed on days 0, 5, 7, 10, and 55 post infection (p.i.) and different organs (pancreas, heart, lungs) were collected for detection of the viral RNA (using RT-PCR and nested PCR) and histological studies.

Results: Pathological changes were absent in hearts of all infected mice. Control mice did not show any histopathological changes. We observed maximum changes in the pancreas of CVB2OVP1 Q164K infected mice, interstitial inflammation of the exocrine pancreas was seen until day 55 p.i. as compared to the other mutants and CVB2O prototype. Infiltration of cells was observed in pancreas of CVB2ORDII VP1 infected mice only until day 10 p.i. We have detected persistent viral RNA only in hearts of CVB2O and pancreas of CVB2OVP1 Q164K infected mice on day 55 p.i.

Conclusion: Strains CVB2OVP1 I118F and CVB2O2C K185R were least virulent. In the strain CVB2OVP1 Q164K, a single amino acid change in the protein VP1 therefore appeared as a virulence factor, which was also associated with viral persistence in the pancreas.

Keys words: CVB2, mouse model, mutations, pancreas, rhabdomyosarcoma cells

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Maternal morbidity as a risk factor for preterm birth in Eastern Croatia: retrospective study

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Introduction: Preterm birth (PTB) is a major problem associated with perinatal morbidity and mortality. It remains one of the most serious problems in obstetrics. The aim of this study is to identify maternal morbidities as a risk factors associated with preterm birth.
Methods: A retrospective study was conducted on 860 cases of PTB compared with 860 cases of term birth (TB). Data was stratified for previous and current medical histories, prepregnancy and complications of current gestation. Gestational age of <37 weeks was defined as PTB and 37–40 weeks as TB.
Results: Common risk factors associated with preterm birth were family history of PTB (12,21 % PTB vs. 2,21 % TB), Caesarean section surgery (5,23 % PTB vs. 0,47 % TB), myoma surgery (1,74 % PTB vs. 0,56 % TB), tobacco use (19,54 % PTB vs. 14,30 % TB). Common morbidities were: vaginal bleeding during pregnancy (5,58 % PTB vs. 1,63 % TB), cerclage (3,84 % PTB vs. 0,23 % TB), pre-eclampsia (9,54 % PTB vs. 1,63 % TB) and diabetes (3,61 % PTB vs. 1,05 % TB).
Conclusions: The main risk factors for preterm delivery were family history of PTB and Caesarean surgery. The most common morbidity was vaginal bleeding during pregnancy and pre-eclampsia.
Keywords: preterm birth, risk factors, maternal morbidity

Assisted reproductive technologies and neonatal intensive care utilization

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Introduction: Over the past three decades, over 4 million births worldwide have been achieved via assisted reproductive technologies (ART). Adverse neonatal reproductive outcomes from ART include congenital anomalies and prematurity. In order to estimate the benefit and cost of ART, we must measure the neonatal outcomes and long term health and development of babies conceived via ART compared with outcomes of individuals from unassisted conception.

Methods: We examined the prenatal, birth, and newborn histories of babies delivered at Cedars-Sinai Medical Center (CSMC) in 2012. Four reproductive endocrinology and infertility centers conducted approximately 1200 IVF cycles in 2012. We examined the prenatal record, OB admission history, and newborn nursery or NICU database. We defined "IVF" as fertility treatments in which both eggs and sperm are manipulated in the laboratory, and non – IVF infertility treatment as controlled ovarian stimulation and intrauterine insemination. This study was approved by the CSMC Institutional Review Board.

Results: IVF pregnancies resulted in ca. 416 newborn nursery admissions, 52 NICU admissions, and 1 mortality (0.24 %). Although the 52 babies represented 10.2 % of NICU admissions, they consumed 1663, or 17.9% of NICU patient-days. 72 % of IVF-NICU patient days represented IVF twins. Morbidities of very low birthweight (VLBW) ART neonates were not significantly different from VLBW neonates from unassisted conception.

Conclusions: ART neonates account for 10.2 % of NICU admissions and 17.9 % of NICU bed-days at CSMC. The majority of NICU utilization derives from IVF premature, multiple gestation neonates, particularly twins. ART VLBW mortality and morbidities appear similar to a birth-weight and gestational age matched contemporary cohort. Thus, ART neonates represent a significant cumulative reproductive and neonatal healthcare expense. Future research will focus on elucidating genetic and/or epigenetic factors that contribute to adverse outcomes of ART, and the site-specific variation in premature births, NICU mortality and morbidities due to ART.

Keywords: assisted reproductive technology, neonatal intensive care, *in vitro* fertilization, healthcare economics

Effects of early hyperglycaemia and insulin treatment on the retinal structure of rat pups

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Aims: In spite of major advances in understanding of the pathogenesis, retinopathy of prematurity (ROP) is still one of the leading cause of childhood blindness in developed countries. Rat pups are applicable to investigate specific role of the factors which are implicated in the pathogenesis of ROP including hyperglycaemia and insulin treatment. The aim of our study was to investigate specific effect of streptozotocin-induced hyperglycaemia, insulin-treatment and intravitreal injection of a potential retinoprotective agent, pituitary adenylate cyclase activating polypeptide (PACAP) on the rat pups' retina.
Methods: We made a comparative analysis between the following treatment-groups: controls (Stz-/Ins-), insulin-treated (Stz-/Ins+), hyperglycemic (Stz+/Ins-), insulin-treated hyperglycaemic (Stz+/Inz+); all animals were treated with intravitreal PACAP or vehicle. Blood glucose levels were monitored. After decapitation (P21) retinas were processed for routine histology and immunohistochemistry for glial fibrillary acidic protein (GFAP), GLUT1 and tyrosine hydroxylase (TH).
Results: Standard histological methods revealed no major differences between the groups. Elevated expression of GFAP - as an aspecific marker of metabolic insults in the retina- was detected from the inner retina in the Stz-/Ins+ group, although hypoglycaemia did not develop. Similar alteration of the GFAP staining was found in the hyperglycaemic (Stz+/Ins-) and insulin-treated hyperglycaemic (Stz+/Inz+) groups. Intravitreal PACAP resulted in suppression of the elevated GFAP expression in the Stz-/Ins+ group, but not in the Stz+/Ins-, and Stz+/Inz+ ones. None of the groups showed alteration in the anti-TH immunoreactivity (dopaminergic amacrine cells) or GLUT1 expression of pigment epithelial cells.
Conclusion: In our model hyperglycaemia or insulin did not induce ROP, however, sign of metabolic insult was detected in the neural retina, which was partly prevented by intravitreal PACAP application.
Keywords: retinopathy of prematurity; hyperglycaemia; insulin; rat model

The fetal splenic vein flow pattern and fetal inflammatory response in preterm prelabor rupture of membranes.

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Objective: To evaluate the intensity of the fetal inflammatory response, characterized by umbilical cord blood IL-6 levels, and neonatal outcome in preterm prelabor rupture of membranes (PPROM) pregnancies with the pulsatile fetal splenic vein flow.

Methods: Women with singleton pregnancies complicated by PPROM in gestational age between 24+0 and 36+6 were included into the study. Doppler evaluation of the fetal splenic vein flow was performed. The flow-velocity waveform pattern was evaluated qualitatively as continuous or pulsatile. The umbilical cord blood interleukin-6 (IL-6) levels were evaluated after delivery.

Results: In total, 75 women were included. The fetuses with pulsatile splenic vein flow exhibited higher umbilical cord blood IL-6 levels than fetuses with continuous flow (pulsatile flow: median 45.9 pg/ml vs. continuous flow: median 8.2 pg/ml; p = 0.005). Pulsatile flow was associated with higher rate of early onset neonatal sepsis (OR 6.0; 95 %CI 1.2-30.1).

Conclusion: The presence of pulsatile splenic vein flow in PPROM pregnancies was associated with a higher fetal inflammatory response and higher rate of early onset neonatal sepsis.

Effects of maternal smoking on the early neurobehavioral development of rat pups

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AIMS: Maternal smoking impairs the developing fetus, which results in delayed cognitive and motor development, retarded locomotor behavior, moreover, an increase in the incidence of psychological abnormalities also has been noticed. The aim of the present study was to investigate the influence of maternal smoking during pregnancy on the early physical and neurological development of the newborn rat pups.

METHODS: Wistar rats were exposed to whole-body smoke exposure for 40 minutes daily from the mating until delivery, except for the control group. After delivery, animals were divided into 4 groups: gestational smoker rat mothers (M+) with pups who were exposed to smoke prenatally (P+) and control pups (P-); gestational non-smoker rat mothers (M-) with pups who were exposed to smoke prenatally (P+) and control pups (P-). After that the offspring were tested for somatic and neurobehavioral development daily for 21 days. On the 4th and 5th weeks of life a motor-coordination test (foot-fault test) was carried out. Data were compared to that of the control group by using ANOVA and T-probe as the statistical methods.

RESULTS: Our results showed significant differences between the data of group P+ vs. P-, independently of the raising mater. Some parameters like eye-opening, ear unfold, incisor eruption, ear twitch, acoustic startle reflex appeared earlier in P+ than P-. On the other hand we observed a delay in the development of some other reflexes like crossed extensor reflex, air righting and hind limb placing. In case of the motor-coordination test there were no differences between the groups.

CONCLUSION: These results suggest that maternal smoking during pregnancy may impair the neurological maturation of rat pups. Our finding may help to reveal the later, adulthood effects of prenatal smoke-exposure.

KEYWORDS: maternal smoking, neurobehavioral test, rat pups

Postpartum anemia and iron administration

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Background: In postpartum the anemia is very frequent, and it should be corrected without delay. Anemia due to heavy bleeding during spontaneous delivery or cesarean section can become significant (< 10 g/dl) or severe (< 8 g/dl). Women with anaemia after childbirth may feel tired and breathless and are at risk of infection.

Objective: The aim of our study was to observe the effectiveness of intravenous iron supplementation is more effective in a shorter period

Materials and methods: It was a prospective study on 188 postpartum women with Hb < 8 g/dl in the first 48 hours after delivery treated with intravenous (i.v.) Iron sucrose. The dose of iron sucrose was between 200 mg Fe (2 ampoules diluted in 250 ml 0.9 % NaCl infused over 60 minutes) at day first until the maximum dosage 800 mg (200 mg/day, 2 ampoules in 4 days). The hemoglobin values, the hematocrit and erythrocytes indices were monitored in the day 4 and 7 after the first perfusion.

Results: Increase in hemoglobin value was 0,92 g/dl after 4 days and 1.96 g/dl after 7 days. The beneficial effect of i.v. therapy was statistic significant ($p < 0,0001$). In the day 7 all the erythrocytes indices were significant improved. The incidence of severe anemia (Hb < 8g/dl) was 73,6 %, and just one needed heterologous blood transfusion immediately after cesarean section because of a severe low proteinemia. The incidence of adverse reactions was very low –tegumentar rush, facial eritema, mild chills during infusion of the second ampoule and one case of venous thrombosis. Comparing this group with a lot of 180 postpartum women with Hb < 8 g/dl in the period 1999–2000, by using i.v. sucrose iron were avoided heterologous blood transfusions in 73 women.

Conclusion: The results suggest that intravenous iron as a total dose infusion is able to replenish iron stores more efficiently, completely and at a faster rate than oral iron therapy, thus providing the fuel for stimulation of full erythopoiesis. Also, it represents an effective alternative to blood transfusions in the treatment of postpartum severe anemia

Keywords: postpartum anemia, iron supplementation in postpartum, i.v. iron.

Soluble TLR2 in amniotic fluid - new marker in management of women with preterm prelabor rupture of membranes (PPROM)

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Aims: To determine amniotic fluid soluble Toll-like receptor 2 (sTLR2) levels in PPROM according to the presence of infectious histological chorioamnionitis(HCA) . To test the sTLR2 cut off level of 222.7 ng/mL.

Methods: Forty four women with PPROM and a gestational age between 24 + 0 and 36 + 6 weeks were included in the cross sectional study (23 women with infectious HCA, 21 without infectious HCA). One hundred sixty nine women with PPROM and a gestational age between 24 + 0 and 36 + 6 weeks were included in a prospective cohort study.

Amniocenteses were performed, and sTLR2 in the amniotic fluid were determined using ELISA.

Results: Women in prospective cohort study with infectious HCA had significant higher sTLR2 levels (median: 311.3 ng/mL) than other women (40.8 ng/mL; $p < 0.0001$). The cutoff level 222.7 ng/mL had a sensitivity of 63 %, and specificity of 98 %, and a likelihood ratio of 40.3 for the prediction of infectious HCA.

Conclusion: Amniotic fluid sTLR2 is a promising predictor of infectious HCA with high specificity in PPROM.

Analysis of risk factors by type of preterm birth

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Background: Prematurity is a major public health concern and is the leading cause of perinatal mortality and long term morbidity. A better understanding of the events that lead to preterm birth and of the modifiable risk factors it is a public health priority.

Objective: The aim of this study was to identify current risk factors associated with preterm delivery, statified by gestational age at birth.The data collection was under the guideline of the RECOOP HST Consortium Mother and Child Health Network Prospective Study and supported by POSDRU/107/1.5/S/82839 project .

Materials and methods: This is a prospective study – part of the larger multinational prospective study MOCHEA Research Network. We analyzed 4078 births at „Dr. I. Cantacuzino Hospital, Bucharest, Romania in 2011–2012. Preterm deliveries were divided into the following sub-groups: extremely preterm (<28 weeks gestation), very preterm (28–32 weeks gestation), moderately preterm (33–34 weeks gestation) and late preterm (35–36 weeks gestation) according the data of the World Health Organization.

Results: Of these 4078 birth, 474 (11,62 %) were preterm births (< 37 weeks): 13 extremely preterm deliveries (0,32 %), 118 (2,89 %) very preterm, 115 (2,82 %) at 32–34 weeks gestation and 228 (5,59 %) were late preterm biths.

Prior spontaneous abortion, vaginal bleeding and abnormalities of the uterus were risk factors for preterm delivery occuring at < 28 weeks gestation.

Risk factors identified for 28–32 and 33–34 weeks of gestational age case subgroups were similar. Diabetes and uterine surgery in antecedents were significant risk factors for delivery in 35–36 weeks gestational group.

The influence of socioeconomic status on preterm delivery is well recognized and in our study this was revealed by the level of investigations during pregnacy.

Conclusion: The determinants of preterm delivery are multifactorial and involve subcategories with different etiologies. The focus should be done on modifieble risk factors in prevention of preterm birth.

Keywords: preterm birth, risk factors, late prematurity

HIF 2β as a marker of oxygenation in human placental explants

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Background: Normal development and function of the placenta to a considerable extent depends on the oxygen concentration, the contents of which change during pregnancy. The pathological limitation of oxygen may lead to the complications of pregnancy (Pringle et al., 2010). Placental cells adapts to hypoxia with the help of specific transcription factors: HIFs (hypoxia inducible factors). It could be used as a marker of tissue oxygenation. HIF-2β gene is actively expressed in the placenta. The regulation of HIF-2β expression occurs at the posttranslational (Patel et al., 2010) and pretranslational levels (Rajakumar et al., 2000). The placental explants represent a recognized approach for the study of biochemical processes in the placenta because of preserved tissue architecture, in contrast to isolated cells or cell lines (Huppertz et al., 2011). However, the favourable oxygenation conditions for explants cultures are not well established.
Methods: The viability of explants during cultivation was estimated by Lactate Dehydrogenase test. Expression of HIF-2β at the mRNA level was determined by RT-PCR. The objects of the study were mature human placental explants. Expression of HIF-2β in explants was compared to it in the placenta shock-frozen no more than 15 minutes after birth.
Results: It was found that after 6 hours of cultivation in low oxygen conditions the mRNA level of HIF-2β in explants increases 7 times compared to its basal level in freshly isolated tissue. While the explants cultivated in 21 % O_2 atmosphere, has shown HIF-2β expression level comparable to fresh placenta. During the whole period of cultivation the activity of lactate dehydrogenase in the medium were the same either in 21 % or in 8 % O_2 atmosphere that proofs the viability of explants in both conditions.
Conclusions: Our studies have shown that the HIF-2 β mRNA level is a sensitive parameter for hypoxia detection. The most suitable conditions for the cultivation of explants require the atmosphere of 21 % oxygen, which is demonstrated with the oxystatus similar to what is observed in the freshly isolated tissue.
Keywords: Placental explants, hypoxia, HIF-2 β, mRNA

Genetic predisposal to pregnancy loss

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AIMS: The immunological mechanisms of pregnancy maintenance are an important issue of the reproduction. Their dis-balance in the genetically determined Th1- and Th2-cytokine levels may be one of the causes of early fetus elimination. Chromosomal anomalies in couples with the recurrent pregnancy loss vary from 4.8 % to 5.2 %. The aim of this study is to analyze cytogenetic features of couples with the recurrent pregnancy loss (RPL) in conjunction with studying the genetic polymorphisms of IL-10 and IFN-γ genes associated with cytokine levels in women with recurrent pregnancy loss, and to measure blood levels of IL-10 and IFN-γ in these women.

METHODS: DNA extraction from peripheral blood cells, PCR, agarose gel electrophoresis, ELISA, *in vitro* PHA-stimulated peripheral blood leukocytes, GTG and CBG chromosomes banding, conventional karyotyping.

RESULTS: Karyotyping of 366 couples (732 individuals) with early recurrent pregnancy losses in the anamnesis revealed chromosomal anomalies in 4.09 % (28 cases), and within them 2.87 % carried reciprocal translocations: 0.82 % – Robertsonian translocations, 0.55 % – numerical and structural gonosomal anomalies, and 0.27 % – marker chromosome of unknown origin. The risk of early reproductive losses in women after excluding the cytogenetic component increases three fold if SNPs 1082GG, 592CC, 819CC of IL10 gene and 875AA + 875AT of IFN-γ are present. ELISA measurement of blood serum IL-10 and IFN-γ showed a significantly higher level of IFN-γ in women with early reproductive losses ($P < 0.05$) in comparison with the reproductively healthy women.

CONCLUSIONS: A complex of cyto- and immunogenetic investigations in couples with the recurrent pregnancy loss is proposed.

KEYWORDS: recurrent pregnancy loss, chromosomal anomalies, single nucleotide polymorphisms, IL-10 and IFN-γ.

Poster Sessions

Cardiovascular Diseases

Differences in plasma content of glucose, C-reactive protein, uric acid and cholesterol in male, female and ovariectomized rats under acute and chronic stress – a pathway to develop cardiovascular diseases

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Aims: Stress has a major impact upon the circulatory system and it plays a significant role in susceptibility to cardiovascular diseases. Epidemiological studies indicate that there is a significant difference in cardiovascular function between men and women. To explore these differences, we analyzed serum levels of glucose, C-reactive protein, uric acid and cholesterol in male, female and ovariectomized rats under acute and chronic stress. Also we performed glucose tolerance test on all rats to see the influence of stress on serum glucose levels. It is known that cardiovascular diseases are more prevalent in males, so we expected to see significant differences between serum biochemical analysis of male, female and ovariectomized rats. Methods: At age of 3 months 69 Sprague Dawley rats were sacrificed after subjecting to chronic and acute stress. 19 rats were chronic and 32 rats were acutely stressed and 18 rats were a control group. 25 females were ovariectomized according to the protocol (Harlan HUS-QREC-PRD-932, Issue: 01, Revision 03). Glucose tolerance test was performed one day before sacrificing. Blood was drawn directly from the heart and serums were separated by centrifugation. Biochemical analyses were performed at the biochemical laboratory of University Hospital Centre Osijek for the serum content of glucose, C-reactive protein, uric acid and cholesterol for all animals. Results: Glucose tolerance test of chronic stressed animals shows that female rats have the lowest glucose tolerance and also the highest serum level of glucose which is much higher if compared to starting value of glucose tolerance test and can be explained as additional stress during sacrificing and collecting the blood from the heart. Males and ovariectomized rats also show higher serum level of glucose than the starting point of glucose tolerance test at chronic stress. Glucose tolerance test of acute stressed animals shows similar trend, but with males having the lowest glucose tolerance and also the highest serum level of glucose. Female and ovariectomized rats also show higher serum level of glucose than the starting point of glucose tolerance test at acute stress. Glucose tolerance test and serum levels of control animals show the same trend as acute stress with males having the worst glucose tolerance and the highest level of serum glucose, which can be explained as glucose tolerance test and drawing the blood from the heart being the first acute stress animals have experienced. C-reactive protein level at acute stress is highest in ovariectomized group, but at chronic stress males and ovariectomized females reach the same level. Serum uric acid at acute stress is highest in male group and rich similar level at chronic stress in both female groups. Cholesterol level shows the same pattern in both, chronic and acute stress – males tend to have the lowest values and ovariectomized rats the highest values of serum cholesterol. Cholesterol control group shows the opposite values where males have the highest values and ovariectomized group has the lowest values of serum cholesterol. Conclusions: We observed significant differences between serum content of glucose, C-reactive protein, uric acid and cholesterol in male, female and ovariectomized rats under chronic and acute stress. Also, we conclude that procedures of glucose tolerance test and drawing the blood from the heart have the same impact as acute stress. In ovariectomized group stress has the major impact on cholesterol levels at both acute and chronic stress. According to our results it seems that acute stress has higher impact on male and chronic stress has had higher impact on female rats.

Vascular responses to acetylcholine- and hypoxia-induced dilation of diabetic ovariectomized rats

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The aim of this study was to determine the effect of ovariectomy at 3rd week of age on vascular relaxation responses in healthy and diabetic female Sprague-Dawley (SD) rats.
Materials and Methods: Twelve healthy, ovariectomized SD rats were divided in 4 groups: a) 12-weeks old control group (N = 3); b) 16-weeks old control group (N = 3); c) 12-weeks old (6 weeks DM) group (N = 3); d) 16-weeks old (10 weeks of DM) group (N = 3). Diabetes mellitus (DM) was induced by streptozocin 60mg/kg i.p. at 6th week of age, and duration of DM was 6 and 10 weeks, respectively. Prior to decapitation, rats were anesthetized with 75 mg/kg ketamine + 2.5 mg/kg midazolam. Thoracic aortic rings were used to test acetylcholine (ACh) response (10^{-9}–10^{-5} M) and response to reduced pO_2 (bath gas mixture containing N2 95 %, CO_2 5 %) after precontraction with noradrenaline (NA) for 5 minutes, in the absence/presence of the NOS inhibitor L-NAME or COX-1,2 inhibitor indomethacin (INDO) in the tissue bath. Viability of rings at the end of the hypoxic protocol was tested with their ability to contract in 95 % O_2– 5 % CO_2 bath solution. To test differences among groups Two-way ANOVA was used. Statistical significance was set at $P < 0.05$ (SigmaPlot v11.2, Systat Software, Chicago, USA).
Results: 6 weeks DM rats had significantly reduced ACh induced relaxation (AChIR) compared to respective age-control group ($P < 0.01$ for 10^{-7} M ACh concentration). L-NAME blocked AChIR in all 4 groups of rats ($P < 0.001$ for 10^{-7}–10^{-5} M ACh concentration), while INDO significantly reduced AChIR only in 10-weeks DM rats ($P < 0.001$ for 10^{-7}–10^{-5} M ACh) compared to respective age-control.
Hypoxia induced significant relaxation (HIR) in all study groups. L-NAME blocked HIR in 12-weeks old control group, while INDO blocked HIR in 16-weeks old control group. In 16-weeks old DM group, both L-NAME and INDO significantly blocked HIR.
Conclusion: These results demonstrate that: 1) vascular reactivity is impaired in diabetic rats; 2) ACh–induced vasorelaxation is mediated by NO in control rats; 3) metabolites of COX-1,2 might play role in ACh- and hypoxia-induced relaxation in DM rats.
Key words: vascular function, acetylcholine, hypoxia, ovariectomy

Epicardial adipose tissue thickness as predictor for adiponectin systemic blood concentration

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INTRODUCTION: Epicardial adipose tissue (EAT) is a known risk factor for CAD and metabolic syndrome. Adiponectin (ADIPOQ) is responsible for the modulation of metabolic syndrome. Also, ADIPOQ exerts its antiatherogenic effects on the vascular endothelium.

The aim of this study is to predict possible concentrations of ADIPOQ, IL6 and TNFa in the aortic root (AR) and coronary sinus (CS) using EAT thickness.

METHODS: In this study 34 subject were included, 16 with CAD and 20 controls all of whom underwent cardiosurgery. Prior to cardiosurgery, EAT thickness was measured by echocardiography and coronarography was performed in all subjects. During cardiosurgery, blood samples were taken from AR and CS. ADIPOQ, IL6 and TNFa concentrations were measured in the AR and CS.

RESULTS: EAT thickness is positively correlated with IL6CS concentration ($r = 0.418$, $p = 0.024$) and negatively correlated with ADIPOQAR ($r = -0.625$, $p < 0.001$) and ADIPOQCS ($r = -0.631$, $p < 0.001$). EAT thickness did not show correlation with TNFa concentration in AR or CS. MARS analysis showed that ADIPOQAR ($R2 = 0.529$) and ADIPOQCS ($R2 = 0.539$) concentration can be predicted with EAT thickness. Also, MARS analysis showed that IL6CS concentration can be predicted ($R2 = 0.159$) but with very low accuracy.

CONCLUSION: EAT thickness, known CAD risk factor, is positively correlated with IL6CS concentration, which indicates that ticker EAT is producing more proinflamatory cytokines. Also, EAT thickness is negatively correlated with ADIPOQAR and ADIPOQCS. EAT thickness can be used, fairly accurately as predictor for adiponectin systemic concentration.

KEYWORDS: adipse tissue, adiponectin, concentration, interleukin 6, tumor necrosis factor alpha

Pain-to-hospital times, cardiovascular risk factors and early intra-hospital mortality in myocardial infarction

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Background: Late admission to hospital and high prevalence of cardiovascular risk factors remain a significant public health problem in patients with acute myocardial infarction (AMI).

Methods: For 778 patients treated for AMI at the Coronary Care Unit (CCU) of University Hospital Split, Croatia in 2010 and 2011, the following data were acquired: outcome during hospitalization (survived, deceased), cardiovascular risk factors (hypertension, diabetes, dyslipidemia, previous myocardial infarction, smoking) and time from the onset of pain to admission at CCU.

Results: Among 778 patients treated for AMI there were 291 (37 %) women and 487 (63 %) men. Forty-five patients (6 %) died during hospitalization, mostly due to cardiogenic shock. An association was found between early intra-hospital mortality and the following risk factors: age > 70 years, female gender, previous myocardial infarction and smoking. Median pain-to-call time was 2 h, and median time from the onset of pain to arrival into CCU was 4 h. There were 59 (7.6 %) patients admitted to CCU within the recommended 90 min. Diabetic comorbidity was not associated with early death or with longer time from pain to the emergency call.

Conclusion: Compared with studies in previous years, the number of patients treated for AMI at University Hospital Split decreased, as well as the hospital mortality, while prehospital delay shortened. However, prehospital delay is still too long. These findings emphasize the need for prevention programs targeted to reduction of cardiovascular risk factors and shortening time from the onset of pain to a patient's call for help.

Key words: prehospital delay, cardiovascular risk factors, acute myocardial infarction

Comparison of coronary artery disease risk factors in coastal and continental Croatia in acute coronary syndrome patients

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Aim: Coronary artery disease (CAD) is the most common clinical manifestation of ischemic heart disease. It is usually manifested in obese persons, on high-fat and high-energy diet, with sedentary lifestyle and smoking. Aim is to determine a difference in CAD risk factors between patients with different life habits, those living in coastal and those in continental part of Croatia.

Methods: This comparison focuses on clinical data concerning CAD risk factors in acute coronary syndrome (ACS) patients from the two centers, Split and Osijek, with different lifestyles. Patients' demographics, symptoms, history, electrocardiographic, laboratory parameters and diagnostic tests were collected and statistically processed. Chi-square, Shapiro-Wilk, Kolmogorov-Smirnov and Mann-Whitney U-tests were used in statistical analysis.

Results: 254 patients with ACS were included in this study. 111 patients enrolled were from Split and 143 patients from Osijek. We found that patients in continental Croatia had more infarction with ST elevation ($p < 0.001$), with higher CK and MB fraction values, as well, and significantly longer hospital stay ($p < 0.001$). Patients with ACS in coastal region were older (76.43 ± 10.32 vs. 66.69 ± 11.68 years; $p<0.001$), had higher total cholesterol ($p = 0.045$) and LDL fraction ($p = 0.021$) levels, and higher triglycerides values ($p < 0.001$), as well. Conservative therapy for those patients was treatment of choice in Osijek and PCI has been more performed in Split. We found no differences in other parameters and tests we followed in these patients.

Conclusion: Habitants in continental part of Croatia have higher occurrence of ACS and more severe clinical course than patients in coastal Croatia, which is correlated with longer duration of hospitalization.

Redox potential of glutathione in erythrocytes of ischemic heart disease patients

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Relevancy "redox hypothesis" suggests that the circulatory system generated more non-radical oxidants than free radicals, confirmed the prognostic value of Cys/SySS ratio in plasma to assess the risk of cardiovascular disease (Go Y.-M., Jones D.P., 2011). The role of the other glutathione (GSH)/oxidized glutathione (GSSG) pair of erythrocyte predetermining blood rheology and redox signaling in the circulatory system has been studied enough.

In the red blood cell of 118 patients with acute coronary syndromes (ACS) (mean age 55,2 ± 0,9 years) and 144 patients with ischemic heart disease (IHD) (stable angina II–III FC) (mean age 55,3 ± 0,6 years), and 89 healthy subjects (mean age 42,2 ± 0,8 years) indicators of the glutathione system were studied based on which (the Nernst equation) its redox potential (Eh) is defined.

It was revealed that in patients with ACS concentrations of total glutathione (tGSH), GSH and GSSG, the value of Eh and the activity of glutathione reductase (GR) were not different form the controls. The activity of glutathione peroxidase (GP) decreased and the concentration of GSSG increased. The above changes are characteristic for the subgroup of patient with-ST-elevation myocardial infarction (STEMI), whereas in patients without-ST-elevation myocardial infarction (non-STEMI) a marked decrease in the tGSH, GSH concentrations, GSH/GSSG ratio was observed. The Eh oxidation increased by 17 mV. In patients with chronic ischemic heart disease, changes in the system of glutathione were similar to those seen in patients with non-STEMI, at that the combination of IHD and type 2 diabetes the falls in the glutathione system was dramatic with the increasing Eh towards oxidation state at 38 mV.

The prognostic role of Eh is suggested contributing to fatal IHD complications and control of therapeutic efficacy.

Keywords: glutathione system, redox potential, erythrocytes, coronary heart disease, myocardial infarction

Comparison of risk factors in female patients with menopause treated in Clinical Hospital Center Split for acute coronary syndrome

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Aim: Aim of this study is to determine the difference in risk factors in female menopause patients treated for acute coronary syndrome in Clinical Hospital Center Split, in two different periods, years 2007 and 2011.

Methods: 187 female menopause patients admitted in Coronary Care Unit Split for the acute coronary syndrome (ACS) were enrolled in the study. Patient's habits, history, demographics, presenting symptoms, electrocardiography, laboratory tests and diagnostic tests data were collected and statistically processed. Categorical variables were analyzed using Chi-square tests, and continuous variables were first analyzed for normality using the Shapiro-Wilk and Kolmogorov-Smirnov tests, and then compared using the non-parametric Kruskal-Wallis (with Bonferroni correction for posthoc analysis) and Mann-Whitney U-tests.

Results: 187 menopause female patients were followed in this study. 86 women were in enrolled year 2007. and 101 patients were enrolled in year 2011. Women with ACS treated in year 2011 were older (70.95 ± 11.44 vs. 67.86 ± 12.93 years), less housewives (45.5 %) and less working (6.9 %), as well. Regarding their social status, women in the year 2007 were more active, less retired (14.7 %). ACS patients hospitalized in year 2007 had more complications, especially hearth failure ($p = 0.002$) and ventricular arrhythmias ($p = 0.008$). Those women had higher LDL cholesterol (5.50 ± 1.26 mmol/L vs. 4.9 ± 1.31 mmol/L; $p = 0.015$) and triglycerides levels ($p = 0.002$), as well. Among women treated for ACS in year 2011 there were more hypertensive patients ($p = 0.016$) and those with periphery artery disease ($p = 0.005$). We found higher incidence of ST-elevation myocardial infarction ($p < 0.001$), with higher values of CK ($p = 0.018$), CKMB ($p = 0.04$) in year 2011. Because more pathological findings on coronary angiography were found [left main stenosis ($p = 0.003$), LAD stenosis ($p < 0.001$), Cx stenosis ($p < 0.001$) and RCA stenosis ($p < 0.001$)], significantly more PCI procedures ($p < 0.001$) and CABG ($p = 0.038$) have been performed in the year 2011. In other tests that were done we did not find any significance.

Conclusion: This clinical study proves that in the year 2011 only patients in life threatening conditions under acute coronary diagnosis were treated in Clinical hospital Split. That means that our primary health care system has been improved since 2007 because less severe were not admitted in hospital, and were treated in the primary care system. More coronary angiographies, PCI and CABG treatments were done in 2011 year, which means that clinical practice and standard procedures in Clinical hospital Split have been improved, especially for treatment patients with acute coronary syndrome.

Variants within HNF1α and ANGPTL4 genes and acute coronary syndrome in Czech population
The GENDEMIP study.

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BACKGROUND: Atherosclerosis is a complex arterial disease involving interactions of multiple genetic and environmental factors. Large number of genetic polymorphisms associated with atherosclerotic diseases was identified in recent years. We investigated the possible association between hepatic nuclear factor (HNF1-α) and angiopoietin-like 4 (ANGPTL4) single nucleotide polymorphisms and risk of acute coronary syndrome (ACS) in the Czech population.

MATERIALS AND METHODS: 1,182 patients (835 males and 347 females) with ACS and 1,200 healthy controls (827 males and 373 females) in both groups younger than 65 years were included in the study. Rs7310409 (A > G within the HNF1-α gene) and rs116843064 (G > A within the ANGPTL4 gene) were genotyped using TaqMan genotyping assays.

RESULTS: The frequency of genotypes in patients with ACS did not significantly differ (AA = 17.1 %, AG = 46.6 %, GG = 36.2 %) from the control group (AA = 14.4 %, AG = 50.3 %, GG = 35.3 %, P = 0.12) neither for rs7310409 nor for rs116843064 (AA = 0.1 %, AG = 3.5 %, GG = 96.4 % vs. controls AA = 0.1 %, AG = 4.2 %, GG = 95.7 %, P = 0.69) polymorphism. There was no interaction with gender. In addition, gene variants were not associated with common cardiovascular risk factors (dyslipidemia, hypertension, smoking, obesity and diabetes).

CONCLUSIONS: We didn´t find any association between polymorphisms within HNF1-α and ANGPTL4 genes and risk of ACS in the Czech populations.

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Heart rate variability reflects the depth of oxidative stress in subjects from various functional groups

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Background: Oxidative stress (OS) is an recognized pathogenetic mechanisms in the development of many disorders. Usually its depth is assessed by biomarkers, which level represent a result of the production and utilization and does not necessarily reflect the intensity of redox processes. The search of integrative parameters adequately reflecting the condition of aerobic metabolism remains to be important.
Aim of present research was to study heart rate variability (HRV) in subjects from various functional groups and its relationships with the pro-/antioxidant balance.
Methods: The time and frequency domain indexes of HRV were studied in short time ECG records in diabetes mellitus (DM) type 1 (n = 16) and type 2 subjects (n = 36), in patients with peptic ulcer (n = 39) and athletes (n = 36) in supine position and during orthostatic test. Healthy volunteers (n = 40) served as a control. The depth of oxidative stress was assessed spectrophotometrically in serum by the levels of TBARS, hydroperoxides, middle mass molecules, oxidatively modified proteins, while the antioxidant capacity was determined by activities of catalase and SOD.
Results: The signs of oxidative stress were found in subjects from all studied groups. In DM type 2 patients increased levels of the oxidative destruction products (ODP), high catalase and SOD activities were accompanied by dramatically low HRV (total power (TP) ranging from 600 to 1200 ms2) with predominance of the central regulatory component in the spectral structure. In athletes similar changes in pro-/antioxidant balance were associated with significantly higher HRV (TP ranged from 4500 to 9000 ms2) on account of the higher activity of the autonomic components. Decreased HRV indexes with less prominent OS signs were demonstrated in peptic ulcer and type 1 DM patients
Conclusions: Different depth of OS was demonstrated by complex study of pro-/antioxidant balance in patients from various functional groups. These changes were reflected by the distinct pattern of HRV. The study of HRV can be used in the clinical practice to monitor the functional metabolic reserve in OS associated disorders.
Key words: heart rate variability, oxidative stress, biomarker, athletes, diabetes.

Cholesterol binding motifs in ABC transporters

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In primary sequence of proteins there are couple amino acid long repeats, named short linear motifs, which can bind various target molecules, e.g. cholesterol. Binding may affect conformation of proteins and consequently their function, as well. The Cholesterol Recognition Amino acid Consensus: CRAC motif, what was the focus of our study, is a good example for such short linear motifs. We were curious: is there any pattern of the distribution of CRAC motifs in proteins interacting with cholesterol or is there any pattern difference depending on their function?

We chose a well-known protein family: the ABC transporters for our investigation. Most of these proteins work as primary pumps, so with the energy of ATP they can transport a wide range of substrates through the cell membrane. First, we explored the number of motifs and their distributions among cholesterol transporters known and transporters operate in low cholesterol-containing intracellular membranes. We could resolve significant differences both in number of motifs and in their pattern within these two groups.

Next, we wanted to see what pattern of cholesterol recognition motifs does show in another subgroup of ABC proteins: in multidrug transporters. In the beginning, we searched for all of the possible binding motifs applying sequence analyzer programs. However, for potential cholesterol binding, some more conditions should satisfy: membrane localization should meet with the appropriate position of the motif. Thus to determine which motif a potential binding site for cholesterol might be we checked the membrane topology of ABC transporters with online topology search programs, as well, and depicted potential binding motifs we have found on protein schemes.

All together we have tested 12 transporters belonging to the mentioned three ABC transporter subgroups. For demonstration purposes we have chosen two interesting transporters out of them. They are the closest homologs in their subgroup, but with opposite functions: one of them the best know cholesterol transporter, while the other does not pump cholesterol at all. The distribution of motif patterns found was in harmony again with the function of proteins. Our results support the importance of CRAC motifs in cholesterol transport and in the view of our work cholesterol may play role in the function of multidrug transporters.

Impact of two polymorphisms in FASN gene on lipids and anthropometrical parameters in Czech population

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The aim of this research is to examine SNPs of candidate gene for enzyme fatty acid synthase – FASN. This enzyme is considered as to be active in de novo synthesis of fat and consequently is suspected of influence on weight and BMI changes.

The study was performed on Czech population databases post-MONICA (n = 2622), which is based on MONICA protocol (Manual WHO/MNC 82.2, Nov-1983) and is designated as study model for cardiovascular disease. We focused on investigation of rs2229422 C>T, 1247Ala and rs2228305 G>A, Val1483Ile. Detected variants were compared with biochemical and anthropometrical parameters.

Genotype frequencies in Czech population were in 1247Ala 45.96 %, 43.82 % and 10.22 % for genotypes TT, TC and CC respectively and in Val1483Ile were 0.23 %, 5.25 % and 94.52 % for genotypes AA, AG and GG respectively. In polymorphism 1247Ala we observed significantly higher level of plasma LDL-cholesterol in T allele carriers compared with CC homozygotes with probability P = 0.012 for men and P = 0.0076 for women. Other anthropometrical and biochemical parameters were not changed. Polymorphism Val1483Ile did not affect any parameters.

Widely, it was confirmed that this gene is important in study of dyslipidemia and it will be subject to our future research.

Keywords: polymorphism, fatty acids synthase, dyslipidemia, anthropometrical parameters

Non-invasive assessment of diastolic myocardial properties in ELBW neonates

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Aim: Recent advances in the understanding of heart failure suggest that the impact of diastolic function cannot be ignored. Extremely low birth weight (ELBW) neonates might be at particular risk for developing diastolic heart failure in view of their immature myocardium. ELBW patients at risk for developing heart failure may benefit from early detection of diastolic compromise. The aim of this study was to determine values of left ventricular diastolic indices in ELBW neonates during the early postnatal transitional period using color-flow Doppler and tissue Doppler imaging (TDI).

Methods: 13 ELBW neonates (GA: 24–29 weeks, BW: 757 g) were studied by serial spectral, color-flow and tissue doppler echocardiography over the first three weeks of life. Mitral inflow velocities (E, A, E/A, A duration, Mitral DT), mitral flow propagation velocity (Vp), and tissue doppler derived myocardial velocities (medial and lateral mitral E', A', S, IVRT) were determined, and E/E', E/Vp were calculated. The values obtained were compared with those previously reported for adults.

ResultS: 25 investigations were performed in the 13 ELBW patients. Mean Vp values (39.9 +/- 10.8 cm/s) were lower than those in healthy adults. The mean transmitral peak E velocity (43.3+/-10.3 cm/s) and the mean value of E/A (0.8+/-0.17) were also lower than those in adults. Myocardial velocities were characterized with lower systolic velocities than the diastolic ones (LatS: 4.82+/- 0.74 cm/s; Lat E': 5.37 +/- 0.95 cm/s; Lat A': 7.24 +/- 1.45 cm/s). In both medial and lateral mitral annuli lower E' than A' were found in contrast to those reported in healthy adults. No significant differences were found in Vp and TDI values over the first three weeks of life.

Conclusions: The use of tissue Doppler and flow propagation velocity are feasible in ELBW neonates. Differences found in echocardiography derived diastolic myocardial properties compared to adults are suggestive for impaired left ventricular relaxation in ELBW neonates. Current approaches to hemodynamic management of ELBW neonates might not be ideal in light of the largely adult- based experience.

Keywords: diastolic function, tissue Doppler, neonate

Poster Sessions

Stress, Obesity and Metabolic Diseases

Impact of mothers' nutrition, gender and nutrition of offspring on motoric characteristics in Sprague Dawley rats

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INTRODUCTION: In this study, we were researching impact of mothers` nutrition, gender and nutrition of offspring on motoric characteristics in Sprague Dawley rats.
METHODS: Ten female rats were randomly divided in 2 groups. One group was fed with high fat diet (HFD) and other with standard laboratory chow (CD). After copulation and lactation, offspring from both groups were randomly divided in 2 groups – HFD (n=70) and CD (n=71) group. Multiple Activity Cage package was used for counting the number of vertical and horizontal movements.
RESULTS: We found significant differences in number of vertical and horizontal movements of offspring in relation with mother's nutrition and gender. Offspring of HFD fed mothers, who were fed with CD, are more physically active than HFD fed offspring. Offspring of CD fed mothers, who were fed with HFD are more physically active than CD fed offspring. Male offspring are more physically active in horizontal plane and less active in vertical plane.
CONCLUSION: Mothers` nutrition and gender with offspring diet are associated with changes in offspring physical activity.
KEYWORDS: obesity, high-fat diet, physical activity

Obesity in high school students in Bratislava region

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AIMS: Aim of the study was to evaluate the prevalence of overweight and obesity among high school students in Bratislava region.

METHODS: Data were obtained within the project „Respect for Health" – a cross-sectional, non-interventional study organized by Bratislava Self-Governing Region and Regional Public Health Authority Bratislava. We analyzed data from 4463 probands (52,2 % girls, 47,8 % boys),

14 – 22 years old. To evaluate prevalence of overweight and obesity, we used 3 different criteria: percentile charts for Slovak population based on national survey from 1991 and from 2001, IOTF (International Obesity Task Force) criteria and waist-to-height ratio as index of central obesity.

RESULTS: according to criteria applied, the prevalence of obesity among boys was 7,7–15,6 %,

and that of overweight 10,5–21,1 %. In girls, the prevalence of obesity was 3,8–10,0 %, and

9,3–14,3 % of girls were classified as overweight. Central obesity (waist-to-height ratio ≥ 0,50) was observed in 13,3 % of boys and 9,1 % of girls. Using different criteria (1991 vs. 2001, 1991 vs. IOTF, 2001 vs. IOTF) in boys' cohort, frequency distribution among the categories normal weight, overweight and obesity differed significantly ($\chi 2$, $p < 0,05$). In girls, frequency distribution did not differ significantly when criteria from 1991 and 2001 were applied ($\chi 2$, $p = 0,22$), in all other cases (1991 vs. IOTF, 2001 vs. IOTF) differences were significant ($\chi 2$, $p < 0,05$).

CONCLUSIONS: Prevalence of obesity and overweight among high school students in Bratislava region is very high, regardless the employed classification criteria. Since applying different criteria leads to diverse results, selection of criteria seem to be crucial in data evaluation and must be paid attention, especially if comparing different studies.

KEY WORDS: obesity, adolescents, BMI, cut-off values

Metabolic corrections of rat liver damage under diabetes

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Prevention of the mitochondrial oxidative damage is a therapeutic strategy in diabetes and mitochondria-targeted antioxidants are to have a therapeutic potential in diabetes. The aim of the present work was to investigate the role of a functional damage in rat liver mitochondria during diabetes as well as to evaluate the possibility of metabolic and antioxidative correction of liver disorders. Earlier we demonstrated an impairment of the antioxidative defence system during long-term streptozotocin-induced diabetes (9 weeks) (Lapshina et al., 2006). We observed some improvement of liver metabolism due to the long-term acetylsalicylic acid (ASA) administration that led to some decrease in the level of haemoglobin glycation and affected the activities of different enzymes in the liver tissue, reverting the decreased GSHPx and G6PDH activities in the diabetic rats.

Melatonin treatment (10 mg/kg b.w., 30 days, daily) under streptozotocin – induced diabetes in rats did not influence the level of hyperglycemia or glycated hemoglobin but partially reversed both the activities of the pentose phosphate pathway enzymes, G6PDH and transketolase, and GSHPx activity in the diabetic liver.

The most pronounced effect of the melatonin administration was prevention of an increase in nitric oxide levels in blood plasma and aortic tissue during diabetes. Melatonin might be considered as a factor regulating glucose metabolism by affecting glucose – metabolizing enzyme activities, restoring tissue redox-balance and nitric oxide bioavailability. The melatonin administration during diabetes reversed the decreased mitochondrial ADP-dependent respiration rate and the acceptor and respiration control ratios, demonstrating mitochondria – specific activity.

The effects of melatonin might be due to its radical scavenging properties, its metabolic effects and specific interaction with complexes of the respiratory chain. Our results suggest that melatonin regulating mitochondrial function may have a therapeutic potential for correction of diabetic liver damages.

Key words: diabetes, liver damage, mitochondria, melatonin, acetylsalicylic acid

Association of diet type with ADIPOR2 expression in hepatic tissue of second generation of rats

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INTRODUCTION: Adiponectin signalization is recognized as an important step in lipid metabolism, energy consumption, anti-inflammatory reactions and cell proliferation. Adiponectin secreted by adipocytes, acts through its receptors ADIPOR1 and ADIPOR2, latter can be found in skeletal muscle, liver and placenta. We aim to study the difference of ADIPOR2 expression in hepatic tissue of rat offspring depending on their diet and the diet during pregnancy and lactation.

METHODS: Ten Sprague Dawley female rats were at the age of 21 days divided in two groups – one was fed high with fat diet (HFD – 86 % lard, 8 % minerals, 6 % proteins), and the other one with standard laboratory chow (CD). At the age of 12 weeks the rats were mated, and their offspring were by the end of lactation (age of 21 days) randomly divided in two groups (HFD2 and CD2) and thus exposed to different diets. After sacrifice, at 40 weeks of age, liver samples were taken and using immunohistochemistry we determined ADIPOR2 expression.

RESULTS: In rats fed with high fat diet ADIPOR2 expression was increased compared to control group.

CONCLUSION: Adiponectin, and its signalling and receptors, which have not been fully researched, could have a protective role in obesity related to liver disease.

KEYWORDS: obesity, adiponectin, hepatic tissue, high-fat diet

Beneficial effects of melatonin and plant flavonoids on hepatic cell energetic during chronic and acute liver damage

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This study provides further information about the mechanism(s) of liver mitochondrial injury induced by the known hepatotoxic agent, CCl_4, and about the efficacy of the antioxidant melatonin and cranberry flavonoids in reducing the hepatotoxicity.

Multiple events, including considerable mitochondrial ultrastructure impairments, inhibition of mitochondrial enzymes (enzymes of electron-transport chain and antioxidative defense), protein modification (GSSP formation) and lipid peroxidation due to free radical attack contribute to development of liver damage and dysfunction during chronic and acute CCl_4 intoxication. We found that acute intoxication of rats (0.8 g/kg) resulted in considerable impairments of respiratory function of rat liver mitochondria without alterations in the GSH level, the high-dose acute intoxication (4.0 g/kg) led to complete uncoupling of respiration and phosphorylation, the loss of respiration control in rat liver mitochondria and decrease of mitochondrial GSH level. In the case of acute intoxication, the level of plasma nitric oxide increased and melatonin administration decreased NO level under intoxication. After 30 days of chronic CCl_4 intoxication, the functional parameters of mitochondria were similar to the control values, despite the considerable changes in redox-balance of mitochondria (rise of the mitochondrial levels of GSH, or GSSP, inhibition of mitochondrial glutathione peroxidase or succinate dehydrogenase) and mitochondrial morphology damage.

Long-term melatonin administration prevented markedly mitochondrial membrane damage and enhanced regenerative processes in the liver. Histopathological examination confirmed the hepatoprotective effects of melatonin and its combination with succinate and cranberry flavonoids. The hepatoprotective effect of melatonin is due to antioxidant, membrane-stabilizing and anti-inflammatory properties. The synergistic action of melatonin, succinate and plant polyphenols may be useful for clinical application.

Key words: mitochondrial dysfunction, liver, intoxication, melatonin, flavonoids

SSAO/VAP-1 activity in the aorta and adipose tissues – the role of gender, obesity and stress

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Semicarbazide Sensitive Amine Oxidase/Vascular Adhesion Protein 1 (SSAO/VAP-1) is a protein with dual function. As an enzyme it metabolizes primary amines to the respective aldehyde, hydrogen peroxide and ammonia, while as an adhesion molecule it participates in the accumulation of leukocytes at the site of inflammation. Its adhesion molecule function is dependent on its enzyme activity likely by using leukocyte surface bound amine as substrate. It is expressed as a membrane-bound extracellular surface protein, as well as in soluble form in the plasma. The SSAO/VAP-1 may contribute to cardiovascular diseases by several mechanisms.

It is highly expressed in the vascular tissues, in both the endothelium and the smooth muscle. The products of the enzyme reaction are more toxic than the amine substrates and may induce oxidative and carbonyl stress in the vasculature thus might promote the development and progression of endothelial dysfunction and atherosclerosis. The adhesion molecule function may also contribute to these processes by participating in the leukocyte infiltration and consequently in the development of low grade inflammation characteristic for the cardiovascular diseases.

It is also highly expressed in the adipose tissues, where it can augment the effect of insulin on glucose uptake, but it may also participate in the development of inflammation in obesity.

In this work we have studied the SSAO activity in the aortae and adipose tissues of rats to examine the gender difference and the effect of obesity and stress. The tissue samples were provided by Marija Heffer and Radivoje Radic from Osijek, Croatia.

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

The levels of S, Cu, Fe and Se in blood plasma of child-bearing age women after optimization of Selenium intake

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Most of the territory of Belarus belongs to the geochemical province on selenium and iodine. Epidemiological studies indicate the prevalence of endemic goiter in the population, iodine and selenium deficiency diseases in farm animals. Correction of the deficit is solved by iodized salt, but Se status remains low.

Thirty six female volunteers aged 18–40 years took part in the investigation. The level of Se in plasma (by absorption spectrometry (AS) method) and the levels of Se, S, Cu, Fe (by emission spectrometry (ES) method) before and after 2–4 weeks of Se-methionine administration (orally 100–200 mcg/day) were studied.

Baseline Se levels in blood plasma were 61–67 mcg/l (by AS) and 39–40 mcg/l (by ES), after the end of Se-methionine reception – 73–98 mcg/l (by AS) or 68–82 mcg/l (by ES). An increase in glutathione peroxidase activity was found. Along with an increase of Se level there were an increase of Fe, fall of Cu and stability in S content in blood plasma.

ES analysis results indicate a low Fe content and increased Cu level in relation to reference values in the plasma of surveyed persons. The trend toward normalization of these parameters, as well as the level of Se, took place at 2-week Se-methionine intake in a dose of 100 mcg/day.

Keywords: microelement status, Se-methionine, Se, S, Cu, Fe in plasma, glutathione peroxidase.

Poster Sessions

Neurological Disorders and Brain Research

Vitamin D deficiency and synaptic transmission

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Background: The key role of vitamin D in mineral homeostasis is well established. Nowadays, there are also a great number of evidence on the association of vitamin D deficiency with a range of non-skeletal abnormalities such as cardiovascular disease, cancer, stroke and metabolic disorders. Vitamin D receptors and key enzymes involved in the metabolism of vitamin D are expressed in the brain suggesting that the central nervous system is also a target organ for vitamin D. The aim of this study was to analyze the interrelations between the key presynaptic processes of neurotransmission and vitamin D deficiency.

Method: The study was performed on rats with nutritional D-hypovitaminosis, which leads to reduced $25OHD_3$, which is a biomarker for vitamin D_3 and disturbances of metabolic processes in bone tissue that correlated with osteoporosis manifestation. The experiments were carried out on rat brain synaptosomes, which retain all features of intact nerve terminals.

Results: Alterations in glutamate transport were demonstrated under conditions of vitamin D deficiency, i.e. (i) significant changes in glutamate uptake by high-affinity Na^+-dependent transporters of plasma membrane; (ii) alterations in stimulated by depolarization of the plasma membrane transporter-mediated release of the neurotransmitter and (iii) significant changes in Ca^{2+}-dependent glutamate release by means of exocytosis. Whereas no differences in tonic release of glutamate in the nerve terminals were detected. Using the cell – free model system of fusion of synaptic vesicles with target membranes, the changes in the final step of exocytosis were demonstrated. Abnormality of membrane cholesterol content in synaptosomes was found at vitamin D3 deficiency state that can create the potential basis for impairing transport of the neurotransmitters and membrane fusion process, and lead to neuronal dysfunction in patients with this pathology.

Conclusion: Our data suggest that alterations in key presynaptic processes of neurotransmission may be one of the causes of cognitive impairment associated with low level of vitamin D. Thus, this study may improve the understanding of the molecular mechanisms underlying the development of neurological consequences under conditions of vitamin D deficiency.

KEYWORDS: vitamin D; glutamate; synaptic transmission

New aspects of endocytic adaptor ITSN1 functioning in neurons

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Aims: ITSN1 is an endocytic adaptor protein that has a considerable impact in functioning of a nervous system. Changes in its expression level or disruption of its function were reported to affect synaptic vesicle endocytosis and dendritic spine morphology. Overexpression of Itsn1 gene is associated with development of Down syndrome and Alzheimer's disease. The aim of our studies is to uncover molecular mechanisms of ITSN1 functioning in neurons and pathways of its regulation.

Methods: *In vitro* binding assays, mass spectrometry, shRNA-mediated knock-down, confocal microscopy.

Results: Previously we have identified novel SH3A-binding ITSN1 partner – microtubule-associated protein STOP. Considering the role of microtubules in formation of dendritic outgrowth, we used shRNA-mediated knock-down assay in order to reveal the effect of ITSN1 level decrease on morphology of cultivated hippocampal neurons. We found that number of dendritic tips and total length of dendrites tend to increase in shRNA-transfected cells. Sholl analysis also showed an increase in complexity of dendritic tree in these cells, suggesting that ITSN1 supresses formation of outgrowths in developing neurons. We also investigated new ways of regulation of ITSN1 functioning. Since ITSN1 is a synaptic protein, we considered that it can possibly undergo Ca-dependent post-translational modifications. This suggestion was supported by bioinformatic predictions. To test this hypothesis, we performed *in vitro* kinase reaction between purified SH3 domains and coiled-coil region of ITSN1 and fraction of calmodulin-binding proteins from mouse brain protein lysate. In the presence of Ca and calmodulin both proteins were phosphorylated. To find the phosphorylation sites, we performed tandem mass spectrometry analysis of phosphorylated proteins and identified 9 sites of serine/threonine phosphorylation, 5 for coiled-coil region and 4 for SH3 domains.

Conclusions. We have identified novel ITSN1-binding protein STOP, revealed ITSN1 effect on neuron morphology and showed that ITSN1 can potentially undergo Ca-dependent phosphorylation in brain.

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Concentration-dependent effect of divalent cations on the mechanical stability of biomembranes

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Cellular and subcellular membranes separating and connecting biological compartements play a crucial role in the maintenance of cell integrity. Biomembranes must withstand forces acting on them in various biological processes and their mechanical properties may be a fundamental factor in membrane-associated mechanosensitive processes.

Here we investigated the effect of Ca^{2+} and Mg^{2+} on the mechanical stability of supported lipid bilayers (SLB-s) by using atomic force microscopy (AFM). SLB-s were prepared by incubating small unilamellar liposomes (d~100 nm) made of dipalmitoylphosphatidylcholine (DPPC) on mica substrate in the presence of 1 to 40 mM Ca^{2+} or Mg^{2+}. Liposomes sedimented, flattened, ruptured and coalesced to form SLB on mica, a process that was facilitated by scanning the region of interest at destructive settings. The formation of SLB was verified by non-contact mode AFM imaging, and at least 300 force curves were registered at different points of the SLB surface at 1 μm/s tip speed at each experimental condition.

In the vast majority of the force curves sudden transitions occured at well-defined force values that may correspond to the puncture of the bilayer by the AFM tip. The tip displacement at these events was approximately 5 nm, which corresponds well to the lipid bilayer thickness. In some cases the break-through took place in two subsequent steps, which may be interpreted as the sequential crossing of the tip through the bilayer leaflets. Detailed statistical analysis of the force spectroscopy data revealed that both Ca^{2+} and Mg^{2+} increased the break-through force in a concentration-dependent manner, with a more expressed effect caused by Mg^{2+}.

In conclusion, divalent cations increased the mechanical stability of DPPC bilayers in a concentration-dependent manner. This effect may be attributed to their ability to orient phospholipid head groups via electrostatic interactions. Because the diameter of Mg^{2+} is smaller than that of Ca^{2+}, its orientation-inducing effect may be more pronounced resulting in a more significant membrane stabilization. The local ionic environment may regulate the mechanical characteristics of phospholipid membranes in live cells as well.

Correlations of neurometabolites in dorsolateral prefrontal areas with ADHD symptoms

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Background: ADHD is the most prevalent neuropsychiatric disorder in childhood with the unknown pathomechanisms. Symptoms of this diagnosis are categorized into three clusters: hyperactivity, impulsivity and inattention. The alterations of prefrontal cortex structure, function and neurometabolite levels are the consistent findings in children and adults with ADHD. Despite number of studies evaluating the neurometabolite differences between ADHD and control subjects, there is the lack of evidence of neurometabolite associations with ADHD symptoms. We aimed to find out the correlations of neurometabolites with ADHD symptoms evaluated by clinicians and parents.

Methods: Twenty medication – naïve ADHD children at the age of 11.4 ± 1.27 years were examined by single-voxel 1H-MRS. The spectra were taken from dorsolateral prefrontal cortex (DLPFC, 8 ml) and white matter behind DLPFC (anterior semioval center, 7.5 ml), bilaterally. Neurometabolites were correlated with ADHD Rating Scale IV (ADHD-RS-IV) score evaluated by clnicians and Conners Parent Rating Scale (CPRS) and Daily Parent Rating of Evening and Morning Behavior (DPREMB) subscale scores evaluated by parents.

Results: NAA/Cr in the right DLPFC positively correlated with the CPRS subscale IV– learning problems and negatively correlated in the left white matter with the DPREMB morning behavior subscale and ADHD-RS-IV score. Glx/Cr positively correlated in the right white matter with the ADHD-RS-IV and negatively correlated in the left white matter with the DPREMB morning behavior subscale score. Cho/Cr in the left white matter negatively correlated with the DPREMB morning behavior subscale and ADHD-RS-IV score.

Conclusion: ADHD symptoms are significantly associated with neurometabolite levels in prefrontal areas. The dysbalancies between the left- and the right-hemisphere prefrontal circuits could play an important role in ADHD.

Keywords: N-acetylaspartate, glutamate, choline, prefrontal cortex

Poster Sessions

Cancer Research

Search of new anticancer thiazolidinones within structure-based design

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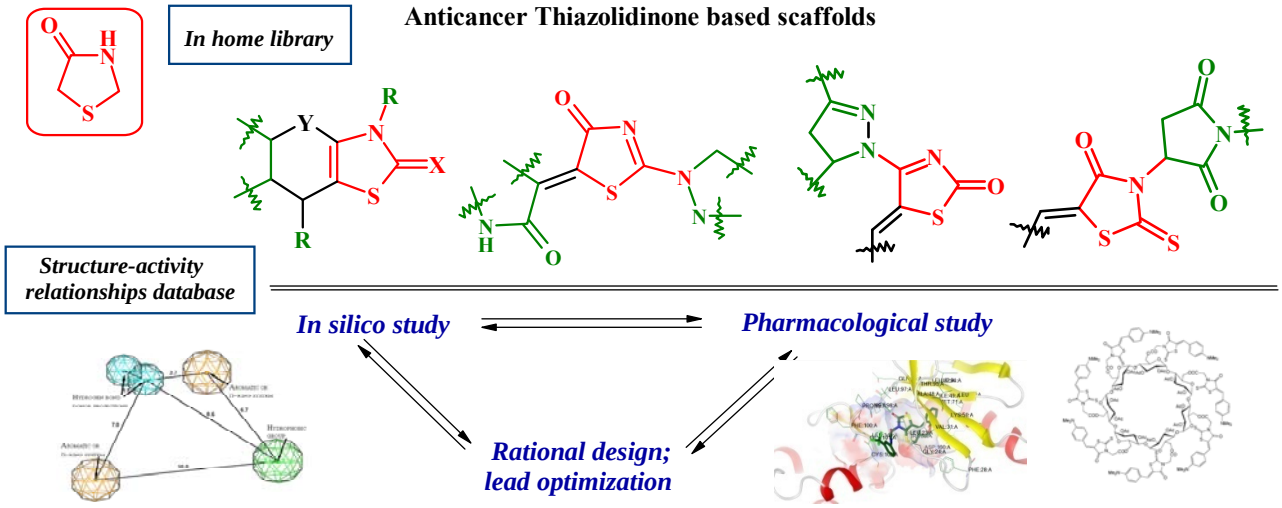
Prospects of new antitumor agents search among 4-thiazolidinone derivatives is caused by the identification of a number of highly active substances as the result of library's high throughput screening; and by the determination of a series of biological targets responsible for the antitumor effects realization in response to the 4-thiazolidinones and related heterocycles action. Main examples of above mentioned targets are PPAR-family, Bcl2 family, Tubulin, EGRF etc.

Proceeding from the multiyear collaboration with the NCI (DTP) a powerful structure-activity database of 4-thiazolidinones and related heterocycles in home library was obtained, lead compounds as well as the promising scaffolds for the further highly anticancer active agents design were identified.



Using the arsenal of modern *in silico* approaches the main search directions of the realization of anticancer effects were outlined for the synthesized compounds. Experimental investigation of the latter directions and molecular modes study allowed establishing kaspase independent pro-apoptotic way to be one of the most probable modes of some 4-thiazolidinones anticancer effects realization.

Aiming to improve drug-like properties and the bioavailability parameters of the active substances the methods of the structures' chemical modification were developed and the expedience of drug-delivery systems usage was tested. So, effective is the use of polymer carriers with the highly active thiazolidones that allows the active nano-particles formation.

On the other hand the use of triterpenoids in the implementation of hybrid pharmacophore approach in the thiazolidone-oleanane conjugates synthesis can significantly improve the bioavailability parameters.

Impact of cyminal and benzylidene groups of 4-thiazolidones on production of reactive oxygen species and induction of apoptosis in human tumor cells

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Introduction: 4-thiazolidone derivatives are a new group of synthetic compounds possessing different types of biological activity, including strong anticancer potential. However, molecular mechanisms of 4-thiazolidones antineoplastic activity remains poorly studied. The main aim of present study was to evaluate an impact of two side groups, namely, benzylidene and cyminal, towards anticancer properties of two related 4-thiazolidinones – Les-3166 and Les-3506. Materials and Methods: Synthesis of 4-thiazolidone derivatives, cell culture, flow cytometry, fluorescent microscopy, Western-blot analysis. Results: We demonstrated that substitution of the benzylidene group of Les-3166 by 2-chloro-3-(4-nitrophenyl)-2-propenylidene fragment (cyminal) in Les-3506 in the pharmacologically attractive position 5 of the 4-thiazolidinone moiety resulted in approximately 2 times higher cytotoxic activity of Les-3506 towards mammalian carcinoma and leukemia cell lines. Flow cytometry has revealed that both compounds arrested growth of tumor cells in S-phase of cell cycle (35.77 % cells in S-phase under treatment with Les-3166 and 45.97 % with Les-3506 compared with control (23.34 %). Thus, 2-chloro-3-(4-nitrophenyl)-2-propenilydene in Les-3506 molecule seems to enhance S-specificity of 4-thiazolidones action compared to benzylidene group in Les-3166.

Cyminal group in Les-3506 molecule seems to be responsible for early activation of the initiator caspase-9 already at 6 h of incubation of Jurkat T-cells with Les-3506, whereas another compound with benzylidene group (Les-3166) leads to this event only at 12 h. Activation of other initiator caspases -8 and -10 and cleavage of their substrates – Bid and FADD, occurred at 12 h after treatment of cells with Les-3506, while in case of using Les-3166, these phenomena were observed only at 24 h after drug treatment. Activation of the effector caspases-3, -7 under the action of Les-3506 also takes place much earlier (6 h) compared to Les-3166 (12 h).

We also tested the ability of Les-3166 and Les-3506 to influence ROS production in the treated cells. H_2O_2-sensitive dye dichlorofluorescein diacetate (DCFDA) and O_2--specific dye dihydroethidum (DHE) were used. They are specific for intracellular generation of hydrogen peroxide and superoxide anions, correspondingly. We did not find any impact of Les-3166 and Les-3506 on production of H_2O_2 radicals at early time points (1-6 h), while later (12, 24 h) they caused a slight increase of H_2O_2 level in treated Jurkat T-cells. In contrast, Les-3506 led to a rapid increase in production of superoxide radicals already at 1 h after the treatment of Jurkat T-cells, while benzylidene group Les-3166 led to much later O_2- generation (3 h). Thus, 2-chloro-3-(4-nitrophenyl)-2-propenylidene fragment in drug structure leads to earlier ROS generation and apoptosis induction compared to the benzthiazole group.

Conclusion: The cyminal group in molecule of 4-thiazolidones is responsible for early generation of the superoxide radical, activation of mitochondrial pathways of apoptosis mediated by caspase-9, and further cell death accompanied by the arrest in S-phase of cell cycle. In contrast, benzylidene group possesses much weaker activity and leads to delayed and less intensive apoptosis. Thus, cyminal-based compound Les-3506 is a promising pro-drug for anticancer chemotherapy.

Effect of histone post-translational modifications at the level of chromatin loops and chromosomes

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Disassembly of chromatin to loop-sized fragments has been observed in a variety of organisms including non-apoptotic mammalian cells and in yeast spheroplasts. Single-strand specific nuclease digestion of intact, chromosome-sized DNA can recapitulate this surprisingly uniform fragmentation pattern, raising the possibility that single-strand discontinouties (nicks) delimit chromatin loops.

In order to elucidate the role of transription in the establishment of these higher-order chromatin structures, we studied the relationship between chromatin fragmentation and histone modifications contributing to the transcriptional landscape. A number of histone gene substitution mutants – defective in post-translational histone modifications – as well as RNA polymerase I, II, III conditional mutants were screened for karyotype changes and loop-size fragmentation patterns.

We found that the amount of nicks – assessed by the S1 nuclease sensitivity of chromatin - positively correlates with the rate of transcription. Our results support the notion that preformed nicks flank the sites of active (coding and/or non-coding) transcription.

Keywords: histone modification, histone mutagenesis, transcription

Crucial role of reactive oxygen species in overcoming drug resistance in tumor cells by novel antibiotics of landomycin family

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Introduction. Tumor cells rapidly gain resistance to gold standard chemotherapy agents (e.g., doxorubicin, cisplatin, vincristin), as well as to novel targeted chemotherapeutics (e.g., sorafenib, vemurafenib, herceptin). This significantly diminishes effectiveness of treatment and worsens prognosis in cancer patients. Landomycins (Ls) belong to a novel family of angucycline antibiotics possessing high anticancer potential that is comparable with such potential of clinically approved drugs (e.g., doxorubicin). Previously, it was reported by us that landomycin E enhanced production of the reactive oxygen species (ROS) in tumor cells. It was also capable of overcoming tumor cell resistance to chemotherapy, however, the molecular mechanisms of its anticancer activity have not been studied yet (Korynevska et al, 2007; Panchuk et al, 2012).

The main aim of present work was to perform in-depth study of anticancer potential of 6 novel landomycins with different side groups in their structure (landomycin A, 11-deoxylandomycin A, landomycin B, 11-deoxylandomycin B, landomycin D, 11-deoxylandomycin D).

Materials and Methods. A panel of 10 carcinoma and leukemia cell lines possessing various mechanisms of drug resistance (over-expression of P-gp, MRP-1, bcrp, isogenic deletion of key genes involved in cell cycle regulation and apoptosis – p21, p53, caspase-3, Bax, ferredoxin) have been treated. MTT assay, fluorescent microscopy and flow cytometry were applied.

Results. The results of MTT assay demonstrated that sensitivity to landomycins in the parental tumor cell lines and their drug resistant sub-lines with over-expression of P-glycoprotein and bcrp did not differ significantly, while cell lines over-expressing MRP-1, demonstrated 2-fold elevation in their resistance to landomycins. In contrast, resistance to doxorubicin (used as a positive control) increased 80-100 folds, thus, indicating high efficiency of landomycins in killing drug-resistant cells. Fluorescent microscopy using annexin V/PI double staining revealed that these antibiotics induced apoptosis in tumor cells that was also accompanied by rapid depolarization of mitochondria and loss of their intactness (revealed by JC-1 staining and rhodamine 123 accumulation) as soon as in 1 h after drug treatment. Such targeting of mitochondria also led to an early oxidative burst in tumor cells that was caused by hydrogen peroxide. In contrast, another anticancer antibiotic doxorubicin led to production of superoxide radicals at much later time-points (12 h). Usage of specific mitochondrial respiratory chain inhibitors (DPI, rotenone, antimycin A, oligomycin) caused further enhancement of production of hydrogen peroxide by the landomycins, while H2O2 scavenger N-acetylcysteine decreased it almost to basal level. Application of NAC also significantly inhibited cytotoxic activity of the landomycins both in sensitive and drug-resistant tumor cell lines.

Conclusion. Landomycins are capable to overcome drug resistance in tumor cells via using hydrogen peroxide that is produced by mitochondria.

Keywords: Landomycins, drug resistance, tumor cells, hydrogen peroxide, mitochondria.

Primary systemic therapy of breast cancer: links between metabolical and pathological remission

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Introduction: The role of metabolic imaging is increasing in the measurement of therapeutic response in the treatment of malignant diseases. FDG-PET-CT is highly recommended for staging and outstanding in therapy monitoring, because this imaging modality is highly sensitive in the detection of viable tumour tissue.

Aim: We analysed retrospectively the relationship between the metabolic response on FDG-PET-CT scans and changes in Ki-67, a proliferation marker.

Method: We report 35 of our patients treated with primary systemic therapy (PST) during 2008–2011. All of them were examined with FDG-PET-CT for staging before the PST and before their surgery. We measured on the FDG-PET-CT scans the SUVs (Standardized Uptake Value) and morphological changes in the primary tumour and axillary lymph node region. Calculated ΔSUV were compared with the changes of the Ki-67 proliferation marker measured by immunohistochemistry in the core-biopsies and surgical specimens.

Results: The decrease of SUV and size were significant in the primary tumour and the axillary lymph node region. Decrease of Ki-67 was significant. Significant correlation was found between Ki-67 and SUV before therapy, initial Ki-67 and ΔSUV, and ΔKi-67 and ΔSUV, so between the metabolical changes and the proliferation of the tumour.

Conclusions: The metabolical changes were more sensitive in the measurement of the therapeutic response than morphological remission, besides they correlate well with pathological response, even in not standardized, clinical conditions. The FDG-PET-CT results may play an essential role in the indication of PST in connection with the proliferation markers of the tumours, and it could be successfully used in the assessing of the early therapeutic response of breast cancers.

Keywords; PET-CT, breast cancer, primary systemic therapy, proliferation, Ki-67

Express diagnostics of dying cells in whole blood samples by their changed glycoprofile

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Detection of dying cells in blood is an important diagnostic approach used at a number of diseases, as well as at the estimation of the effect of negative environmental factors (toxins, poisons, etc). Currently existing technologies require separation of nucleated blood cells from enucleated – erythrocytes (RBCs), and subsequent conduction of the analysis.

Aim: To use the described phenomena of altered glycoprofile at cells death [1, 2] to develop methods of detection of apoptotic and necrotic cells in populations of leukocytes (lymphocytes, monocytes and granulocyte) in human whole blood samples.

Methods: Fluorescent microscopy, flow cytometry, fluorescent labeling of lectins, lectin staining of blood samples.

Result: Altered glycotopes of dying cells are stable markers exclusively present on cells with damaged membranes and can be effectively used for express detection of apoptotic cells in whole blood samples by combining lysis procedure with lectin detection technology. In current work we describe a technology of express diagnostics of dying cells in whole blood samples, which need the collection of 0.1 ml of venous blood and after subsequents lysis (destruction) of erythrocytes and staining of leucocytes for the presence on their specific surface of glycans typical for dying apoptotic cells [1] and simultaneous staining of necrotic cells with PI allow estimation of amount of apoptotic/necrotic cells by means of fluorescent microscopy or flow cytometry. The duration of all diagnostic procedures is about 1 hour; and the duration of data analysis is from 5 to 20 min (depending on the applied equipment).

Conclusions: This technology is adapted for express detection of dying cells in the whole blood samples by utilizing an efficient erythrocyte lysis system and its combination with glycan detection technology in a single step reaction.

Key words: apoptotic cells, flow cytometry, whole blood, lectin.

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Recombinant synthesis of Anuroctoxin in *Escherichia coli*

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Background: The voltage-gated Kv1.3 potassium channel plays a key role in the activation of T lymphocytes by maintaining a negative membrane potential. By blocking these channels the proliferation of T cells can be inhibited. This may result in immunosuppression, which has a great potential in the therapy of certain autoimmune diseases. Anuroctoxin (AnTX), a 35-amino-acid peptide isolated and characterized previously from the venom of the scorpion Anuroctonus phaiodactylus, blocks Kv1.3 with high affinity (Kd = 0.7 nM).

Aim: Producing recombinant AnTX in large quantity, usage of the produced peptide for structure-function studies and finally application in *in vivo* animal experiments.

Methods: AnTx is produced as a fusion-protein in E. coli transformed by pPAL7 vector in M9 minimal medium supplemented with N15, selected with ampicillin and induced by 0.5 mM IPTG (isopropil-tio-galactoside) at OD_{600} = 0.6 value. The tagged toxin was purified with low-pressure affinity chromatography after lysing the cells. The conjugated toxin as substrate binds to subtilisin enzyme immobilized on the solid-phase. During the cleavage step the peptide separates from the solid-phase, and AnTX can be extracted using an eluation solution for further application.

Results: From 1 liter bacterial culture we purified about 300–500 μg peptide with this method. With high-performance liquid chromatography (HPLC) we identified the recombinant peptide, the structural analysis is currently under progress by the means of Nuclear Magnetic Resonance spectroscopy (NMR). We also plan to test the biological activity of the recombinant toxin on Kv1.3 channels using patch clamp.

Our future plans are to modify and analyse different peptide structures produced for therapeutic application.

Keywords: T-lymphocyte activation, voltage-gated Kv.1.3 potassium channel, anuroctoxin, fusion-protein, affinity chromatography, HPLC, NMR, patch clamp

Study of interaction of uropathogenic *E. coli* cells with mannose-rich apoptotic cells-derived microvesicles

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Introduction: Escherichia coli cells causing urophathogenic infections and Crohn disease utilize the same mechanism of binding with host cells – namely through mannose-specific lectin (adhesion) FimH on the tip of type 1 fimbriae. Recently we demonstrated that apoptotic cells release distinct types of apoptotic blebs; some of them derived from endoplasmic reticulum (ER) or plasma membrane. Both exhibit desialylated glycotopes resulting from surface exposure of immature ER-derived glycoproteins or from surface-borne sialidase activity [R.Bilyy, T. Shkandina, et al, JBC, 2012].

Aim: Taking into account the nature of uropathogenic infections which arises during immunocompromised conditions (like cooling or dabetis) which are usually associated with the increase number of apoptotis here we studied the possibility of E.coli cells to use the mannose-rich apoptotic cells-derived microvesicles (ACMV).

Methods: microscopy, cell and bacteria cultivation, HeLa cells were used as experimental model.

Result: We used annexinV-FITC as a marker of apoptotic cells and propidium iodide as a marker of necrotic cells and demonstrated that FimH induce apoptotic, not necrotic, cell death. We demonstrated that ER-derived ACMV are bound by FimH lectin. Also by using combination of fluorescent and DIC microscopy and fluorescent stains of E.coli cells we demonstrated the direct binding of E.coli with mannose-rich ACMV of human HeLa cell after co-incubation period of 2 h.

Conclusions: Apoptotic blebs can be the site of bacterial entrance point into the epithelia of urinary tract.

Key words: apoptosis, apoptotic blebs, uropathogenic infection

Regulation of CHI3L1 expression by p53 in glioblastoma cells

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Aims. Wide-known functions of p53 protein are related to its role in DNA damage, cell growth arrest, senescence, and apoptosis. p53 is stimulated by stress, activating a wide range of transcriptional targets. Low-intensity stress activates p53, stimulating antioxidant response, thus protecting against senescence. On the other hand, high-intensity p53 activation increases oxidative stress by activation of pro-oxidant targets, increasing the rate of ageing, but protecting against cancer. Chitinase-like protein 1 (CHI3L1) acts as survival factor for glioblastoma cells on different types of stress. CHI3L1 level is increased after irradiation of U87 glioblastoma cells, treatment with etoposide and ceramide, as well as treatment with antioxidants, also stimulate CHI3L1 expression. Specific p53 inhibition increases CHI3L1 expression in U87 cells, bearing wild-type p53, thus the aim of our study was to check whether p53 could regulate the expression of CHI3L1.

Methods. To check the effect of p53 transfection on CHI3L1 level, U87 cells were transfected with plasmid construct, containing p53 cDNA, levels of endogenous CHI3L1 and transfected p53 were measured by Western blotting. *In vitro* analysis of p53 binding to CHI3L1 promoter was performed using modified electrophoretic mobility shift assay. Briefly, U87 cells were transfected for 48 hours by plasmid construct, containing p53 cDNA. Next, p53 protein was immunoprecipitated with anti-p53 antibodies from total cell lysate, incubated with CHI3L1 promoter DNA and separated in 1 % agarose. The total lysate of non-transfected U87 cells was used as negative control.

Results. Transfection with p53 significantly decreased CHI3L1 level in U87 glioblastoma cells. p53 induced gel shift when incubated with CHI3L1 promoter DNA.

Conclusions. CHI3L1 could be repressed by p53 in direct or indirect manner in glioblastoma cells, thus, CHI3L1 could be involved in p53-mediated stress responce in these cells.

Keywords. p53, glioblastoma, chitinase 3-like protein 1

A novel aspect of arginine starvation: impact on expression of glycosyltransferases in melanoma cells

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Despite of the recent progress in chemotherapies, prognosis for patients with advanced melanoma remains poor. As melanoma cells frequently lack argininosuccinate synthetase, the key enzyme of arginine biosynthesis, enzymotherapy based on arginine-degrading enzymes is considered as a promising anti-melanoma approach.

Two isogenic human melanoma cell lines – primary WM793 and its derivative metastatic WM1205Lu were used as models. We observed that, as expected, arginine starvation abrogated proliferation of both cell lines. Supplementation with arginine precursors citrulline or ornithine did not support cell proliferation confirming that these melanoma cells are auxotrophic for arginine. The capacity of WM793 and WM1205Lu cells to reestablish growth after arginine resupplementation decreased in a time-dependent manner. The decline in viability under arginine starvation was accompanied by induction of apoptosis. Importantly, metastatic cells were more sensitive to arginine starvation relative to the primary cells.

We previously demonstrated that glycosylation profile of melanoma cells is specifically altered under arginine deprivation. Here we addressed the question, whether arginine deprivation affects expression of glycosyltransferases that may affect external glycosylation pattern. RT-PCR analysis revealed expression of β-galactoside-α2,3-sialyltransferase-III (ST3Gal III), β-galactoside-α2,3-sialyltransferase-IV (ST3Gal IV), Galβ1,3GalNAc-R α2,6-sialyltransferase (ST6GalNAc II), β1,6-N-acetyl-glucosaminy-ltransferase (GnT V) and N-cadherin mRNAs in both tested cell lines. The significant decline of ST3Gal III and ST6GalNAc II mRNAs was observed in arginine-starved cells versus cells starved for lysine. It is important to mention that these enzymes sialylate cancer associated cell surface T antigen and their status positively correlates with tumor prognosis and metastasis.

Our results demonstrate for the first time that changes in glycosylation profile of melanoma cells under arginine deprivation are due, at least in part, to altered regulation of certain glycosyltransferases at transcriptional level.

Function of Bcr-Abl and PLCε is complex and does not include direct interaction

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Bcr-Abl, the product of a chromosomal translocation t(9;22), has been demonstrated to be a key protein responsible for the pathogenesis of Ph-positive leukemia. Three forms of Bcr-Abl proteins have been observed: p190 Bcr-Abl, p210 Bcr-Abl, and p230 Bcr-Abl. The only structural difference among various Bcr-Abl chimeras is the presence of Dbl homology (DH) and pleckstrin homology (PH) domains in p210 Bcr-Abl and p230 Bcr-Abl, and their absence in p190 Bcr-Abl. p210 Bcr-Abl and p230 Bcr-Abl are responsible for chronic myelogenous leukemia while p190 Bcr-Abl is associated with 20-30% of acute lymphoid leukemia cases suggesting the important role of the DH and PH domains in leukemogenesis. Our recent data demonstrated that Bcr-Abl PH domain binds a number of proteins including phospholipase Cε (PLCε). Also we have shown that full-length PLCε co-localizes with p210 Bcr-Abl in perinuclear area and with p190 Bcr-Abl in cytoplasm of Cos-1 cells. Analysis of PLCε expression in leukocytes obtained from patients with different blood cancers revealed that PLCε expresses in all of them.

Aims: The aim of our study was to prove the interaction between full-length PLCε and two forms of Bcr-Abl as well as to find which domain of PLCε is responsible for interaction with PH domain of Bcr-Abl. Methods: Different expression constructs carrying full-length sequences for p190 and p210 Bcr-Abl and PLCε as well as different domains of PLCε and PH domain of Bcr-Abl were used. Hek293T cells were used for transfection studies. For analysis of interactions between full-length proteins co-immunoprecipitation technique has been applied. For studying the interaction between domains of PLCε and PH domain of Bcr-Abl recombinant proteins have been purified and pull-down approach has been used. Results: We have found that full-length PLCε does not interact directly neither with p190 nor with p210 Bcr-Abl. Also we have found that PLCε RA1RA2 domains region and core domain of PLCε do not interact with PH domain of Bcr-Abl. Conclusion: Our results suggest that there is no direct interaction between PLCε and both forms of Bcr-Abl. Some non-stable complexes involving these proteins might exist (for example, complex with DLC-1, a protein which also is in the list of proteins interacting with PH domain of Bcr-Abl). RhoA might act as an intermediate protein in formation of complex PLCε-RhoA-DLC-1-Bcr-Abl. Also some post-translational modifications might change the complex formation. But it requires further experiments.

Poster Sessions

NanoBioTechnology

Silica-coated superparamagnetic nano- and microparticles for cell labeling

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Magnetic silica particles are widely investigated in new applications in biomedicine, biotechnology, data storage, etc. In this study, both nonporous and porous magnetic silica particles were developed. Iron oxide (γ-Fe_2O_3) nanoparticles were synthesized by co-precipitation of Fe(II) and Fe(III) salts followed by oxidation of Fe_3O_4 with sodium hypochlorite. Surface of maghemite nanoparticles was modified using tetramethyl orthosilicate and the resulting nanoparticles were called as γ-Fe_2O_3@SiO_2. In order to attach amino groups on the surface, γ-Fe_2O_3@SiO_2 was coated with a secondary silica shell using (3-aminopropyl) triethoxysilane to obtain γ-Fe_2O_3@SiO2-NH2 nanoparticles. Optionally, iron oxide was precipitated inside the mesoporous microspheres obtained by sol-gel method using hydrolysis and condensation of tetraethyl ortosilicate.

The number of average particle diameter increased from 9.7 nm in neat γ-Fe_2O_3 to 19 nm in γ-Fe_2O_3@SiO_2 and 192 nm in γ-Fe_2O_3@SiO_2-NH_2 nanoparticles. The particles were superparamagnetic and the content of iron decreased from 66.1 wt. % in γ-Fe_2O_3 to 27.7 and 19.8 wt. % in γ-Fe2O3@SiO_2 and γ-Fe_2O_3@SiO_2-NH_2 nanoparticles, respectively. The presence of amino groups on the surface of particles was confirmed by Fourier-transform infrared and elemental analysis.

In biological experiments, both γ-Fe_2O_3@SiO_2 and γ-Fe_2O_3@SiO_2-NH_2 nanoparticles were investigated. Particles were recognized and quickly engulfed by the murine macrophages of J774.2 line without any toxic event. γ-Fe_2O_3@SiO_2 and γ-Fe_2O_3@SiO_2-NH2 particles could thus provide a perspective *in vivo* tool by which the magnetic resonance imaging will monitor involvement of macrophages in the inflammatory processes.

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Synthesis and optical properties of ultrasmall nanocrystals as optical markers based on lanthanides emission controlled by ion-ion interactions

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Background: Introducing to medicine and biology concept of optical markers in tremendous way has changed the recent status of these two important disciplines. This was mainly due to strong development in imaging techniques which recently allow us to investigate both static as well dynamic properties of living cells, their components and their interactions with external factors.
One of the alternatives for recently used molecular markers are inorganic quantum dots. However, even if they are much better from physico-chemical point of view, from the application point of view the high risk of their toxicity makes them still limited in use. One of the solutions combining advantages of both concepts is to make nontoxic inorganic nanocrystals doped with lanthanide ions.
Methods: In this work, we will present optical (absorbance, photoluminescence (PL), PL excitation, PL decay, Raman) and structural (HRTEM, XRD, DLS) results obtained for NaYF4, NaGdF4 and GdF nanocrystals doped with different lanthanide ions and their combinations. The aim of this work was to design and to synthesize optical markers emitting in VIS as well in IR spectral range at different excitation wavelengths from UV to NIR, and to understand the physical processes responsible for their emission and excitation. In particular, we focused our attention on more detailed analysis of ion-ion interaction between the pairs of the ions and the influence of the nanocrystals surface and their influence on optical properties of the marker.
Results: The average size of obtained nanocrystals, determined from TEM measurements differs for various dopant concentrations and matrix composition and take values between 5 and 100 nm. It has been shown that obtained nanocrystals emit light from 450 nm up to 1550 nm depending on their composition, size and excitation conditions.
Conclusion: Using different dopant concentration and matrix composition emission spectrum, size and morphology of NCs can be adjusted. This open many possibilities in using fluoride-based nanocrystals in bio-applications.

Synthetic polymer-covered nanoparticles of magnetite and native protein-covered magnetic nanoparticles for usage in nanoneurotechnology

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AIMS: Magnetic nanoparticles attract increased attention because of their usage in magnetic resonance imaging, drug delivery, selective/local hyperthermia, tissue repair and cell separation. The aim of this work was to study interaction of synthesized polymer-covered superparamagnetic nanoparticles and native protein-covered magnetic nanoparticles with rat brain nerve terminals, and to analyze their effects on the key characteristics of synaptic transmission.

METHODS: Novel polymer-covered superparamagnetic nanoparticles were synthesized at Semenenko Institute of geochemistry, mineralogy and ore formation (Kyiv, Ukraine), and Institute of Macromolecular Chemistry (Prague, Czech Republic). Binding of nanoparticles with nerve terminals was studied by photon correlation spectroscopy, flow cytometry and radiolabeled assay. Spectrofluorimetry with potential-sensitive and pH-sensitive fluorescent dyes were applied for characterization of functional state of nerve terminals.

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

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

KEYWORDS: polymer-covered magnetic nanoparticles, ferritin, synaptosomes.

Toxicological studies of sodium fluoride based nanocrystals for biomedical applications

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Upon entering into the world of medicine, nanomaterials brought along the promise of spectacular improvement in nearly all of its aspects. There are many publications presenting their use in e.g. MRI as contrast agents, drug delivery systems, specific cell tracking, hyper-thermic therapy, optical imaging as markers. For all of these applications there are some fundamental requirements to be fulfilled, namely the biocompatibility and low toxicity. In order to gain these abilities, nanoparticles should be stable in aqueous media and over wide range of pH values. In this work, we focus on inorganic nanocrystals with sodium fluoride core doped with various lanthanide ions and of different diameters.

These ions are responsible for optical properties unmatched by commercially available dyes or even quantum dots: no luminescence bleaching and blinking, no autofluorescence, atomic-like photoluminescence (PL) spectrum, long PL decay times. Additionally, their surface was functionalized to render them hydrophilic. Water-dispersibility is achieved through Ligand Exchange method, during which hydrophobic ligands are substituted with hydrophilic one. This allows further bio-conjugation via functional groups now available at the surface.

We have conducted cytotoxicity measurements of both types of nanocrystals (hydrophobic and hydrophilic) to determine their influence on human peripheral blood cells. Cytotoxicity of nanocrystals was measured using 3H-thymidine incorporation assay. Immunosafety was assessed by measurement of proliferative response of lymphocytes *in vitro* and phagocytic activity and respiratory burst of leukocytes.

Preliminary results indicate relative low toxicity of nanocrystals to human peripheral blood cells. However, for some samples, toxic effects were observable, but only for high concentrations (75 ug/cm^2). Toxic effects were milder for hydrophilic nanocrystals. Our findings showed that nanocrystal size does not significantly influence toxicity in any obvious way.

Keywords: nanocrystals, ligand exchange, toxicology

Fluorescent carbon dots: synthesis and spectroscopic properties

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Carbon dots are novel prospective nanomaterials for science and technology. They are promising for biosensing and bioimaging applications. Greatest interest to carbon dots in comparison with other fluorescent nanoparticles (e.g. quantum dots) are due to their easy production (“green synthesis”), high brightness, absence of toxicity, broad range of possibilities for modifications and functionalizations.

We studied carbon dots obtained by hydrothermal treatment of starch and sucrose ("blue dots") as well as by the method of microwave treatment of glycol ("green dots"). These materials were characterized by the light absorption spectra, and the spectra of fluorescence excitation and emission. We have shown that by using different methods of synthesis, carbon dots with different spectroscopic properties can be obtained: the fluorescence band maximum occupies different wavelength positions within the blue-green range of the visible spectrum at the same excitation. The mechanisms of light absorption and fluorescence emission are not fully understood. In this respect, we performed special experiments on fluorescence excitation over extended range of absorption wavelengths. We studied the excitation-emission correlations and marked a strong Stokes shift that signifies some still unknown relaxation phenomena in the excited states. The time-resolved studies demonstrate heterogeneity of fluorescence decays occurring in nanosecond time range. The studied carbon dots demonstrate high temperature (10–80^{o}C) and pH (2–13) stability.

The work on carbon dots continues. We are trying to obtain the hybrid structures that combine fluorescent and magnetic properties with the prospect of further functionalization. In our view, carbon dots are very promising platforms for assembly of multifunctional nanocomposites for using for *in vivo* diagnosis and drug delivery.

Synthesis of $NaGdF_4:Yb^{3+}$, Er^{3+}nanocrystals for biomedical applications

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BACKGROUNDS: Among the various types of fluorescent nanoparticles that are currently used in bio-imaging, lanthanide-doped up-converting nanocrystals (UCNCs) attract considerable attention. Low photodamage of biological specimens, deep tissue penetration, reduced photobleaching and lack of autofluorescence make them excellent optical markers. Many different host matrix have been investigated so far and it is known that fluoride host matrix $NaYF_4$ is the most common one. This is mainly because of the low-phonon frequency and highly efficient Up-Conversion energy transfer phenomena. Promising alternative is $NaGdF_4$ matrix, where yttrium ions are substituted by gadolinium ions. This replacement can even more decrease phonon energy in system, reduces the size of NCs and opens new attractive application paths for nanocrystals as multifunctional optical and magnetic markers.

AIMS: The aim of our work was to obtain fluoride nanocrystals co-doped with erbium (Er^{3+}) and ytterbium (Yb^{3+}) ions.

METHODS: $NaGdF_4:Yb^{3+}$, Er^{3+} nanocrystals have been synthesized from trifluoroacetate salts by co-thermolysis method in the presence of oleic acid (ligand/solvent) and 1-octadecen (solvent). $NaGdF_4:Yb^{3+}$, Er^{3+} nanocrystals have been prepared in a function of dopant and ligand/solvent ratio. The structural properties of the products were characterized by X-ray diffraction (XRD) and transmission electron microscopy (TEM). The luminescent properties of the NCs were investigated by photoluminescence spectroscopy.

RESULTS: The average size of obtained nanocrystals, determined from TEM measurements depends mainly on various dopant concentration. Also it has been found that ligand/solvent ratio influence signinficnantly NCs morphology.

CONCLUSIONS: Using different dopant concentration and ligand/solvent volume ratio both NCs emission spectrum and NCs size and morphology can be adjusted to properties enables their efficient application in bio-medicine.

KEYWORDS: nanocrystals, rare earth ions, optical markers

Functionalization of $NaGdF_4:Yb^{3+}$, Er^{3+} nanocrystals for their biomedical applications

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BACKGROUND: Up-converting nanocrystals (UPCNCs) that are doped by lanthanide ions because of the possible excitation in "tissue optical window" region represent an interesitng alternative to conventional organic dyes and quantum dots. Lower toxicity, reduced photobleaching and lack of autofluorescence pose additional advantages. So far the most suitable host matrix for lanthanide ions was $NaGdF_4$, in which the presence of Gd^{3+} ions opens new attractive application paths for nanocrystals as multifunctional optical and magnetic markers. Due to organic solvents used in synthesis, obtained NCs are hydrophobic and there is a need to change surface ligands or surround them by another material, which will make them water dispersible.

AIMS: The aim of our work was the synthesis of fluoride nanocrystals doped by erbium Er^{3+} and ytterbium Yb^{3+} ions and adapting their surface to water environment.

METHODS: $NaGdF_4:Yb^{3+}$, Er^{3+} nanocrystals have been synthesized from trifluoroacetate salts by co-thermolysis method in the presence of oleic acid (ligand/solvent) and 1-octadecen (solvent). The structural properties of the products were characterized by X-ray diffraction (XRD) and transmission electron microscopy (TEM). The luminescent properties of the NCs were investigated by excitation, emission and absorption measurements. The structure and size of $NaGdF_4$: Yb^{3+}, Er^{3+} NCs were controlled by variable dopant concentration and different ligand/solvent ratio. In order to transfer NCs to aqueous solvent two procedures were applied: ligand exchange and surface coating methods.

RESULTS: The average size of obtained nanocrystals, determined from TEM measurements differs for various dopant concentrations. Also ligand/solvent ratio influence on NCs morphology was observed. Both procedures of NCs surface modification have been successful.

CONCLUSIONS: Using different dopant concentration and ligand/solvent volume ratio emission spectrum, size and morphology of NCs can be adjusted. What is more, successful NCs water transfer makes them applicable in biological media.

KEYWORDS: nanocrystals, rare earth ions, ligand exchange