Poster Sessions
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Abstract Review

CROATIA

Women's Health and Cardiovascular Diseases

Female stroke

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Aim: Stroke has mostly been considered a male disease, and male gender as a risk factor for cerebrovascular diseases. However, recent research shows that women suffer from stroke more often, and that it is more lethal for female than male patients. The aim of this pilot study was to investigate the situation in Osijek area.
Methods: A group of 100 patients, average age 70.5 years; 45 females and 55 males with stroke treated in Department of neurology, Clinical hospital center Osijek was included. Patients were grouped according to gender and the following were investigated in each group: distribution of stroke according to groups of age, frequency of hemorrhagic and ischemic stroke, frequency of ischemic stroke subtypes, presence of certain risk factors, occurrence of complications during hospitalization and final outcome at discharge.
Results: In average, women were older than men at the time of first stroke. Differences in occurrence of risk factors were also noticed. The frequency of complications was almost equal, except for uroinfections, which were, as expected, more frequent in women due to specific structure of female urinary tract. Women had poorer outcome of stroke and higher case–fatality rate than men.
Conclusions: Biological differences like hormonal status (effect of estrogen and testosterone on endothelium and vascular system), influence of risk factors present only in women (oral contraceptives usage, hormone replacement therapy, and pregnancy), specific life-styles, comorbidity (migraine, thrombophilia), and longer life-span of women can explain some of the gender differences, however, a lot of details still remain unclear.
Key Words: women, stroke, risk factors, complications, outcome

Anxiety, depression and stress in acute myocardial infarction: a pilot study

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Aim: The aim of our study was to detect the connection between depression, anxiety and stress level with the course of acute coronary syndrome. A comparison of psychological test results obtained from cardiology ward patients was conducted to determine differences between patients with acute myocardial infarction and angina pectoris.

Methods: The sample included 25 patients hospitalized for acute coronary syndrome in 2012 in Osijek.The general questionnaire, Beck Depression Inventory, Beck Anxiety Inventory, Type D Personality Test and Life Event Stress Scale were used to assess levels of depression, anxiety and stress. The patients were divided into two groups – the one with acute myocardial infarction (MI) and the other with angina pectoris (AP), and were further compared and contrasted by psychological parameters.

Results: Study included 14 male and 11 female patients, average age 61 yrs (SD=9.5). There were 12 patients in MI group and 13 in AP group. Certain level of depression was reported by 11 patients, while 5 achieved middle score on anxiety. In comparison to AP patients larger portion of MI patient were both – middle score on anxiety and showing characteristic of social inhibition. The results of anxiety and depression tests suggest differences between two groups of patients, which are not significant due to small number of examinees. Two fifths of patients in AP group had a middle score on Life Event Stress Scale and none in MI group. Type D Personality Test and Life Event Stress Scale showed difference between MI and AP patients.

Conclusions: Beside already known risk factors for cardiac diseases such as hypertension, dyslipidemia and diabetes, patients who show also symptoms of depression, anxiety and stress have greater risk of developing acute myocardial infarction rather than angina pectoris.

Keywords: depression, anxiety, stress, acute myocardial infarction

Coronary artery disease risk factors in coastal and continental Croatia

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Aim: A comparison of clinical data obtained from cardiology ward patients in two major regional hospital centers (UHC Osijek and UHC Split) was conducted to determine differences between patients with CAD in continental and coastal Croatia.

Methods: Data was obtained from patients hospitalized for CAD in 2007 in Osijek and Split, respectively. The variables included age, gender, BMI, diagnosis, duration of hospitalization, history of arterial hypertension and diabetes, laboratory findings, ECG and coronarography data. The sample included 295 patients from UHC Osijek and 195 patients from UHC Split.

Results: Patients from the coastal region (UHC Split) were found to be older than patients in continental Croatia (UHC Osijek; $p<0.001$), mainly due to the difference in the age of male patients in the two regions. The patients in Osijek had a higher BMI than patients in Split – 28.1±4.9 versus 23.91±2.02 ($p<0.001$). Arterial hypertension was found more often in UHC Split male patients, compared to UHC Osijek male patients ($p=0.011$), but a difference was not found when comparing all patients' data or female patients' data. Dyslipidemia was found more often in Osijek in all patients and separately in males and females ($p<0.001$, respectively). The laboratory data analysis revealed that UHC Split patients had higher HDL ($p=0.005$), but also higher LDL ($p=0.012$) and triglyceride levels ($p<0.001$) than UHC Osijek patients. Gender-related differences were observed in both centers. In Osijek, females were older (68.62±10.77 versus 62.25±11.25 years, $p<0.001$) and had higher HDL levels (1.27±0.36 versus 1.13±0.34 mmol/L, $p<0.001$) than men, but in Split the differences were not observed. Unlike in Osijek, men in Split had higher BMI than females (24.78±1.69 versus 22.83±1.89, $p<0.001$).

Conclusions: Patients from coastal Croatia have less risk regarding some traditional cardiovascular risk factors than those from the continental region, delaying the onset of clinically apparent CAD. Furthermore, dissimilar patterns of gender-related differences between clinical data from Split and Osijek also suggest a discrepancy between CAD patients in two Croatian regions.

Keywords: Coronary artery disease, obesity, dyslipidemia, BMI

Peripheral expression of adipocytokines and role of sympathetic nervous system in function of perivascular adipose tissue and vascular reactivity

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Aim: Visceral (VAT) and perivascular adipose tissue (PAT) are active endocrine and paracrine organs. Two opposite functions are assigned to PAT. It has antiatherogenic and proatherogenic effects. In this research, changes of PAT and sympathetic nervous system will be measured also influence of mother`s nutrition during gestation on function of PAT and VAT in offspring will be determined.

Methods: 10 female rats will be randomly divided in 2 groups. One group will be fed with high content of saturated fatty acid – FD group (n=5), and the other will be fed with standard laboratory chow – CD group (n=5). After coupling and lactation offspring will be randomly divided in 2 groups – one will be fed with high content of saturated fatty acid (FD-FD2 and CD-FD2), and other with standard laboratory chow (FD-CD2 and CD-CD2). We will have 4 groups of offspring genetically similar, but exposed to different intrauterine and postnatal nutritional environments. Also, blood concentrations of adiponectin, insulin, glucose, LDL, HDL, triglycerides, TNF-α and IL-6 in four separate groups of animals will be measured. After decapitation, expression of *ADIPOQ* and *β3-AR* gene will be measured in samples of VAT and PAT, and expression of *SLC6A2* and *DβH* will be measured in coeliac ganglion. Role of PAT will be tested by measuring vascular reactivity after norepinephrine perfusion through isolated branches of superior mesenteric artery.

Results: By measuring expression of *ADIPOQ* gene and *β3-AR* gene in adipose cells of PAT in regard to different environment of offspring we expect to confirm that lipolysis mediated by sympathetic nervous system in predisposed rats is decreased. By analyzing offspring of fat diet mothers which are on control diet we will attempt to determine role of postnatal diet on sympathetic nervous system activity in PAT and synthesis of adiponectin in PAT and VAT. We will try to link the changes in *ADIPOQ* gene expression in PAT with changes in expression of *SLC6A2* gene and *DβH* gene in coeliac ganglion. Also, we expect to comfirm protective role of PAT in control diet rats and lack of that protection in fat diet rats by measuring vascular reactivity of superior mesenteric artery with and without PAT.

Conclusions: Understanding relationship between PAT and sympathetic nervous system will enable us to create more rational pharmacotherapy for obesity and metabolic syndrome. In general, it could enable great social, economic and health benefits for individuals and society.

Cyp450 Ω-hydroxilase expression in hyperbaric oxygenation

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Aim: Our previous studies showed that hyperbaric oxygenation could affect vascular sensitivity and reactivity on vasodilators and vasoconstrictors in healthy and diabetic rats. CYP450- Ω- hydroxilaze **(**CYP450-4A) is one of the oxygen sensors in blood vessels, producing increased amounts of vasocontrictor metabolite 20-HETE in positive correlation with increase in pO_2. The aim of this study was to evaluate the effects of intermitent hyperbaric oxygenation (HBO) on expression of CYP450-4A in aortic and cerebral vessels of rats.

Methods: Type I diabetes mellitus (T1DM) was induced in Sprague-Dawley (SD) rats by a single intravenous injection of streptozocin (60 mg/kg). Groups: SD control healthy rats, control healthy rats that underwent HBO (2 h for 4 days), T1DM group and T1DM rats that underwent HBO. The expression of CYP450 -4A was determined by Western blot in aortic and cerebral vessels samples.

Results: Densitometrical analysis of the protein bands demonstrated that the CYP450-4A aortic/cerebral vessel expression has increased in the control group of rats treated with HBOT, as well as in diabetic rats group treated with HBO, compared to their respective controls.

Conclusions: This study, for the first time demonstrated the increased expression of CYP450-4A enzyme in control and T1DM rats after hyperbaric oxygenation, thus confirming previous functional studies on the role of CYP450-4A metabolites in vascular responses to various physiological stimuli.

Keywords: hyperbaric oxygenation, cyp450 Ω-hydroxilaze, Western blot, diabetes mellitus

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Epicardial adipose tissue is associated with central adiposity but not with CAD

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Aim: Increased amount of visceral adipose tissue (VAT) and epicardial adipose tissue (EAT) have been linked to coronary artery disease (CAD). Clinically central adiposity is used as proxy for higher amounts of VAT. The aim of this study is to determine if central distribution of adipose tissue and CAD are associated with higher amounts of EAT.
Methods: In this study 55 patients were selected into the peripheral and central group using waist circumference. All 55 patients were subjected to coronarography and echocardiography. According to the results patients were selected into the CAD group or control group. EAT thickness was determined by ultrasound.
Results: In regional distribution groups, selected by standard borderline values of waist circumference, no significant difference was found in thickness of EAT ($p=0.847$). When tested in regional distribution groups selected by adjusted criteria for Caucasians a significant difference was found ($p<0.001$) in thickness of EAT between peripheral and central group. There was no significant difference ($p=0.330$) in thickness of EAT between CAD and control group. Weak correlation is found between the waist circumference and the EAT ($r=0.238$; $N=55$; $p=0.080$).
Conclusions: Central distribution of the adipose tissue has higher amount of EAT which is a known risk factor for CAD. Waist circumference did not show a strong correlation with thickness of the EAT which was expected due to its undiscriminating nature between subcutaneous and visceral adipose tissue.
Keywords: epicardial adipose tissue, cardiovascular disease, ultrasound, waist circumference

Mother and Child Health

Bone loss among premenopausal women on long-term suppressive therapy with thyroid hormone

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Introduction: It is controversial whether the long-term treatment with thyroid hormone given at suppressive doses has a negative effect on bone metabolism. Aim of this prospective study was to determine whether the long-term thyroxin therapy in the premenopausal period is a risk factor for the development of secondary osteoporosis, and whether those women have increased bone loss during the premenopausal period.

Patients and methods: There were a selected group of women in premenopausal period (N=19) aging 39,0±8,0 suffering from differentiated thyroid gland carcinoma. To all of them, the total thyreoidectomy was done and the thyroxin suppressive therapy was introduced. The duration of the suppressive therapy from the beginning of the research was 9,4±6,4 years. Laboratory results have excluded other possible factors for secondary osteoporosis. The prospective study of bone densitometry was done during four years to all examinees using the method of dual photon x-ray absorptiometry (DXA) of the spine and the femoral neck, and also by the method of single-photon absorptiometry (SPA) of the distal radius.

Results: In the beginning of this study, while the patients were treated for 10 years osteopenia was found in the spine and femoral neck in two examinees for every region. On the distal radius osteopenia was found in 4 examinees. The total was 8 out of 19 with osteopenia. One year later, after the second measurement no statistically significant loss of bone mass was in any region of skeleton. However, looking at the individual scores, osteopenya was found in 6 examinees on the distal radius but on the spine and the femoral neck there were bone loss in several women not to the point that would create osteopenia. But, after the 4 years of measurement t-test had shown some significant bone loss.

Conclusion: after careful examination, our conclusion is that women who begin with a long-term thyroxin therapy (about 10 years) in the premenopausal period can develop osteopenia at the beginning of menopause. In that case, those women should complete mammography testing and bone densitometry before hormone replacement therapy is used.

Keywords: menopause, osteopenia, thyroxin, absorptiometry

Impact of high-fat diet on fertility in Sprague Dawley rats

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Aim: Pilot study examined the impact of high-fat diet on fertility.
Methods: 10 female Sprague Dawley rats from the same litter were at puberty (age of six weeks) randomly divided into two groups (N=5 for both groups) and fed with standard chow or high-fat-diet (20% lard, enriched with vitamin and mineral-mix, mixed with standard laboratory chow) ad libidum. At the age of twelve weeks the female rats were placed in separate cages with one male per cage for mating. The male Sprague Dawley rats were also all from the same litter to minimize genetic-dependent diversity in future brood size and quality. The number of pregnancies and the size of litter were recorded for each group of females.
Results: In the control group fed with standard chow the birth dates were spaced between 23rd and 39th day with a mean size of litter of 14, and a standard deviation of 1.414. In experimental group all females also gave a birth between 23rd and 40th day, but conception was delayed for two weeks in the case of 3 females. Although mean size of litter was also 14, standard deviation was higher, 2.646.
Conclusions: All female control group-rats besides one gave birth within a week time from the date of expected birth. In comparison, only two HFD rats had their litter in that week. Although the mean litter size was the same, the HFD rat-litter showed a greater diversity in number of pups. The prolonged study with a bigger number of animals should further investigate influence of high-fat diet on fertility.
Keywords: High-fat diet, obesity, fertility

Metal contamination and its effect on reproductive system

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The War in1991 in Croatia resulted with a significant release of contaminants into the environment as a result of the use of combat assets.

Aim of the study was to investigate the concentrations of metals and metalloids in serum, hair, urine, soil, water and vegetables on 11 locations (8 exposed to heavy and 3 exposed to mild combat activities) of eastern Croatia, in order to create biobank for further research.

Methods: Total of 391 participants (204 male, 187 female) gave samples of urine , hair and serum in order to determine levels of metal for each settlement and answered an questionnaire in order to establish level of their professional exposure, war or environmental exposure.

Water was collected form 14 different sites, soil from 28 sites, vegetables from 41 sites.

Inductively coupled plasma mass spectrometry was made to measure 66 elements.

Results: All results suggest an association between the intensity of war activities and the degree of contamination by metals used in ammunition. Significantly higher concentrations of Al, As, Ba, P and V in the blood, significantly higher concentrations of As and Cd in urine and Al, Fe, Cd, Pb and V in hair of all subjects exposed to war operations were found.

Furthermore, environmental biomonitoring has shown high levels of As, Fe and Cd in drinking water of some settlements and also higher levels of Cd, As, Hg , Sb and Mg in soils and vegetables collected from number of sampled locations (even exceeding maximum allowed concentrations) .

Conclusion: By making full environmental and population biomonitoring for selected settlements a necessity for further research emerged. Many of elevated metals have toxic effects, either on male or female reproductive system or on pregnancy outcomes. Cadmium is one of the most interesting in this study , since is known “metallohormone” activity on uterus and on impaired sperm production. Further extended research is planned .

Key words: war, contamination, metals, metalloids, Croatia

Cardiovascular medications during pregnancy

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Aim: The course of pregnancy is associated with a number of changes in the woman's body. Literature data indicate that 1%-3% of pregnant women develop some cardiac disorder. The aim was to assess the prevalence of cardiovascular drug use and the rate of congenital malformations in neonates. One arm of the study (one-month study) was performed at four maternity hospitals in Zagreb, Croatia and the other arm of the study (one-year study) was performed in Novi Sad, Serbia.

Methods: The study was designed as cross-sectional study, and included 893 pregnant women from Zagreb and 6099 pregnant women from Novi Sad.

Results: According to data collected in the Zagreb arm, the prevalence of cardiac disorders in pregnancy was 3.6% (n=32), whereas data from the Novi Sad arm indicated a prevalence of 1.7% (n=102), yielding a predominant use of β-blockers in Zagreb (n=32; 3.6%) and of antihypertensive agents in Novi Sad (n=64; 1.0%). In Zagreb, malformations were detected in 26 (2.9%) fetuses and newborns in the general population and in 3 (9.4%) fetuses and newborns *in utero* exposed to cardiovascular agents. The agents taken during pregnancy were almost exclusively from FDA C and D classes. The prevalence of congenital malformations in fetuses at *in utero* exposure to FDA class D drugs was 3.1% (n=1). In Novi Sad, malformations were detected in 326 (5.35%) fetuses and newborns in the general population and in 5 (4.9%) fetuses and newborns *in utero* exposed to drugs for cardiovascular disorders. Pregnant women most frequently used FDA class C drugs. Class C and D drugs were most commonly taken in first trimester, when the fetus is most sensitive to the action of teratogenic factors. A severalfold increase in the use of methyldopa and slight increase in the sporadic use of furosemide was recorded by the end of pregnancy, whereas the use of other drugs was reduced or completely discontinued. One case of a major malformation, palatoschisis, was found in a woman having taken a C class drug.

Conclusions: For most cardiac disorders, the risk posed by the disease itself for both the mother and the fetus generally exceeds the postulated risk of medications used to treat the disease. If a pregnant woman requires such therapy, a respective agent with the best safety profile should definitely be prescribed. The exact cause of these malformations cannot be positively identified because the fetus is generally not exposed to a single teratogenic factor but to a combination of such effects.

Keywords: pregnancy; cardiovascular medication; Zagreb, Novi Sad

Nanobiotechnology and Cancer Research

Expression of TFF3 in epithelia of mouse fetus

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Aim: TFF3 protein has an important role in maintenance of epithelial integrity by acting through various mechanisms. In this work we wanted to get insight in TFF3 expression in mouse fetus
Methods: Mouse fetuses, day 15 to 17 were isolated, fixed in 4% paraformaldehyde, paraffin embedded, cut (6μm), and processed for immunohistochemical staining. Primary polyclonal rabbit anti-TFF3 antibody was used, and PBS as a negative control.
Results: TFF3 expression was found in keratinocytes that constitute basal and middle layers of stratified epithelia in developing skin of fetuses and in keratinocytes at the vestibulum of the oral cavity. Goblet cells of the nasal cavity, bronchi and gut also stained positively. Mild signal was found in the epithelium of kidney collecting ducts. Negative controls showed no signal.
Conclusions: TFF3 has been shown to promote migration of epithelial cells *in vitro* and *in vivo*, partly by reducing cell-to-cell adhesion. It participates in immune response, and activates various signaling cascades. TFF3 is also known to affect apoptosis. Since it is expressed in various epithelial tissues during fetal development, it could play an important role in these actively proliferating tissues. Cancers which express or overexpress TFF3 probably only exploit the physiological properties of TFF3 in growth and metastasing, which has diagnostic and potentially therapeutic implications.
Keywords: epithelial cells, fetal development, mouse, neoplasms, TFF3 protein

Translational Research and Drug Development

Novel monomethine derivatives as detection tools

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Aim: Development of new monomethine derivatives based on cyanine dyes and their application in molecular biology as detection tool is highly prospected and promising thanks to their fluorescent ability. Depending on their model of interaction with nuclear acids and preference for AT or GC region they can be used for nuclear acids visualization and as marker for cell staining.
Methods: Fluorescent microscopy were used for evaluation and detection of cell localization of monomethine cyanine derivatives no.1,2,3 on live HeLa cells with Hoechst 33258 as control. Agarose gels post staining was applied for DNA/RNA visualization with SybrSafe as positive control.
Results: Evaluated cyanine derivatives no. 1,2,3 are spread all over the cell expressing green fluorescent signal. 30 minutes after incubation signal from derivatives no.1 and 2 is strong and easily detected. Signal is stable during the next 2h. Derivative no.3 need 90 – 120 minutes to enters in to the cell and localize. Signal is weaker then for other two mentioned before. 15 and 45 minutes gel post staining showed that derivatives no.2 and 3 gives good visualization of DNA/RNA under the UV and trans white light. Results are comparable to SybrSafe. Derivative no.1 gives no visual results of DNA/RNA on gel.
Conclusions: Monomethine derivative no.1 is the best candidate for fluorescent microscopy since it enters quickly giving strong signal. It can be used in combination with other dyes for multiple detection. Derivatives no. 2 and 3 are promising candidates for further development as visualization tool.
Keywords: monomethine cyanine derivatives, visualization, cell localization, DNA/RNA, SybrSafe

Association of ACE DD-genotype in women and renal disease

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Aim: To investigate the significance of insertion/deletion polymorphism of angiotensin-converting enzyme (ACE) as a possible contributing factor in the development of diabetic nephropathy.
Methods: Genomic DNA was extracted from whole blood of 100 patients with diabetic nephropathy and 102 diabetic patients with normal renal function (urinary protein excretion rate less than 300 mg/day and creatinin clearance level ≥ 80 ml/min). Genotyping was carried out using primers and fluorescent probes in a LightCycler System (Roche). Statistical analysis was performed using the SPSS 19.0 software (SPSS Inc, Chicago, IL, USA).
Results: Genotype frequencies of the ACE I/D polymorphism were in accordance with the Hardy-Weinberg equilibrium. Females with end stage renal disease showed higher frequency of DD genotype than those with preserved renal function (41% vs. 28%; p=0,038). In addition, the D allel frequency was significantly higher in women with diabetic nephropathy compared to controls (χ^2=6,345, p=0,011), respectivly, only one of ten women does not posses the D allel.
Conclusions: Based on obtained results could be concluded that the ACE DD genotype has an impact for the development of renal disease in females with type 2 diabetes, indicating a possible DD genotype-associated gender effect in renal disease.
Keywords: diabetes mellitus type 2; diabetic nephropathy; angiotensine converting enzyme, insertion deletion polymorphism

CZECH REPUBLIC

Women's Health and Cardiovascular Diseases

Influence of SNP in gene for prepro-orexin on prevalence of obesity in the Czech population

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The obesity is current world problem that reaches pandemic proportions. OrexinA and orexinB are produced by neuron cells of lateral hypothalamic area, also known as "hunger center". Involvement of these neuropeptides in control of feeding behavior was previously confirmed.

We investigate a role of single nucleotide polymorphism in gene for prepro-orexin (rs760282). SNP was monitored in population study Czech postMONICA (N=2500). This first study provided information on the frequency of mutations in this SNP in the general population. Second study was realized on the obese children group – patients (N=349) before and after spa intervention. Investigated genotype (-909T/C) is substitution T (wild-type allele) for C (mutant allele).

Distribution of genotype in population TT, TC and CC: 53.93, 39.44 and 6.63% respectively. Database of patients exhibited 55.30, 39.54 and 5.16% respectively. Genotype distribution between databases is not statistically differ (P=0.57).

Data in male and female postMONICA groups were tested for BMI, waistline (WL), hips (H) and cholesterolemia (CHOL). In female group was found significant increasing trend in investigated parameters. The trend is most markedly in WL (85.99±13.36, 85.81±13.32 and 84.43±13.34cm) and H (105.69±10.49, 105.49±10.61 and 105.18±10.91cm) for TT, TC and CC allele respectively. In male group significant difference between monitored parameters was not found.

Results in patients for BMI before spa intervention was 30.52±4.35, 31.02±4.66 and 30.57±4.78kg/m^2 for TT, TC and CC allele respectively and after spa intervention 28.17±4.09, 28.77±4.38 and 28.29±4.39kg/m^2. Change of BMI before and after intervention was – 2.31±0.73, – 2.30±0.76 and – 2.28±0.78kg/m^2 for TT, TC and CC allele respectively.

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Mother and Child Health

Role of MTHFR gene in spontaneous abortions

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Introduction: According to estimates, up to 20% of pregnancies end in the first trimester by spontaneous abortion (SA). Apart from a negative impact of some environmental factors on the embryonal development, it is evident that genetic factors also play an important role. One of the candidates is methylenetetrahydrofolate reductase gene (MTHFR) which is associated with disorders in folate dependent homocystein metabolism and DNA methylation.
Material and methods: We collected DNA from 538 samples of spontaneous abortions and genotyped MTHFR variant C667T by PCR-RFLP method. The frequencies of genotypes were compared with known population sample of adults (N = 2 555). For the statistical analysis we used $\chi 2$ test.
Results: Genotype frequencies which are within the HW equilibrium are similar to the neighbouring populations. There was a significantly lower frequency of the TT genotype in SA in comparison with the controls (8.7% vs. 12.1%) when we compared CC genotype carriers (44.4% in SA and 42.7% in controls) with t-allele carriers (32.2% in SA and 34.7% in controls, P=0.028, OR=1.071**,** CI=0.89-1.29).
Conclusions: Our results suggest that TT genotype in MTHFR may have a protective effect on embryo development.
Keywords: spontaneous abortions, methylenetetrahydrofolate reductase gene

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Translational Research and Drug Development

Verification of novel potential biomarkers of intraamniotic infection and inflammation in preterm birth patients

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Aim: We have recently performed global quantitative proteomic analyses of amniotic fluid from preterm birth patients to identify novel potential biomarkers of intraamniotic infection and inflammation (IAI). This phase of the project, the discovery phase, pointed on several amniotic fluid proteins with altered levels, which could be thus associated with the presence of IAI. Our mail goal was to verify these initial findings using complementary techniques.
Method: Except the antibody-based approach ELISA, which is the golden standard in protein quantitation, we used a novel perspective proteomic tool for targeted protein detection and quantitation – multiple reaction monitoring mass spectrometry (MRM MS). Without the need for a specific antibody, this approach can detect and quantify multiple proteins in complex matrices with high sensitivity, accuracy and precision.
Results: Using ELISA and MRM, we targeted and quantified selected proteins, which showed statistically highly significant dysregulation in our initial discovery phase experiments. We were able to confirm altered levels of all of these selected candidates. Most of these proteins showed correlation with the presence of IAI.
Conclusions: Our findings show, that protemics is indeed capable of providing novel insights into various pathologies, with one of the goals being the identification of novel biomarkers. We sucessfully demonstrated this using a two stage strategy, where we idetified and verified several novel potential IAI biomarkers.

Keywords: proteomics, biomarkers, intraamniotic infection and inflamation

HUNGARY

Women's Health and Cardiovascular Diseases

Oxidative contractile dysfunction in human cardiomyocyte

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Aim: Oxidative myofilament protein alterations have been shown to contribute to myocardial contractile dysfunction. We aimed to characterize the effects of myeloperoxidase (MPO) on cardiomyocyte contractile function and to identify the related myofilamentary protein modifications in left ventricular human myocardium.
Methods: Ca^{2+} dependent (F_{active}) and independent ($F_{passive}$) forces were measured in permeabilized human cadiomyocytes before and after applications of hydrogen peroxide (H_2O_2), MPO and in the presence or absence of an MPO-inhibitor (MPO-I) and the reducing agent dithiotreitol (DTT). Ellman's and sulfhydryl (SH) group biotinylation assays were used to quantify the extent of the SH oxidation of myofilament proteins.
Results: Application of H_2O_2 significantly decreased cardiomyocyte F_{active} (from 23.1±3.7 kN/m^2 to 16.0±2.8 kN/m^2, n=7, P<0.01) and increased $F_{passive}$ (from 3.5±0.9 kN/m^2 to 4.0±0.9 kN/m^2, n=7, P<0.01). When H_2O_2 and MPO were applied together, a reduction in F_{active} and an additional increase in $F_{passive}$ were observed. The MPO-I could partially prevent the effects on F_{active} and $F_{passive}$ (from 16.3±3.4 kN/m^2 to 11.1±1.6 kN/m^2; and from 1.8±0.4 kN/m^2 to 2.3±0.5 kN/m^2 (n=5), respectively). Combined application of H_2O_2 and MPO significantly decreased the relative SH content of myofilament proteins (to 87.04±1.2% from 100%, P<0.05), which effect was reversed by the reducing agent DTT. DTT also reversed the MPO induced increase in $F_{passive}$ (from 2.4±0.3 kN/m^2 to 1.4±0.2 kN/m^2, n=6). H_2O_2 and MPO significantly decreased the number of SH groups of the actin and a recently unidentified ~60 kDa molecular weight protein.
Conclusions: MPO-derived oxidants may contribute to myocardial contractile dysfunction via decreasing cardiomyocyte force production and increasing $F_{passive}$ of human cardiomyocytes. These effects are mainly attributed to myofilament protein oxidation and could be partially prevented by MPO-inhibition.
Keywords: myeloperoxidase, hydrogen-peroxide, SH oxidation

Adverse effects of statin on skeletal muscle cells

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Aim: Statins are the most widely used drugs in the treatment of hyperlipidaemia. Their side-effects on skeletal muscle, however, have been reported with increasing frequency. Although the pathomechanism of the statin associated myopathy has been studied in detail and several theories do exist, there is still no real consensus.
Methods: To the study of the effects of statin on skeletal muscle rats were fed with a special diet to achieve high blood cholesterol levels and fluvastatin was administered to them orally. Fluvastatin and rosuvastatin were also applied on rat primary cell cultures. Morphometric analysis was used to detect effects on cell proliferation and differentiation. Effects on the cytoskeletal structure of muscle cells were visualized using immunofluorescence.
Results: Cholesterol levels rose more than seven fold (from 1.5±0.1 to 10.7±2.0 mmol/L; n=15 and 16) with a dramatic increase in LDL/HDL ratio (from 0.29±0.02 to 1.56±0.17). While the latter was reversed by statin treatment, an elevation in blood creatine kinase level remained. In the control cell cultures the number of myogenic nuclei increased, as compared to the end of the 1st culturing day, by 58% after 24 hours and by further 54% after an additional day. In contrast, the number of myogenic nuclei in the fluvastatin-treated group rose by only 3% after 24 hours and fell by 18% (decrease not significant) after another 24 hours. Cytoskeletal components of the cells were visibly degraded; most profound effects were seen on the distribution of fibrillar actin.
Conclusions: Our results clearly demonstrate that statins elicit skeletal muscle related adverse effects in rats therefore such an animal model could be used to test their side-effects. The effects of statins on the proliferation, differentiation and structure of developing muscle cells may explain certain adverse effects seen in clinical use.
Keywords: skeletal muscle, cholesterol, statin, cell proliferation

Twins and veins

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Aim: To estimate the heritability of venous biomechanics on a twin sample.
Methods: 79 monozygotic twin pairs (age 43.0±1.3 years, mean±standard deviation/SD/) and 38 dizygotic twin pairs (age 46.7±2.0 years, mean±SD) were involved. Anteroposterior and mediolateral diameters of the common femoral vein at both sides were measured by B-mode ultrasound. Measurements were made both in supine and standing body positions, with or without controlled forced expiration (Valsalva test).
Results: Genetic factors play a decisive role in determining the geometrical properties in its distended state, that is, in the erect body position and with substantial Valsalva pressures (Clark's heritability indices between 0.41-0.57 for capacity in the erect position). Environmental factors (shared and unshared) determine geometry at lower pressures. Both environmental and genetic factors shape distensibility. Correlations of capacity between twin pairs are high in all age groups, especially between monozygotes in the aged group.
Conclusions: Venous biomechanical behavior is strongly influenced by genetic factors at high pressure, while environmental factors shape the venous biomechanics at low pressure. Elucidation of the genes that influence venous biomechanics at different pressures may provide important new insight in the pathophysiology of venous diseases that are associated with altered venous biomechanics.
Keywords: venous biomechanics, heritability, genetics, Valsalva manouver

Do you see double?

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Aim: To estimate the relationships between intraocular pressure, arterial stiffness and central blood pressure.
Methods: As part of the International Twin Study 2009, intraocular pressure (Goldmann applanation tonometer), arterial stiffness (aortic augmentation index, Aixao, aortic pulse wave velocity, PWVao) and central systolic blood pressure (SBPao) of 155 twin subjects (mean age 43±16,2 years, 26% men) were investigated. Regression coefficients (β) between intraocular pressure, arterial stiffness and central blood pressure were calculated. Possible statistical biases originating from familial relationship were corrected.
Results: Age- and sex-adjusted β and significance (p) values are shown in the table.

	Right ocular pressure		Left ocular pressure	
	β	*p value*	β	*p value*
AIxao	-0,093	*<0.001*	-0,097	*<0.001*
PWV_{ao}	0,163	*0,237*	0,212	*0,05*
SBP_{ao}	0,035	*0,037*	0,046	*0,01*

Conclusions: Significant relationships between the intraocular pressure, Aixao and SBPao were estimated bilaterally, but only in one side concerning the PWVao, however the direction of β was the same. Ophtalmological follow-up of patients with hypertension, and detailed cardiovascular check-up in case of ocular hypertension is recommended.
Keywords: Arterial stiffness, central blood pressure, intraocular pressure, hypertension

Genetic relation between arterial haemodinamics and exhaled nitric oxide

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Aim: Nitric oxide, measured by fractional exhaled nitric oxide (FE_{NO}) has an important role in the airways and vessel walls. Several factors are known to influence its level and arterial stiffness in the general population, but the association between them is unknown.

Methods: 117 adult twin pairs (48 American, 35 Hungarian and 34 Italian; 83 monozygotic and 34 dizygotic pairs; 48±16 years /mean±standard deviation/) were investigated. FE_{NO} was measured by an electrochemical sensor-based device and the markers of arterial stiffness (brachial and aortic augmentation index, Aix_{bra}, Aix_{ao}; aortic pulse wave velocity, PWV_{ao}) by the oscillometric TensioMed Arteriograph. Cholesky decomposition models were applied.

Results: Genetic effects accounted for 59% (95% confidence interval[CI]: 43%, 71%) of the variation in FE_{NO}. Shared environment accounted for 0%, while non-shared environmental influences explained 41% of the variation (95% CI: 29%, 57%). Significant negative within-twin correlation was observed between FE_{NO} and Aix_{bra} ($r=-0.19$, $p<0.05$), Aix_{ao} ($r=-0.16$, $p<0.05$) but no significant correlation was found between FE_{NO} and PWV_{ao} ($r=-0.08$, $p=0.27$). The genetic covariances of FE_{NO} and Aix_{bra}, FE_{NO} and Aix_{ao} were 100%.

Conclusions: Variations in FE_{NO} are explained by genetic and non-shared environmental effects. There is a strong genetic covariance between FE_{NO} and augmentation index providing a basis for further studies on the cardiovascular effects of airway diseases.

Study of postischaemic glucose uptake and glucose metabolism in isolated rat hearts by various NMR spectroscopy methods

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Aim: The glucose uptake of heart increases after ischaemia. The extent of this increase is modified by experimental and cardioprotective agents. The study was aimed to trace the metabolic fate of the glucose taken up in excess.

Methods: The rate of glucose uptake was studied *in situ* in Langendorff-perfused rat hearts by ^{31}P NMR spectroscopy after the administration of 2-deoxyglucose.

The metabolic fate of the glucose was traced by ^{1}H-^{13}C heteronuclear single quantum coherence 2D NMR measurements of tissue extracts of perfused rat hearts after the administration of universally ^{13}C-labelled glucose to the perfusion liquid.

These studies were performed for normoxic and postischemic hearts both in the presence and in the absence of poly(ADP-ribose) polymerase (PARP) inhibitors as known cardioprotective agents.

Results: The postischaemic increase of glucose uptake is moderated by most of the studied PARP inhibitors. HO-3089, in contrast, further boosts the increase.

When comparing the metabolite maps of the heart extracts of different groups, HO-3089 treated hearts showed among others (a) protection against lactate accumulation caused by ischaemia (b) further increase of aspartate accumulation caused by ischaemia (c) a glucose concentration between the normoxic and postischaemic values.

Conclusions: HO-3089 appears to improve mitochondrial function in postischaemic rat hearts as reflected by increased amounts of high-energy phosphate metabolites, decreased amounts of lactate and increased amounts of aspartate.

Keywords: ischaemia-reperfusion, perfused rat heart, glucose metabolism, NMR spectroscopy.

Mother and Child Health

The impact of maternal hypertension on neonatal adaptation

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Aim: To evaluate the relationship between the hypertension of the mother during pregnancy and the respiratory and metabolic adaptation of their infants after birth during their hospital stay.

Methods: We analyzed the data of neonates who were born between the 1st of January, 2009 and the 31st of December, 2011 at the Department of Obstetrics and Gynecology, Medical School, University of Pécs, Hungary. The data were collected data from medical records of e-MedSol platform and we used t-test, Mann-Whitney-test, Kolmogorov-test, relative risk calculation with 95% confidence interval to compare neonatal outcome.

Results: In this period 257 out of 3748 women suffered from hypertension during pregnancy. It leads to preterm birth 1.34 (RR:1.34 CI:1.219-1.471) times higher than in the healthy group. We found that the need of oxygen therapy (RR:1.18 CI:1.108-1.260) and transfusions (RR:1.06 CI:1.019-1.103), the incidence of hypoglycemia (RR:1.2 CI:1.115-1.300), hyperbilirubinemia (RR:1.06 CI:1.010-1.103), apnea (RR:1.05 CI:1.007-1.084) were significantly higher as compared to those without the maternal disease. The neonatal infection does not have significant connection with maternal hypertension.

Conclusions: Women who suffer from hypertension during pregnancy have higher risk to preterm delivery and maternal hypertension is a risk factor to respiratory and metabolic adaptation of their newborns.

Keywords: maternal hypertension, preterm delivery, neonatal adaptation

Morbidity data of early-term birth

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Aim: Timing of deliveries is an important issue, especially just before (34-36 weeks, late-preterm) and after (37-38 weeks, early-term) the 36th gestational week. We compared the morbidity data of early-term neonates to those who born on the 40th week of gestation.

Methods: We examined the data of neonates who were born between January 1, 2009 and December 31, 2010 at the Department of Obstetrics and Gynecology, Medical School, University of Pécs, Hungary. We compared the morbidity data of early-term (n=963) to term infants who were born in the 40th gestational week (n=1235) using t-test, Mann-Whitney-test, Kolmogorov-test, relative risk calculation with 95% confidence interval. We analyzed the birth weight, rate of respiratory difficulties, oxygen therapy, infection, apnea, hypoglycemia and hyperbilirubinemia.

Results: The risk is 2 times higher for mechanical ventilation in the group of early-term infants (RR:2,00 CI:1,06-3,80) compared to those who were born at the 40th week of gestation. There is no significant difference between the two groups with regard to apnea and respiratory problems, but the risk of respiratory difficulties is 2.6-fold if we take only the babies born on the 37th week into account (RR:2,57 CI:1,40-4,73). To be an early-term baby means 2.0-fold risk for hyperbilirubinemia (RR:1,97 CI:1,39-2,79), 2.3-fold risk for hypoglycemia (RR:2,28 CI:1,74-3,00), 1.4-fold for infections (RR:1,43 CI:1,26-1,62). We examined the infants who conceived by in vitro fertilization and found 1.5-fold risk for being delivered on the 37-38th weeks (RR:1,55 CI:1,31-1,83) and 2.8-fold risk for late-preterm delivery versus late-terms (RR:2,81 CI:2,31-3,41).

Conclusions: Early term infants have more difficulties with regard to respiratory and metabolic adaptation compared to late-term infants. Our results draw attention to the magnitude of the monitoring of the early-term neonates' adaptation.

Keywords: early-term delivery, neonatal adaptation

Screening of pregnant women for *Streptococcus agalactiae* at University of Debrecen

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Background: *Streptococcus agalactiae* or group B streptococcus (GBS) is a Gram-positive bacterium which may cause invasive diseases in newborns or in pregnant and postpartum women. It is considered to be the leading cause of neonatal sepsis and meningitis in the United Sates and Western Europe. Vaginal or cervical GBS colonization is detected by a culture-based screening strategy, which should be carried out in 35-37 week of gestation to indicate intrapartum antibiotic prophylaxis.

Aim: The aim of the present study was to analyse the results of GBS screening among pregnant women at University of Debrecen between 01.January 2011 and 31. December 2011.

Methods and patients: Screening for GBS colonizaton and susceptibility testing were performed according to the recommendations of CDC 2010. During the examined period, we tested the cervical samples of 293 pregnant women for *S. agalactiae*. The bacteria were isolated on blood agar (Oxoid). The beta-heamolytic, catalase negative strains were identified to species level by slide agglutination. The antibiotic susceptibility was performed by disk diffusion test according to the Clinical Laboratory Standards Institute (CLSI, 2010) recommendations.

Results: The prevalence of *S. agalactiae* among the pregnant women was 13.7 % (40/293). Heavy colonization was detected in 29 cases (72.5 %), while in 11 cases a low level of colonization was recorded (27.5%). All isolated strains proved to be susceptible to penicillin, and beta-lactam. 73 % and 75 % of the strains were susceptible to clindamycin and erythromycin, respectively.

Conclusion: As a conclusion it is noted that detection of the colonization status for *S. agalactiae* and the antibiotic susceptibility of the isolated strains are important data for determination of the appropriate intrapartum antibiotic prophylaxis.

Keywords: GBS, prevalence, pregnant women, colonization

Peripheral Th1/Th2/Th17/regulatory T-cell balance in asthmatic pregnancy

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Aim: Asthma is a common chronic disease that may complicate pregnancy and a risk factor for complications; however, immunological mechanisms of the bilateral interactions between asthma and pregnancy are not fully understood. Healthy gestation is characterized by a sensitive balance of Th1/Th2/Th17/regulatory T (Treg) cells that may be altered in asthmatic pregnancy. The aim of this study was to describe the prevalence of these cell subsets in asthmatic compared with healthy pregnancy.
Methods: The prevalence of Th1, Th2, Th17 and Treg lymphocytes was identified by cell surface and intracellular marker staining in blood samples of 24 healthy non-pregnant (HNP), 23 healthy pregnant (HP), 15 asthmatic non-pregnant (ANP) and 15 asthmatic pregnant (AP) women using flow cytometry.
Results: The Th1/Th2 cell ratio was decreased in both HP and ANP compared with HNP women; however, no further decrease was observed in the AP group. The Th17/Treg ratio was decreased in HP, but not in AP women, compared with HNP data. Healthy pregnancy increased Treg cell prevalence compared with HNP data (4.64% versus 2.98%; $P < 0.05$), and this pregnancy-induced elevation was absent in AP women (2.52% versus 4.64%; $P < 0.05$). Th17 cell prevalence was similar in the HP and HNP groups (2.78% versus 3.17%; $P > 0.05$). Asthma increased Th17 prevalence in non-pregnant patients (3.81% versus 3.17%; $P < 0.05$), and this asthma-specific increase of Th17 cell prevalence was also observed in AP patients (AP versus HP: 3.44% versus 2.78%; $P < 0.05$).
Conclusions: The abnormal asthma-dependent Th17 elevation together with blunted Treg increase may play a role in the compromised immune tolerance characterizing asthmatic pregnancy.
Keywords: asthma, pregnancy, Tc17, Th17, Treg

Nanobiotechnology and Cancer Research

Primary systemic therapy of breast cancer and monitoring the tumour response

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Introduction: Neoadjuvant or primary systemic therapy (PST) is a commonly used therapy for treating locally advanced breast cancers nevertheless its application has increased in the therapy of primary operable breast tumors as well. PST has many advantages: increasing the chances of breast-conserving surgeries, the tumour response for the chemotherapy drug can be monitored *in vivo*, in case of complete pathological response (PCR) the length of disease free survival period is longer.
Aim: To compare the tumour response monitored by ultrasonography (US) and physical examination (PE) and examine breast cancer subtypes likely to respond to PST.
Methods: We have processed the data of 81 patients of Semmelweis University Department of Diagnostic Radiology and Oncotherapy who received PST between 1997 and 2009. The database was analyzed retrospectively. The statistical analysis was performed by a SPSS Stat 17 program.
Results: Most of the patients discovered their tumors by self-examination (67%), X-ray mammography was performed at 60.3%, US was performed at 71.8% of all the patients. The tumours were typically IDCs. Grade 3, high proliferation rated and HER2 and oestrogen receptor positive lesions were dominant. The patients received antracycline and taxane based treatment, typically 6 cycles. The decrease in the size of the tumours was significant by both US and PE ($p=0.003$ and $p=0.001$), mammographical monitoring data were not available at the majority of the patients. PCR could be reached at 8 % of the patients, which was more frequent at triple negative and high proliferating lesions. Mastectomy was avoidable at 40.5% of the patients. During the monitoring period (average 5.5 year) distant metastasis appeared at 21.8 % of the patients, and we lost only 1 patient because of breast cancer.
Conclusions: PST is efficiently applicable on patients with breast cancer. Both PE and US can be used in monitoring of response to therapy.
Keywords: PST, ultrasonography, physical examination.

Examination of primary breast cancers and their distant metastasis

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Aim: Breast cancer is the most common malignant tumorous disease among women. Nowadays it can be treated succesfully but after the appearance of distant metastases it is considered to be incurable. Our aim is to examine primary breast cancers compared to their distant metastasis and also to monitor the patients' survival rate related to oncological treatment.
Methods: We reviewed the autopsies done in the 2nd Department of Pathology (Semmelweis Univ.) of patients who died in metastatic breast cancer between 2001 and 2011. We examined 18 out of the 82 cases we found who had primary breast cancer and their complete clinical documentary was available. Beside the primary tumors we examined 61 distant mets. For analysing the datas we used Statistica for Windows and SPSS Stat 17.
Results: The average age of the patients at the time of diagnosis was 59. The overall survival was on the average 5 years. The elapsed time until the appearance of the first distant metastasis was on the av. 3 years, the survival time after this was on the av. 1.33 years. 66.7% of the primary tumors were IDC, 27.8% were ILC., medullar carcinoma occured only in one case. We could only determine the immunohistochemical subtypes of the tumors in 15 cases. 2 were HER2 positive, 5 were triple negative, 4 were luminal A and 4 were luminal B. Poorly differentiated grade III tumors prevailed (53.34%) among the cases. Distant mets were mostly found in liver (61.1%), bones (50%) and lung (38.9%). The expression of Ki67 in the primary tumors were significantly higher than in distant mets (15.23 % vs. 3.31% p= 0.001). The hormone receptor status in the primary tumor and the distant mets were in occurence of ER 56.25% and PR 60% concordant. In the other cases the receptors were identified in the primary tumors but the distant mets were proved to be negative. The patients were likely treated with multiple lines of chemotherapy.
Conclusions: According to our research metastatic breast cancers are principally invasive ductal carcinomas. Nearly half of the tumors don't express estrogen receptor and a phenotype change can be occured in the distant metastasis. After the hematogenous spread of the cancer, the survival rate is expressly bad. To learn more about the biological characteristics of the examined tumors, we are planning to do their deeper molecular pathological examination.

Translational Research and Drug Development

PP1 dephosphorylates the retinoblastoma protein

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Aim: The phosphorylation level of retinoblastoma protein (pRb) plays an important role in the regulation of cell proliferation and cell cycle progression, and therefore has a major impact on the survival of malignant cells. While the phosphorylation of pRb by kinases is well characterized, our knowledge on the dephosphorylating phosphatases is limited. Protein phosphatase-1 (PP1) has been considered as one of the pRb phosphatases, however its direct role in the dephosphorylation has not been fully established. The goal of our study was to investigate the role of PP1 and possible PP1 inhibitory proteins on pRb phosphorylation.
Methods: The catalytic subunit of PP1 (PP1c) was silenced by transfection of siRNA in HeLa cells. Vectors coding for Flag-tagged KEPI (kinase enhanced phosphatase inhibitor) and LIM-kinase-2 (LIMK-2) were transfected into MCF-7 and HeLa cells, respectively. The efficacy of the transfections was checked by Western blot experiments and immunofluorescence using anti-KEPI, anti-LIMK-2 and anti-Flag antibodies. The viability of cells was determined by MTT assay.
Results: Silencing of PP1c by pan-siRNA reduced markedly the amount of PP1c and in turn it resulted in increased phosphorylation level of pRb. Both KEPI and LIMK-2 include a phosphorylatable peptide sequence implicated in the inhibition of PP1. Expression of KEPI in MCF-7 cells led to its phosphorylation by endogenous kinases and inhibition of PP1 reflected in enhanced pRb phosphorylation. Similarly, expression of LIMK-2 in HeLa cells decreased protein phosphatase activity leading to increased phosphorylation of pRb.
Conclusions: Our results indicate the essential role of PP1c in determining the phosphorylation level of pRb and suggest that PP1 is implicated in the regulation of cell cycle progression and survival of malignant cells, thereby PP1 could be considered as a drug target with therapeutic means.
Keywords: Protein phosphatase-1; retinoblastoma protein (pRb); pRb dephosphorylation; LIM-kinase-2; kinase enhanced phosphatase inhibitor (KEPI).

Inhibiting PARP prevents experimental demyelination

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Aim: Mitochondrial dysfunction has been indicated to play a role in loss of oligodendrocytes in multiple sclerosis (MS). A nuclear-mitochondrial crosstalk dependent on poly(ADP-ribose) polymerase (PARP) activation is critical in determining the fate of injured cells. Here, we investigated activation of PARP in MS lesions, and the effect of PARP inhibition on experimental demyelination.
Methods: MS lesions were studied by poly (ADP-ribose) (PAR) and apoptosis inducing factor (AIF) immunohistochemistry in 13 MS patients. In a primary demyelinating mouse model induced by cuprizone myelination of the corpus callosum (CC) was investigated by in vivo serial 9.4 T MRI and in vitro quantitative MBP immunoblotting/immunohistochemistry. On CC of mice also electron microscopy, PAR and AIF immunohistochemistry, MAP and Akt kinase and caspase-3 immunoblotting was performed.
Results: Strong PAR reactivity reflecting PARP activation was observed in apoptotic oligodendrocytes in pattern III MS lesions. AIF co-localized with anti-PAR staining in condensed nuclei. The same pathology was observed in the cuprizone model: PARP activation in CC; apoptotic morphology with enlarged mitochondria in oligodendrocytes; caspase-independent apoptosis with the nuclear translocation of AIF. 4HQ, a potent inhibitor of PARP blocked cuprizone induced poly(ADP-ribosyl)ation. Inhibition of PARP attenuated oligodendrocyte depletion and decreased demyelination, suppressed JNK and p38 MAP kinase phosphorylation, increased the activation of the cytoprotective PI-3/Akt pathway and prevented caspase-independent AIF-mediated apoptosis.
Conclusions: In summary, PARP activation may play a crucial role in the pathogenesis of pattern III MS lesions. Since PARP inhibition also reduces inflammation by attenuating activation of NF-κB, it may target all subtypes of MS: either by preventing oligodendrocyte death or attenuating autoimmune inflammation.
Keywords: multiple sclerosis, cuprizone, demyelination, PARP, MRI

Production and testing of anti-ErbB2 F(ab)2-s

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Aim: Trastuzumab, a humanized anti-ErbB2 antibody is a specific targeted therapy against ErbB2 positive tumors, with a history of both success and a high rate of therapy resistance. Another humanized antibody, pertuzumab inhibits ErbB2 heterodimerization. Combined application of the two antibodies may improve treatment outcomes by either enhancement at the molecular level (e.g. by hypercrosslinking-based enhanced internalization of ErbB2), or offering sterically complementary docking sites for NK cells implementing ADCC. Distinction between these routes can be ascertained by also testing the F(ab)2 fragments of these antibodies. In the first phase, we have generated these fragments and compaired them in vitro.
Methods: F(ab)2 from trastuzumab and pertuzumab was produced with pepsin-agarose and separated with gel filtration. Affinity and lack of Fc fragment on F(ab)2 was tested with immunofluorescence in flow cytometry. In vitro Ki was assessed with an MTT based assay. ADCC in the presence of the whole antibodies and absence thereof with F(ab)2 was tested in a real time adherence assay.
Results: Since protein A and protein G still showed binding of cleaved F(ab)2, chromatography optimized for exclusion size and ionic strength was used to produce proper F(ab)2 variants with intact antigen binding but no Fc region taggable with polyclonal anti-Fc. The effect on proliferation of in vitro sensitive and resistant cell lines BT-474 and JIMT-1 was not affected by removing FC region. The only measurable difference was a slightly enhanced dimerization inhibition by the smaller pertuzumab F(ab)2 as opposed to its intact parent antibody. Also, intact antibodies mediated ADCC-based killing of both tested cell lines, while F(ab)2 fragments did not.
Conclusions: The set of trastuzumab and pertuzumab whole antibodies and their F(ab)2s are now ready for in vitro and in vivo testing of their possible synergistic effects.
Keywords: ErbB2, trastuzumab, pertuzumab, F(ab)2

POLAND

Nanobiotechnology and Cancer Research

Functionalization and optical properties of $NaYF_4$ based nanocrystals doped by lanthanide ions for biomedical applications.

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Background: In the field of optical biomarkers for biomedicine applications lanthanide doped nanocrystals (NCs) are most interesting not only due to their optical properties, which are significantly better in comparison to organic dyes and even quantum dots, but also because of the low toxicity and low phonon vibrational host lattices, which promote strong fluorescence. However, in order to make them biocompatible and water soluble thier surface must be functionalized.

Aim: The aim of this work was to conduct surface functionalization of $NaYF_4$ based NCs doped with Europium in order to make them water soluble and ready for bio-conjugation.

Methods: $NaYF_4$ NCs were obtained by the co-thermolysis method with trioctylphosphine oxide (TOPO) serving both as a solvent and a surface ligand. Among various approaches (Ligand Oxidation, Surface Silanization, Ligand Attraction) we chose Ligand Exchange to change the phase of NCs from hydrophobic to hydrophilic. In a typical experiment we used dimercaptosuccinic acid (DMSA) solved in dimethyl sulfoxide (DMSO) as the hydrophilic ligand, which substituted TOPO on the surface. The functionalization process was carried out tens of degrees above the room temperature under nitrogen atmosphere with vigorous stirring. After transferring to water the optical properties of NCs were measured by the photoluminescence (PL), PL excitation and PL decay times experiments.

Results: Luminescent, surface functionalized (and thus water soluble) sodium fluoride nanocrystals doped with lanthanide ions with nanometer size diameter have been obtained.

Conclusions: There are numerous factors influencing the success of surface functionalization in NCs. It is determined that the most important ones are: ligand and solvent used in synthesis, process temperature, time and atmosphere, the ligand/solvent ratio and sonication of NCs prior to Ligand Exchange method. Control over them is crucial in order to prevent aggregation, increase the transfer yield and maintain high luminescence. Proper optimalization of these factors requires time, but it will result in unlocking full potential of NCs as optical biomarkers for application in medicine.

Keywords: nanocrystals, lanthanide ions, functionalization, DMSA

Synthesis of GDOF:Eu^{3+} nanocrystalline optical bio-probes by co-thermolysis method using a new kind of envarionmental friendly solvent

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Background: Considerable interest has been focused on the rare earth doped nanocrystals (NC's) due to their interesting optical properties (arising from the 4f electron configuration), and large possibilities in bio-medical applications as optical probes in different diagnostic approaches. Because of low phonon vibrational energy and low toxicity fluorides and oxyfluorides have been recognized as highly efficient fluorescent host materials for rare earth ions.
Aim: The aim of our work was to obtain nanometer fluoride phosphor with single and narrow emission band using a new kind of solvent.
Methods: GdOF:Eu^{3+} nanocrystals with hexagonal or orthorombic structure have been obtained from trifluoroacetate salts by co-thermolysis method in the presence of cheap and nontoxic solvent. This method is based on thermal decomposition of precursors compounds in high temperatures and leads to highly uniform, nanometer-sized particles. The structural properties of the products were characterized by X-ray diffraction (XRD) and transmission electron microscopy (TEM). The luminescent properties of the nanoparticles were investigated by excitation, emission, emission decay and absorption measurements. The structure and size of GdOF:Eu^{3+} nanocrystals were controlled by variable time of synthesis ranging from 15 to 60 minutes and different reaction temperature. **RESULTS:** Highly luminescent nanoparticles with controlled size in nanometer range have been obtained by using low cost and environment friendly method.
Conclusions: It is found that with decreasing size of nanocrystals, surface to volume ratio increases, increasing the number of ions placed at the NC's surface. In consequence, due to different local environments around the rare earth ions (surface or volume), their relative emission peaks intensity ratio changes. In consequence the color purity of nanocrystals can be controlled and optimized by nanocrystals size modifications by changing time and temperature of synthesis. In this way, new and optically efficient nanocrystalline bio-probes have been obtained.

Keywords: nanocrystals, rare earth ions, GdOF

ROMANIA

Mother and Child Health

Maternal, fetal and neonatal outcomes of pregnancy in women with type I diabetes

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Aim: the aim of this study was to investigate maternal, fetal and neonatal outcomes of pregnancy with type I diabetes who delivered in 'Dr. I. Cantacuzino' Clinical Hospital, Bucharest, Romania. Postal code: 73206

Methods: this was a prospective study. We enrolled 20 women with type I diabetes, who presented at our clinic before 10 weeks of gestation. We excluded two of them, one due to first trimester pregnancy loss, and one was lost to follow up. The data was collected from medical records of pregnant women with type I diabetes, from questionnaires filled in by the women and from the medical records of the neonates. All participants gave written informed consent.

Results: the mean age at delivery was 28,1 years. The mean duration of diabetes was 13 years. 22% (4/18) of women presented diabetes chronic complications at the beginning of pregnancy. 11,1%(2/18) presented also hypothyroidism. 88,88% (16/18) used multiple insulin injection and 94,4% (17/18) human insulin. The mean concentration of glycosylated hemoglobin at 10 weeks of gestation was good: 6,3 %, in most women 72,2% (13/18). Preconceptional folic acid supplementation was adequate in 22,2% (4/18) of the patients. Only 61,1% (11/18) of women performed daily home monitoring of blood glucose at conception, and only 27,7% (5/18) sought preconceptional guidance. The rate of preeclampsia was 22,2% (4/18), the rate of preterm delivery:27,7 % (5/18), the rate of cesarean section was 66,6% (12/18) of which 58,3% (7/12) were delivered by emergency CS. Maternal mortality rate was 5% (1/18). The rate of macrosomia was 38,88 (7/18), neonatal morbidity was high, the commonest morbidities were hypoglycemia 55,5% (10/18) and hyperbilirubinaemia 44,4% (8/18), congenital malformations rate was 11,1% (2/18),neonatal mortality rate was 5% (1/18).

Conclusions: Women with type 1 diabetes have a poor outcome compared with women without diabetes, despite the overall good early glycaemic control. They have increased rates of congenital malformations, preeclampsia, premature delivery, perinatal mortality and risk of delivering a macrosomic baby. The targets of the St Vincent Declaration of 1989 have not been met. Improvements may be gained by increased prepregnancy care and in the proportion of pregnancies that are planned.

Keywords: pregnancy, type 1 diabetes, preconceptional care.

Risk factors for preterm birth – epidemiologic analysis of 1000 deliveries in 2011 in "Dr.I.Cantacuzino" Ob-Gyn Department – preliminary results of a prospective study

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Background: Preterm birth is a major clinical problem associated with perinatal mortality and morbidity. Several factors may increase the risk of preterm birth and some of them are purported to predict it.
Objective: The aim of this study was to identify current risk factors associated with preterm delivery. Analysis of a retrospective study performed in our clinic in 2007-2009 did not show statistically significant data on the factors involved in premature birth. We found necessary to immediately start a prospective analysis to highlight the hierarchy of factors involved in premature birth. The data collection was under the guideline of the RECOOP HST Consortium Mother and Child Health Network Prospective Study.
Results: This is a prospective study which started in January 2011 – part of the larger multinational prospective study MOCHEA Research Network. We analyzed 1000 births at Dr. I. Cantacuzino Hospital, Bucharest, Romania. Of these 137 (13.7%) were preterm births (<37 weeks).
Mean maternal age was 28,2 years. Mean BMI was 25,6.
The factors that remained significantly associated with preterm birth were smoking (42.3%), anemia (24.8%), vaginal bleeding (16.4%). Prior spontaneous abortion was found in 29.1%. The medical history revealved urinary tract infections in 14.5% of cases, diabetes (9.4%) or uterine surgery in antecedents (9%). Twenty mothers (14.5%) have positive family history of preterm birth.
On the other hand, persistent psychosocial and behavioral factors continue to negatively influence birth weight. 31% of women who delivered prematurely did not seek or receive medical care during pregnancy.
Conclusion: Screening tests with prediction model for preterm delivery risk should be used for all pregnant women.
Prospective analysis allows more accurate identification of risk factors in preterm birth. This identification is not enough of them; it is necessary to develop predictive models to help early identification and possibly correction of these risk factors.
Further studies are required to understand the causes of the epidemic of preterm births in Romania.
Keywords: preterm birth, risk factors, prematurity

SLOVAKIA

Women's Health and Cardiovascular Diseases

Vitmin D and systolic blood pressure

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Aim: There are some evidence on the association of vitamin D (VD) with cardiovascular risk factors, including hypertension, independently of adiposity. Furthermore, low VD levels have been reported to predict future hypertension. The aim of our study was to analyze the interrelations between VD and blood pressure (BP) in healthy subjects.
Methods: The study was carried out during january to december on 429 healthy subjects (170M/259F). Body mass index (BMI) was calculated. Standard biochemistry, VD and intact parathormone (PTH) levels were measured. All participants were normotensive.
Results: Subjects were divided into 3 groups according the month of collecting data (A:January-April, B:May-August, C:September-December). Groups did not differ in biochemical parameters, PTH and BMI. VD levels variabled in dependence on season. The highest levels of VD (43.1 ng/ml) and the lowest SBP (117.7 mmHg) were found in grup B, and on the contrary, the lowest VD levels (29.6 ng/ml) and the highest SBD (122.9 mmHg) were found in group A. These differences were significant ($p<0.002$ for VD and $p<0.005$ for SBP). VD levels did not significantly correlated with SBP.
Conclusion: The significant relationship between VD and SBP was not confirmed, but our data suggest some possible role of low VD levels in SBP arise. The study was limited by number of participants, thus the future extensive study is needed. The confirmation of the hypothesis, that VD deficit is linked to the hypertension, would offer a cheap and effective method in reducing blood pressure or preventing of hypertension developmnet by optimizing vitamin D status. Considering the prevalence of VD deficit worldwide, even a modest effect on SBP would be of clinical importance.
Key words: vitamin D status, systolic blood pressure
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Mother and Child Health

Determinants of mental rotation in gifted children

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Aim: Mental rotation is one of the cognitive domains often studied in context of gender specific testosterone effect.. Low levels of testosterone are associated with higher scores in mental rotation tests in men but lower scores in women. It is currently unknown whether mental rotation is also associated with prenatal testosterone or with testosterone-related genetic polymorphisms. The aim of our study was to analyze associations between prenatal exposure or acurate testosterone effects and mental rotation in intellectually gifted boys and girls.
Methods: One hundred forty seven boys and eighty girls aged 10-18 years with IQ>130 were enrolled. Saliva samples were collected and used for ELISA of actual levels of salivary testosterone. The 2D/4D finger length ratio as an indicator of prenatal testosterone was measured on both hands and averaged. Amthauer mental rotation test was used for the assessment of this spatial ability. The CAG repeat polymorphism in exon 1 of the androgen receptor gene was analyzed using PCR and capillary electrophoresis.
Results: In boys, mental rotation coefficient was significantly influenced by 2D/4D finger length ratio (r^2=0.029; $p<0.05$) and the number of CAG repeats in the androgen receptor gene (r^2=0.048; $p<0.01$). Actual levels of testosterone did not correlate significantly with mental rotation. However, MANCOVA revealed that after adjustment of age as a confounding variable, only the effect of the genetic polymorphism was significant (r^2=0.046; $p<0.02$). In girls, only actual levels of testosterone significantly correlated with mental rotation (r^2=0.093; $p<0.01$). MANCOVA after adjustment of age revealed no association.
Conclusion: In intellectually gifted boys mental rotation is determined by genetic polymorphism of the androgen receptor and not by prenatal or actual testosterone levels. No significant impact of testosterone exposure or genetic variability in androgen receptor was proved in intellectually gifted girls.
Keywords: gifted children, mental rotation, testosteron

Metabolic changes in mothers during lactation

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Aim: Accumulation of fat, changes in lipid profile and decreased insulin sensitivity represent typical metabolic changes in maternal organism during pregnancy. Lactation promotes the complete restoration of these physiological changes. The aim of this study was to compare parameters of insulin sensitivity, lipid metabolism and bone turnover in three groups of healthy mothers.
Method: In 106 healthy mothers of healthy 5-to-12-month olds infants insulin sensitivity (HOMA index), lipid profile, concentrations of calcium, inorganic phosphorus and alkaline phosphatase activity were determined. Mothers were devided into 3 groups according to their lactation status in time of investigation. Sixty-nine mothers were breast-feeding (exclusively or partially), the second group of mothers weaned about 3 months before the examination (n=19), and the third group ceased with breast-feeding about 6 months prior to the study (n=18). Three groups of mothers did not differ significantly by age, BMI and weight gain during pregnancy.
Results: Breast-feeding mothers had lower plasma fasting glucose (p=0,040) and were more insulin sensitive (p=0,007) compared to the other 2 groups. Lactating mothers presented with the most favorable atherogenic profile: they had the lowest plasma triglyceride concentration (p=0,019), the lowest LDL- (p=0,020) and VLDL-cholesterol (p=0,019). Groups did not differ significantly in plasma calcium concentration, however breast-feeding mothers had significantly higher plasma levels of inorganic phosphorus (p=0,019) and higher activity of alkaline phosphatase (p=0,002).

Conclusions: In the mothers, breast-feeding promotes favorable lipid profile and insulin sensitivity but it increases bone turnover. These metabolic changes slowly vanish within a few months after the cessation of lactation.

Key Words: insulin sensitivity, lipid profile, bone metabolism, breast-feeding

Attention deficit hyperactivity disorder: Neurometabolite changes after treatment

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Aim: Attention deficit hyperactivity disorder (ADHD) is the most common neurobehavioural childhood disorder with the unknown ethiopatogenesis. The cortical-striatal pathway with the disturbances in catecholaminergic neurotransmission is the most discussed. Magnetic resonance spectroscopy brought a new light into ADHD neurobiology by detecting some neurometabolites. The aim of our work was to find out the 1H MRS neurometabolite changes after two months of atomoxetine or methylphenidate treatment in children with the ADHD-combined type.
Methods: Twenty-one children (mean age 12.3 years, range 6.1 to 16.8) underwent 1H MRS examination before and after two months of treatment with methylphenidate (n=10) or atomoxetine (n=11). The spectra were taken from the dorsolateral prefrontal cortex (DLPFC, 8 ml) and white matter behind the DLPFC (anterior semioval center, 7.5 ml), bilaterally using single-voxel 1H spectroscopy (1.5 T Siemens Symphony, PRESS, TE/TR = 30/3000 ms, 128 averages), and evaluated with the LCModel.
Results: After atomoxetine treatment NAA/Cr (N-acetylaspartate/creatine) decreased in the left (m0=1.560, SD0=0.121; m1=1.477, SD1=0.158; p=0.021) and Cho/Cr (choline) increased in the right (m0=0.194, SD0=0.027; m1=0.227, SD1=0.029; p=0.026) DLPFC. Glx/Cr (glutamate/glutamine/GABA) increased in the left white matter (m0=7.252, SD0=0.742; m1=7.787, SD1=0.697; p=0.013) after methylphenidate medication.
Conclusion: Decreased NAA/Cr could indicate the reduction of cortical-striatal circuit hyperactivity sustained by „self-sustaining loop“ after atomoxetine medication. Methylphenidate could have the effect on tonic dopamine release in mesocortical pathway via the increased activation of glutamatergic projections with the representation in the increased Glx/Cr.
Keywords: ADHD, neurometabolite, atomoxetine, methylphenidate
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Candidate gene variants in Slovak autism patients

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Aim: Autism belongs to one of the most genetically influenced neuropsychiatric disorders. However, the detailed genetic basis is far from being clear. Whole genome studies pointed out a number of candidate genes. These are mostly involved in synaptogenesis and neuroendocrine regulations. In this study we have focused on polymorphisms of oxytocin (OT), oxytocin receptor (OXTR), gamma-aminobutyric acid receptor (GABRG3), neuroligin (NLGN) and reelin (RELN) in autistic patients from Slovakia. All these genes were previously shown to be in association with autism.
Methods: After informed consent 90 autistic boys and 85 healthy boys were enrolled into the study. DNA was extracted from saliva. Four SNP polymorphisms were assessed in genes OT, OXTR, GABRG3, NLGN4X by RFLP method. In RELN gene $(GGC)_n$ STR polymorphism was genotyped by fragment analysis.
Results: We have found no significant differences in genotype distribution of examined polymorphisms in OT, OXTR, GABRG3 and NLGN4X genes. Significant difference in number of GGC repeats in 5´UTR of RELN gene between autistic and control boys (P=0.001) was found. Slovak autistic boys had higher number of GGC repeats (9.63±0.082) in comparison with controls (9.18±0.100).
Conclusions: Our finding confirms previous studies showing that higher number of GGC repeats in the RELN gene was associated with autism. Higher number of these repeats was found to be associated with decreased RELN expression that may have severe consequences on brain development and function. This is the first genetic study of autism in Slovakia. Further genotyping is needed to gain insight about genetic causes of this neuropsychiatric disorder in Slovakia.
Keywords: autism, candidate genes, reelin

Nanobiotechnology and Cancer Research

Nephrotoxic effects of Fe_3O_4 nanoparticles

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Aim: We evaluated the potential nephrotoxic effects of a single dose of i.v.administered oleic acid coated Fe_3O_4 nanoparticles (NP) in rats.
Methods: Female Wistar rats were administered either placebo (10% v/v rat serum in 0.9% NaCl), suspension of TiO_2 NP (positive control, bimodal 84/213 nm distribution), or Fe_3O_4 NP (bimodal 31/122 nm distribution) in doses of 0.1, 1.0 or 10.0 % LD50. Rats were sacrificed 24h, 7-, 14- and 28-days after NP injection (n=9-10/each group).
Results: Renal function of NP administered rats, as monitored by plasma creatinine and urea concentrations, creatinine clearance and protein excretion rate, did not differ significantly in either interval from rats administered placebo. There was no change in plasma concentrations of uremic toxines (i.e.: asymmetric dimethylarginine, advanced glycation end-products determined fluorimetrically or as carboxymethyllysine). No significant changes in the expression of pro-inflammatory (tumor necrosis factor -α) and pro-fibrotic (transforming growth factor-β1, collagen IV) genes in renal cortex were revealed. NP administration did not alter significantly the levels of kidney injury molecule-1, the marker of acute tubular damage. Correspondingly, renal histology did not reveal significant differences between groups. NMR measurement of T_2 relaxation times of kidney homogenates indicated no NP accumulation.
Conclusions: In young female rats, no obvious nephrotoxic effects were observed after a single i.v. dose of Fe_3O_4 NP.
Keywords: Fe_3O_4 nanoparticles, nephrotoxicity, female Wistar rats
Aknowledgement: *Study was supported by 7FP EC EU: NanoTEST (Development of methodology for alternative testing strategies for the assessment of the toxicological profile of nanoparticles used in medical diagnostics.), Grant No.: 201335.*

Translational Research and Drug Development

Role of CCN3 in experimental glomerulonephritis

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Aim: In contrast to factors promoting mesangial cell proliferation, little is known about their endogenous inhibitors. During experimental mesangioproliferative nephritis, glomerular CCN3 (also known as NOV or nephroblastoma overexpressed gene) expression is reduced prior to the proliferative phase and overexpressed in glomeruli and serum when mesangial cell proliferation subsides.
Methods: To further elucidate its role in mesangioproliferative glomerulonephritis, CCN3 was systemically overexpressed by muscle electroporation in healthy or nephritic rats. This increased CCN3 serum concentrations more than 3-fold for up to 56 days.
Results: At day 5 after disease induction, CCN3-transfected rats exhibited an increase in glomerular endothelial area and in glomerular mRNA levels of the pro-angiogenic factors VEGF and PDGF-C. In the mesangioproliferative phase (day 7), CCN3 overexpression decreased mesangial cell proliferation including expression of α-smooth muscle actin and matrix accumulation of fibronectin and type IV collagen. In progressive nephritis (day 56), overexpression of CCN3 resulted in decreased albuminuria, glomerulosclerosis and reduced cortical collagen type I accumulation. In healthy rat kidneys, overexpression of CCN3 induced no morphological changes but regulated glomerular gene transcripts (reduced transcription of PDGF-B, PDGF-D, PDGFR-β and fibronectin and increased PDGFR-α and PDGF-C mRNA).
Conclusions: The above data identify a dual role of CCN3 in experimental glomerulonephritis with pro-angiogenic and anti-mesangioproliferative effects. Manipulation of CCN3 may represent a novel approach to help repair glomerular endothelial damage and mesangioproliferative changes.
Keywords: mesangial proliferation, glomerulonephritis, NOV

Antiviral activities of natural extracts on coxsackieviruses

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Aim: The aim of the present work was to systematically study the effect of natural extracts – Ridostin and Ferrovir for studying their antiviral activity in vitro against coxsackievirus.
Methods: First the effect of the two preparations on the growth curve of different cell lines (GMK, Hep-2, L929) was studied, and to determine the optimum concentration for the antiviral study. The cells were also studied for gross macroscopic changes. The effect of these antiviral agents on eGFP-CVB3 was studied. Viral titrations were done in cells pretreated for 24 hrs with the either Ridostin or Ferrovir. Untreated cells infected with different virus dilutions served as controls.
Results: Different concentrations of Ridostin and Ferrovir did not have an influence on the attachment efficiency of three cell lines. We observed that the growth of human epidermoid cell line (Hep-2) was most sensitive to the preparations and mouse cell line L929 was least sensitive. Ridostin and Ferrovir did not have a negative effect on the cell lines of human, monkey and mouse origin. Optimal concentrations of these extracts were evaluated for each cell culture system. Further, antiviral activity of Ridostin and Ferrovir against coxsackievirus eGFP was studied. Our results demonstrate Ridostin and Ferrovir exhibited antiviral activity against eGFP-CVB3 in cell line L929, no antiviral effect was observed in GMK and Hep-2 cell lines. Our results suggest that only mouse fibroblast cell line L929 was able to induce interferons in the presence of the interferon inducers, Ridostin and Ferrovir in contrast to GMK and Hep-2 cells.
Conclusion: We conclude that the mechanism of the antiviral effect of the natural extracts, Ridostin and Ferrovir protecting the cells from coxsackievirus-infection was mediated via interferon induction.
Keywords: antiviral activity, ridostin, ferrovir, coxsackievirus

Cytokines in brain of Coxsackievirus infected mice

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Aim: To investigate the presence of cytokines IL-4, IL-1β, TNFα in brains of mice infected with isolates from different origins and coxsackievirus B5 (CVB5) prototype.
Methods: CD-1 mice were orally infected with patient's isolate (aseptic meningitis) and an S1B5 isolate from treated sewage sample (from the area of domicile of the patient) both identified as CVB5 and the CVB5 (Faulkner) prototype strain. Brains were collected on days 5, 10 and 45 post infection (p.i.), one part was snap frozen and other part was fixed in formalin and embedded in paraffin. Presence of cytokines was analyzed in tissue sections by immunohistochemical staining using polyclonal/monoclonal cytokine specific antibodies. Viral RNA was detected by reverse transcriptase PCR and nested PCR.
Results: IL-1 β was present in the brains of all the infected mice at day 5, 10, 49 p.i. All 3 cytokines IL-4, IL-1β, and TNFα were detected at day 5 and 10 p.i. in S2 B5 infected mice and day 45 p.i. of S1B5 infected mice. CVB5 showed mild induction of cytokines IL-1 and TNFα. Viral RNA was observed in 1/15 mice in CVB5 infected mice whereas 3/15 in the S1B5 infected mice and 7/15 in S2B5 infected mice.
Conclusion: Cytokine induction depended on the virus origin. Mice infected with environmental isolate S1B5 and patient's isolate S2B5 induced proinflammatory cytokines as well as the IL-4, which can be related to the presence of RNA in the brain of these mice.
Key Words: brain, coxsackievirus, isolates, cytokines.

UKRAINE –AMOSOV

Women's Health and Cardiovascular Diseases

Plethysmography analysis according to age and gender

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Pulse waves (PW), recorded on the small blood vessels of extremities, give an integral information about the CVS and other organs associated with the CVS. In particular, important task is to determine parameters values of the CVS and PW for both men and women in the norm and pathology for different ages. Measured parameters are - blood pressure (systolic and diastolic), heart rate (HR), peripheral blood oxygen saturation (SpO2) and the pulse wave dynamics. The purpose is to build normal parameters of a cardiovascular system CVS for people with different age and sex for further creation computer express diagnostic system. The **aim** was to develope method for PW forms analysis and to build quantitative measures for pulse wave shape. The research of the need to consider gender as important information in cardiovascular parameters classification was made. Pulse waves registration is provided by using the pulse oximeter UtasOxi 200. Sensors are placed on index fingers vessels of both hands of the patient. Pulse waves averaged over selected ensemble and transformed to normalized amplitude and duration for further classification of forms of PW. **Methods:** The research involved 188 people, including 87 women and 101 men. Cluster analysis and regression analysis were used to study the dependence of age and cardiovascular parameters in healthy people. These procedures were provided for further solving classification task with use of artificial neural networks, which makes it possible to estimate the biological age according to indicators of cardiovascular and other anthropometric data. NN are also used for evaluating normal parameters for further pathologies detection. As a quantitative measure of pulse wave shape changes proposed an index of pulse wave shape, which is calculated as the ratio of the harmonic components of Fourier expansion for normalized PW. **Results:** Proposed index of pulse wave shape gives a numerical estimate of changing the shape of PW of men and women with age, and pulse wave deviation from the norm. When using statistical methods to determine the optimal number of parameters for building a linear model for assessment of biological age, gender was not important criterion, and only had a high correlation with anthropometric indices and the level of SpO2. But further cluster analysis and attempts to build an assessment of biological age by training neural networks identified the need to consider gender as required parameter to construct an accurate model of age changes based on CVS classification. Error of biological age estimation on test data was near 3 years and significant parameters were index of PW shape, SpO2, diastolic arterial pressure and body mass index. **Conclusion:** Ability to analyze changes of PW form index with other parameters gives background information to assess the functional state of subjects and formation of diagnostic conclusions. Gender should be considered as required parameter to construct an accurate model of changes in PW forms with age. Taking in account this difference of norm for CVS parameters for man and woman diagnosis classification should consider this to provide reliable result. To increase the accuracy of classification there is a need to build bigger training set.

Gender based post CABG circulatory aspects

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Aim: To study the influence of morpho-functional circulatory gender aspects in the usage of compensatory resources of heart and vessel regulation in critical conditions in early period after CABG.

Methods: We used the database of patients in early period after CABG, 95 were men and 20 were women. We used ECG, ECHO, and blood pressure test and biochemical blood readings. We also used a control group of 96 patients: 48 men and 38 women. We compared 24 main values of systemic and coronary circulation and oxygen delivery value.

Results: The average age of investigated women was slightly higher (nearly by 20%) and was 57,4 an 52,8 years. Average height, weight, and body surface area in men were higher by 6, 19 and 12%.The main difference was in absolute geometrical values of the LV: end systolic and end diastolic values, and in the thickness of the posterior wall, interventricular septum and stroke volume. The average reading of LV beating in women was approximately 22% lower than in men. Women had a 7% decrease in DAP. This is explained by 39%, decrease in general peripheral resistance in women. Due to the differences in heart rate the heart index and the LV index minute work did not differ greatly. But the LV beating work index was higher in men by 24%.Pressure growth speed in the LV in the isometric phase was higher in women by 33%.Peripheral resistance index, was higher in men by 10%. In both groups it exceeded physiologically normal value. The oxygen extraction was higher in women. The myocardium energy usage coefficient was by 27% lower in women and the compensatory conditions were by 20% lower.

Conclusions: The study proves that the myocardium mass index of the LV in women is smaller by 10-15 g/m^2. In conditions of critical compensatory mechanisms the combination of decreased general peripheral resistance index and increased heart rate allows to create same integral readings in both genders due to 33% smaller LV beating rate in women.

Keywords: CABG, peripheral resistance index, heart rate.

Gender based hemodynamics regulatory reserves

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Aim: The aim of this work study the gender differences in correlation of time intervals of mechanical systole and diastole of the left ventricle depending on the heart rate, which show the regulatory reserves of coronary circulation.

Methods: The materials were based on screening of patients in early postoperative periods after CABG and heart valve replacement, including 517 men and 193 women. We used ECG, ECHO. We also used a control group with practically healthy men and women who didn't have any circulatory disorders. The control group consisted of 96 patients: 48 men and 38 women.

Results: We used the *EVR*-endocardial valuation ratio as a measure of adequate extracardial coronary circulation,

$$EVR=\frac{DPTI}{SPTI},$$

where *DPTI* – diastolic "pressure time" index; *SPTI* – systolic "pressure time" index, яwhich is the energy measure that the myocardium uses during one contraction cycle.

Compensatory condition of myocardium energy usage:

$$DPTI = D\cdot(AP_{cp.} - EDP_{LV}).$$

Work coefficient during one contraction cycle

$$SPTI = Sm\cdot ASP,$$

Conclusion: We created regression equations for men and women *Sm*/*D*(HR) THE correlation coefficient *R in all cases was statically significant* ($p < 0{,}01$).

Resulted in

$\underline{Men}$ *:* $Sm/D = 0{,}0143\cdot HR - 0{,}487;\ R^2 = 0{,}61; N = 517$

$\underline{Women}$*:* $Sm/D = 0{,}012\cdot HR - 0{,}5708;\ R^2 = 0{,}71;\ N = 193$

Coronary circulation in women is more endured and tolerant to increase in heart rate. Prior to surgical treatment of valve disorders and IHD gender differences in circulation control mechanisms are not seen due to heart disorders, but adequate surgical treatment restores gender differences in control. The main circulatory control mechanism in women in early postoperative stages is to control integral traits of circulation, specifically heart productivity, minute blood flow, left ventricle productivity. Women are more sensitive to diastole/systole circulatory disorders

Key Words: circulation, systole, diastole, heart rate

Gender based circulatory aspects in cardiosurgery

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Aim: To study the gender differences in hemodynamic in early postoperative period (PoP) in patients with valve disorders with increase of left ventricle(LV) size.
Methods: In early PoP we studied the hemodynamics in men and women with increased or normal LV. We evaluated following groups 93 women, of which 27 – normal (*group 1*), 20 – moderate increase (*group 2*) and 46 – severe increase (*group 3*) preoperative end diastolic index(EDI) of LV; 207 men, of which 47 – normal (*group 1*), 35 –moderate increase (*group 2*) and 125 –severe increase (*group 3*) preoperative EDI of LV. We used ECG and ECHO.
Results: In the analyzed groups 60% men and 49% of women had increased EDI of LV. Heart failure in group 1 was 34% men and 59.3% women, group 2 17.1% and 30%, group 3 28.8% and 34.8% In female groups 2 and 3 compared with group 1 the beating index increased by 10-14%, in combination with high heart rate it lead towards high heart index in all groups. Contraction activity in groups 2 and 3 was 32% higher than group1. The frequency of heart failure in men was 28 and 40% in women, thus, in women it was almost one third. In all three groups EDI and heart rate was by 20-30% lower in men.
Conclusions: The main reason of formation of gender-related hemodynamic specifics, without regards of preoperative changes is considered to be the mass of contracting myocardium. Its smaller size is compensated by the growth of heart rate and contracting activity of the heart. But, in case of dilatation of LV women tend to have higher frequency of heart failure, which shows smaller compensatory reserves. Most characteristics in men and women tend to be the same, mainly due to compensatory mechanisms.
Keywords: end diastolic index, heart rate, left ventricle

Nanobiotechnology and Cancer Research

Influence of nanocomplex and electromagnetic field on nonlinear dynamics of carcinosarcoma walker-256

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Aim: The study examines the effects of anticancer treatment by nanocomplex of nanodiamonds with anthracycline antitumor antibiotic doxorubicin and alternating electromagnetic field on nonlinear dynamics of the growth of carcinosarcoma Walker 256.

Methods: Nanodiamonds with mean diameter < 10 nm was obtained by a detonation wave. Mehano-magneto-chemical synthesis of nanocomplex from nanodiamonds with doxorubicin (Pfizer, Italy) was processed in mechanomagneto reactor «MMR» (NCI, Ukraine). The magnetic properties were studied by magnetometer using a «Vibrating Magnetometer 7404 VSM» (Lake Shore Cryotronics , Inc., USA) .The animal tumors irradiated locally by 40 MHz with an initial power of 100 W by «Magnetotherm» (Radmir, Ukraine).Nonlinear kinetics of tumor volume was evaluated by growth factor according to autocatalytic equation. Statistical processing of numerical results was carried out using Statistic 6.0 (© Stat Soft, Inc. 1984–2001) computer program with Student`s test.

Results: Detonation nanodiamonds were weak soft ferromagnetic with saturation magnetic moment m_s=0.15842 emu/g. Anthracycline antitumor antibiotic doxorubicin after mechano-magneto-chemical treatment was paramagnetic with m_s=+0. 06836emu/g in contrast to conventional drug which is diamagnetic with m_s= -0.01200emu/g. Complex from nanodiamonds with doxorubicin after mechano-magneto-chemical synthesis was weak soft ferromagnetic with m_s=0.058182 emu/g. In the research of animals with Walker 256 carcinosarcoma was shown, that nanocomplex on the basis of detonation nanodiamonds with doxorubicin after mechano-magneto-chemical synthesis had a greater antitumor effect on 10% ($p < 0.05$) than conventional doxorubicin during combined treatment by local electromagnetic irradiation of tumor.

Conclusions: Nanocomplex from nanodiamonds with doxorubicin after mechano-magneto-chemical synthesis was weak soft ferromagnetic with saturation magnetic moment m_s=0.058182 emu/g and initiated greater antitumor effect on 10% than conventional doxorubicin during combined treatment by local electromagnetic irradiation of tumor.

Translational Research and Drug Development

Gender influence on professional orientation and progress of bachelors education in technical university

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Some women have psychological barriers in chose of professions, inclines toward no technical fields. Frequent asked questions are: does gender influence on choose of profession? Does gender important for progress in career and education? What are competitive characteristics of male and female groups?

Aim: To approve females competitive capability in all professional fields in compare with males and to destroy female's psychological barriers in choose of any professions.

Methods: Evaluation of engendered influence on chooses of profession and on the progress in education was performed on the group of 1919 students of seventh faculties at technical university. Criteria for study with an application of correlation analysis included engendering structure of departments, excellent and send down results in education, stipendiary and non stipendiary students in 14 male and female groups at 7 faculties (covered medical technical oriented departments) included: Medical-Engineering Faculty (IMEF); Faculty of Electronics (FE); Information and Calculating Technologies Faculty (ICTF); Radio Engineering Faculty (REF); Device – Building Faculty (DBF); Engineering and Physical Faculty (EPF); Faculty of Heat and Power Engineering (FHPE).

Results: Significantly more male students choose technical professions, twice predominate females. More female in technical professions choose medical oriented specializations, if they have this opportunity. Despite of their medical prefers, female results significantly much better by all investigated parameters of education at technical faculties (even in heavy industry), then in mail group of study. Medical engineering faculty contained highest per centage of women (>50%) and twice more scholarship (grant) holder in female group in comparison with mails, what is higher then at all other medical and technical oriented faculties. In mean time, male easier reach "excellent" results at this faculty then at others, and have almost the same "excellent" level with female;

Conclusions: In spite of women have psychological barriers in choose of engineering profession and more often go in to medical fields, they have more capabilities then male groups of students as for medical and as for technical disciplines during period of education. Female's competitive capability in education superior to male in all professional fields.

Keywords: Engendering, psychology, capability, profession, education

UKRAINE – DHLNMU

Mother and Child Health

Glycodelin and prognosis of pregnancy

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Aim: stable high frequency of miscarriage (10-25%) requires new approaches to the prediction of this pathology.
Methods: A definition of the content α-2-microglobulin of fertility (Glycodelin), which is produced by endometrial glands and is considered as a possible marker of complications of pregnancy, the serum in 6 - 10 weeks of pregnancy and the threat of premature delivery in 22 - 25 weeks of gestation. Quantifying the glycodelin in serum was conducted by ELISA.
Study involved 69 patients, 10 applied for abortion at will, 20 with miscarriage that began, 19 with pregnancy that does not develop, verified by ultrasound, 20 pregnant with threat of preterm delivery in 22 - 25 weeks of gestation.
Results: serum level of glycodelin in women with physiological pregnancy in 6 - 10 weeks amounted to 120,4 ± 6,85 ng / ml, with the threat of pregnancy loss was reduced to 98,6 ± 3,45 ng / ml ($p < 0,05$ compared with control and pregnancy, that does not develop - 136,3 ± 7,35 ng / ml). With the threat of early preterm delivery was observed to increase glycodelin 139,5 ± 6,45 ng / ml ($p < 0.05$ compared with control and risk of miscarriage in the first trimester).
Conclusions: Changes in production of glycodelin in pathological pregnancy in trimesters I. and II. are going in different directions: in the I. trimester miscarriage occurs in background reduction, and in II. trimester - against the backdrop of increasing production of glycodelin. Women with pregnancy not developed in the I trimester was characterized by normal levels glycodelin in serum. Determination of glycodelin serum for I and II trimesters is an important non-invasive method of monitoring the course of gestational process.

Key words: preterm delivery, glycodelin, α-2-microglobulin of fertility, miscarriage

The current status and perspectives of umbilical cord blood banking in Ukraine

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Due to the high rates of hematologic malignancies the development of cord blood banking is of crucial medical and social importance in Ukraine.

Aim of the study was to evaluate the current status of cord blood banking and clinical application in Ukraine.

Methods: literary review and sociological (opinion poll).

Results: The first cord blood transfusions for managing blood lost in Ukraine are dated 1939 and the first cord blood bank in our country was created in 1984 in Kharcov, being perhaps the first European experience of cord blood banking. But it existed 5 years and again the cord blood banking in Ukraine launched in 2004. At the moment 4 cord blood banks offer their services to expecting parents in Ukraine but all are autologous. Hence, in Ukraine cord blood is being collected only in about 0.3% births. The only report on umbilical cord blood hematopoietic stem cell transplantation in Ukraine with its 47 million population is dated 2009 and concerns a 2 year old patient with hypoxic brain injury, whereas literary data gives evidence that autologous umbilical red blood cells and plasma are currently used for cardiopulmonary bypass in neonates undergoing surgery due to the congenital heart defects.

The opinion poll on the subject “cell therapy”, “cord blood banking” (2010), which included 1000 respondents (aged 18-60; males and females 1:1) as well as 1000 pregnant women and 200 obstetricians from different regions of Ukraine showed that both population and specialized medical staff realize the biologic value of cord blood and support the umbilical cord blood banking.

Conclusions: The Ukrainian population and medical community are ready to support the creation of the public cord blood bank. The necessity of its integration with the world registries is emphasized by the national peculiarities of HLA-phenotype and high rates of migration of the Ukrainian population to Europe and USA.

Keywords: umbilical cord blood, hematopoietic stem cells, hematopoietic stem cell transplantation, cord blood bank

Experimental hypothyroidism increased exposure of LFuc and DGlcNAc sugar determinants in rat adrenal gland

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Aim: To investigate influence of experimental hypothyroidism on tissue glycoconjugates of rat adrenal gland.

Methods: Hypothyroidism was induced in 150-180 g male Wistar rats by supplementation of their daily food allowance with 5 mg/kg of antithyroid drug mercazolil (1-methyl-2-mercapto-imidazole) during 30 days. Control rats were maintained in the same conditions on standard diet. Animals were sacrificed by diethyl narcosis overdose. Adrenal glands were removed immediately, fixed in 4% neutral formalin and embedded in paraffin. Sections 5-7 μm thick was subjected to routine lectin-peroxidase-diaminobenzidine staining protocol. Lectin set included Laburnum anagyroides bark agglutinin (LABA, specific to LFuc), Sambucus nigra agglutinin (SNA, specific to NeuNAc(α2-6)DGal), and wheat germ agglutinin (WGA, specific to DGlcNAc > NeuNAc).

Results: In control rats it was detected selective LABA affinity to vascular endothelium of adrenal medulla. Under experimental hypothyroidism endothelium of adrenal cortex, non reactive in control rats, was strongly labelled with LABA. Moreover, semiquantitative evaluation of medullary vessels endothelial reactivity revealed increased LABA binding. Hypothyroidism induced reduction of SNA, and enhancement of WGA reactivity apparently due to impairments of sialilation in association with unmasking of DGlcNAc residues. Hovewer, remodelling of SNA and WGA receptor sites was less significant in comparison with that for LABA.

Conclusions: Hypothyroidism has significant influence on glycoconjugate processing in rat adrenal gland, inducing enhanced exposure of LFuc and DGlcNAc determinants in association with reduced terminal NeuNAc residues. Vascular endothelium of adrenal cortex and medulla demonstrated heterogeneity in lectin binding.

Keywords: hypothyroidism, adrenal gland, lectin histochemistry, fucosoglycans.

Impairments of rat ovaria and endometrium in experimental hyper- and hypothyroidism as detected by lectin probes

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Aim: To investigate influence of experimental hyper- and hypothyroidism on carbohydrate determinants of rat ovaries and endometrium by means of lectin histochemistry.

Methods: 20 lectins of different affinities, including 5 new lectin preparations, purified from fungi, were used for the histochemical examination of carbohydrate determinants in rat ovary and endometrium under experimental hyper- and hypothyroidism. Lectin histochemistry was supplemented with general morphology and estrous cycle studies.

Results: Hyperthyroidism induced ovarian luteinisation, increased content of decidual-like cells and of collagen fibers in endometrium with no reliable influence on estrous cycle. Hypothyroidism was accompanied with retardation of estrous cycle, leucocyte infiltration of ovarian and endometrial stroma. Lectin binding in the ovaries of control rats was restricted predominantly to zona pellucida and corona radiata of growing follicles, luteocytes and leucocytes. Within endometrium lectins strongly labeled its luminal surface, epitheliocytes and secretion of uterine glands, decidual-like cells, leucocytes and collagen fibers. Both hyper- and hypothyroidism induced significant and specific redistribution of DMan, LFuc, NeuNAc, DGlcNAc, DGalNAc and DGal sugar determinants of ovaria and endometrium; hyperthyroidism induced more severe alterations of tissue glycoconjugates in comparison with hypothyroidism; endometrium was more susceptible to thyroxin-modulated impairments compared to ovaria.

Conclusions: The data give new insight into pathogenic mechanisms of thyroid disorder influences on female reproductive organs demonstrate suitability of lectin histochemistry methods for monitoring the efficacy of hormonal disballance correction therapy, as well as applicability of new lectin preparations for the selective labeling of ovarian and endometrial constituents.

Keywords: hyperthyroidism, hypothyroidism, rat, ovary, endometrium, lectins.

Nanobiotechnology and Cancer Research

Novel thiazolopyridines synthesis as anticancer agents

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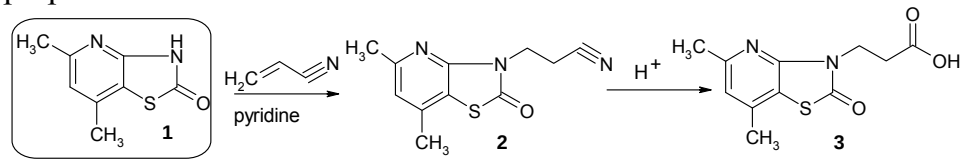
The synthesis innovations that are enabling the development of the condensed heterocycles with the antitumor efficiency are essential challenge of nowadays. Thiazolo[4,5-b]pyridines structural variants syntheses with an eye toward the discovery of compounds having improved or novel properties as drugs is a perspective and relevant strategy.

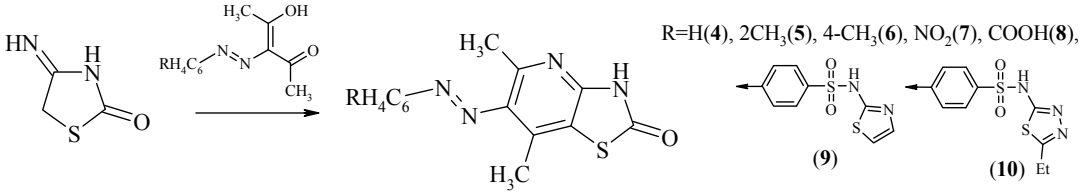
Aim:3*H*-thiazolo[4,5-*b*]pyridines synthesis by 5,7-dimethyl-3*H*-thiazolo[4,5-*b*]pyridine-2-one structural modification in 3rd and 6th core heterocycle positions and their antitumor action evaluation.

Methods: organic synthesis, ^{1}H-NMR spectroscopy, pharmacological screening.

Results: 5,7-dimethyl-3*H*-thiazolo[4,5-*b*]pyridine-2-one (**1**) was selected as the starting compound its synthetic route development by [3+3]cyclocondensation of acetylacetone with 4-iminotiazolidon-2 has been previously reported. The acidic character of its 3rd proton position led us to design 3-(5,7-dimethyl-2-oxo-thiazolo[4,5-*b*]pyridine-3-yl)-propionitrile (**2**) under cyanoethylation reaction which has been further utilized by hydrolysis for 3-(5,7-dimethyl-2-oxo-thiazolo[4,5-*b*] pyridine-3-yl)-propionic acid (**3**) preparation:



The next stage includes the core heterocycle structural modification in its 6th position. 5,7-dimethyl-6-arylazo-3*H*-thiazolo[4,5-*b*]pyridine-2-ones were yielded by appropriate α-arylazoacetones treatment with 4-iminothiazolidone-2:



The compounds structures were established by ^{1}H-NMR spectroscopy. Their antitumor activity evaluation was carried out in National Cancer Institute, Bethesda, MD USA on 60 human cancer cells lines. Compound **4** was shown to posses the highest cytostatic effect on some cell lines of non-small cell lung cancer, CNS and breast cancer, and melanoma the growth inhibition being 7.76% (cell line UACC-62), 18.2% (NS 578T) and 23.89% (EKVX).

Conclusions: The core heterocyclic system can be used as a promising framework for the anticancer candidate drug development.

Generation of novel nogalamycins through targeted gene disruption

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Aim: Among anthracycline derivatives, nogalamycins showed superior cytotoxicity and antitumor activity and also proved to be effective against breast cancer clinically. We aimed in generation of novel nogalamycins by targeted gene disruption of *snoaE, snoaM, snoaL* genes from *Streptomyces nogalater* Lv65.
Methods: Enzymatic manipulation of DNA and Southern hybridizations were carried out according to manufacturer's directions (Stratagene, NEB, MBI Fermentas, Boehringer Mannheim). Oligonucleotide primers were purchased from Sigma-Genosys. PCR was performed in a thermocycler MiniCycler (MJ Research). Intergeneric *Escherichia coli – Streptomyces* conjugation was used to introduce plasmid DNA into *S. nogalater* strains.
Results: We were interested in applying gene replacement techniques to *sno*-genes controlling cyclisation steps of the initial polyketide backbone of nogalamycin, thus leading to novel bioactive compounds. To disrupt *snoaE*, the spectinomycin resistance cassette *aadA* was used. The *S. nogalater* mutant with a replaced *snoaE* gene was obtained. The recombinant strain was shown to accumulate novel compounds. Complementation results suggest that *snoaE* disruption had a polar effect on downstream *sno*-genes. SnoaM and SnoaL disruption was achieved after a second crossover introducing the deleted *snoaM, snoaL* genes into the chromosome. In this case nogalamycin was not detected in extracts of the recombinant strains. Additional copies of the *snoaE, snoaM, snoaL* genes, cloned in pKC1218E plasmid, increased nogalamycin production in the wild-type strain *S. nogalater* Lv65.
Conclusions: Our work has showed that the cyclisation in the nogalamycin biosynthesis occurs prior to the three aglycon cyclases, and that their targeted disruption leads to dramatic effects on antibiotic production and leads to generation of novel antitumor compounds.
Keywords: breast cancer, antitumor antibiotics, anthracyclines, nogalamycin.

Translational Research and Drug Development

Biological activity of α-alkylacroleines derivatives

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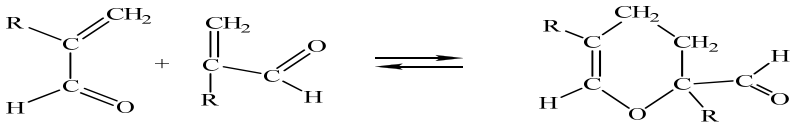
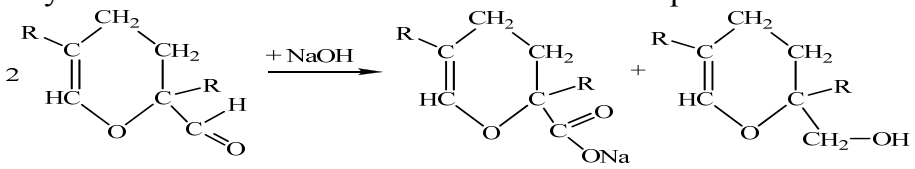
Aim: There are many important biologically active substances among heterocyclic compounds. Thus, acrolein dimer is used as starting material in antibiotics preparation. Our research is concerned with α-Alkylakrolein dimers and their derivatives investigation.

Methods: α-Alkylakrolein dimers were obtained by corresponding aldehyde dimerization at 170-190 °C:



where R = $-CH_3$, $-C_2H_5$.

2,5-dimethyl-3,4-dihydro-2H-pyran-2-methanol (1) and 2,5-diethyl-3,4-dihydro-2H-pyran-2-methanol (2) were obtained *via* Cannizzaro mechanism from corresponding α-alkylakrolein dimer reaction with NaOH 40% aqueous solution at 50 °C:



Structure of synthesized compounds was confirmed by IR– and NMR–spectroscopies.

The bacteriostatic and bactericidal activities were determined towards Gram-negative (*Escherichia coli, Salmonella enteritidis*) and Gram-positive (*Staphylococcus aureus 209 and Streptococcus*) cultures by the diffusion method in agar. As the standards amikacin and ciprofloxacin were used. Fungicidal activity was determined towards *Candida albicans, Aspergillus fumigatus and Penicillium chrysogenum* using a Saburaud medium. A standard disc with pimafucin served as the control.

Results and Conclusions: The data obtained showed that α-alkylacrolein dimers and their derivatives have acceptable biological activity in comparison with standards that allow using them as antiseptic agents. Using PASS program it was determined that investigated substances have high probability of broad spectrum anticancer activity (85% prediction accuracy).

Table. Results of predictable screening of biological activity

Probable activity / Substance	Apoptosis agonist, %	Anticarcinogenic, %	Antioxidant, %	Antineoplastic, %
α-Alkylakrolein dimers	71	52	53	–
1, 2	–	–	82	78
Na salts of 2,5-dialkyl-3,4-dihydro-2H-pyran-2-carbonic acid	68	69	78	–

Alterations of rat liver carbohydrates in streptozotocin-induced diabetes mellitus as detected by lectin histochemistry

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Aim: To use a set of lectins with different carbohydrate affinities for the investigation of rat liver glycoconjugate impairments under the influence of streptozotocin-induced diabetes mellitus.
Methods: The lectin panel included 7 conventional lectins – Con A, SNA, RCA, WGA, PNA, SBA, and HPA, supplemented with the original fucose-specific Laburnum anagyroides bark agglutinin (LABA). Tissue samples were fixed in 4% neutral formalin, embedded in paraffin and subjected to routine lectin-peroxidase-diaminobenzidine staining protocol.
Results: In control rats it was detected strong reactivity of Con A, LABA, SBA and SNA with cytoplasmic granularity of hepatocytes, of RCA, WGA and HPA - with vascular endothelium, of WGA and HPA - with bile capillaries. Experimental diabetes was associated with the redistribution of Con A and LABA receptor sites from centrolobular hepatocytes to hepatocytes with peripheral localization. Diabetes mellitus induced exposure of lectin reactivity within the hepatocyte and endothelial cell nuclei. Endothelial lining of sinusoidal hemocapillaries, of central veins and portal tract vessels also demonstrated significant and differential rearrangement of carbohydrate determinants. Diabetes-induced activation of Kupffer cells was associated with exposure of SNA, PNA and SBA receptor sites within these cells cytoplasm, completely non-reactive in control specimens.
Conclusions: These results give new insights into the pathogenic mechanisms of diabetes induced impairments of hepatic carbohydrates, and demonstrate applicability of original fucose-specific lectin preparation for experimental histopathology research.
Keywords: Streptozotocin, Diabetes mellitus, Rat liver, Lectins

Prooxidant activity of amaranth oil provides its membrane protective effects under normal and adrenalin induced stress conditions

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Aim: to study the physical chemical properties of plasmatic membranes (PM) of rat hepatocytes and pro-/antioxidant balance in blood of rats supplemented for one month with Amaranth oil (AmO) under normal conditions and after adrenalin induced stress.
Methods: white male rates were divided into two groups: 1, control; 2, supplemented with AmO 60 μl/kg per day for one month. Half of the animals from each group underwent adrenalin (350 μg/kg) induced stress 30 min before decapitation. Monolayers of membranes were prepared by I. Langmuir. Time of stable membrane functioning (t_{st}), surface pressure ($\Delta\pi$) and boundary potential jump ($\Delta\varphi$) were evaluated during interaction with neurotensin under normal and stress condition. Pro-/antioxidant balance was assessed spectrophotometrically in rat blood samples by the levels of TBARS, conjugated dienes, oxidative modification of proteins, catalase and SOD activities.
Results: Increase in t_{st} of PM of rat hepatocytes was noted after AmO use under normal conditions and after adrenalin induced stress. Intensity of neurotensin molecules incorporation into PM from "Amaranth" rats increased and remained at the control level even after adrenalin stress. More distinct biphasic distribution of peptide-membrane interaction process was clearly observed with AmO supplementation. That effect was abolished by adrenalin, more prominently in control animals. The study of pro-/antioxidant balance in blood showed modulation of antioxidant enzymes activity aimed at mild prooxidant effect with AmO use that was further maintained after adrenalin stress exposure.
Conclusions: AmO was shown to have membrane protective action both under normal and adrenalin-induced stress conditions. Modification of membrane fatty acid composition during activation of free radical reactions can be an essential mechanism involved in these effects, as it was underpinned by slight prooxidant activity triggered by AmO. Demonstrated AmO actions can be important for improvement of adaptive potential and heart rate variability in clinical settings.
Keywords: Amaranth oil, plasmatic membrane stability, neurotensin, adrenalin induced stress, mild prooxidant activity

Mouse lacking pituitary tumor transforming gene show elevated exposuure of DGalNAc sugar determinants

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Aim: To investigate influence of pituitary tumor transforming gene (*pttg-1*) knockout on glycome of murine parenchimal organs by means of lectin histochemistry.

Methods: Kidney, liver, lungs and testes of murine strain BL6/C57 with *pttg-1* knockout (*pttg*-KO) were compared to the wild type animals (*pttg*-WT). Animals for both groups were kindly provided by Dr. S.Melmed (Cedars Sinai Medical Center). Tissue samples were fixed in 4% neutral formalin and embedded in paraffin. General morphology was studied after haematoxylin and eosin staining. Carbohydrate determinants were labelled with soybean agglutinin (SBA, specific to DGalNAc), wheat germ agglutinin (WGA, specific to DGlcNAc > NeuNAc), both lectins conjugated to peroxidase, with subsequent diaminobenzidine visualization.

Results: General morphology studies revealed no significant changes in liver and lungs of *pttg*-KO mice in comparison to control. Kidneys of experimental animals were characteristic with glomerular compactization and enhanced urinary space of Bowmans capsules. In testes it was detected dislocation of Sertoli cells, decreased population of spermatogenic cells, impairments of syncytial complexes in between them. Kidneys and testes thereafter were subjected to lectin histochemistry investigation. It was identified enhanced exposure of DGalNAc sugar residues (SBA receptor sites) within Golgi complex of secondary spermatocytes, in brush border of renal tubules and on the lumenal surface of collecting ducts. Redistribution of WGA receptors included reactivity shifting from basal spermatogonia to adlumenal spermatogenic cells, apparently due to the enhanced exposure of DGlcNAc residues on the final stages of spermatogenesis.

Conclusions: This study suggests that *pttg-1* gene plays pivotal role in carbohydrate processing in mammalian organism.

Keywords: *pttg-1* knockout, glycoconjugate processing, lectin histochemistry.

UKRAINE – IMBG

Women's Health and Cardiovascular Diseases

Polymorphisms of ESR1 gene involvement in stroke

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Estrogen is known to play role in vascular function and lipid metabolism regulation and to be a neuroprotector. Two single nucleotide polymorphisms in *ESR1* gene – c.454-397C>T (*Pvu*II) and c.454-351 A>G (*Xba*I) - are thought to influence gene transcription and thus change cell sensitivity to estrogen action.

Aim: To establish possible involvement of c.454-397 C>T and c.454-351 A>G polymorphisms into stroke development we investigated this SNPs in: case group - patients with ischemic stroke (n=183), control group I - individuals from the general population (n=100), control group II - healthy individuals elder then 65 years (n=88), from different regions of Ukraine.

Methods: Blood samples for DNA analysis were obtained after informed consent. Genotyping was performed by PCR followed by RFLP analysis.

Results: Homozygous -397C genotype frequency was significantly higher ($P<0.05$) in women from case group (34.1%) compared to women from control I (20.8%) and control II (20.8%) groups.

There was a significant difference ($P<0.05$) in -351G/G genotype frequency between women (17.6%) and men (10.6%) from case group. Frequency of -351G/G genotype was significantly higher ($P<0.05$) in women from case group (17.6%) comparing to women from control II (5,7%). Linkage disequilibrium between -397 C>T and -351 A>G was shown ($P<0.0001$); CG and TA alleles are in phase ($r^2=0.557$). Pairwise comparisons using Fisher exact test revealed significant difference ($P<0.05$) of CG/CG genotype frequency in women from case group (17.7%) comparing to women from control II (5.7%). Individuals with this genotype have 3.5-fold higher risk of stroke development (OR = 3.54; CI-95%: 1.043 – 16.03).

Conclusions: Our findings suggest a possible role of studied ESR1 gene polymorphisms in stroke development in female.

Keywords: ESR1 gene, polymorphism, stroke

Discovering inhibitors of protein kinase ASK1

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Aim: Apoptosis signal-regulating kinase 1 (ASK1) has recently emerged as an attractive therapeutic target for treatment of several cardiovascular diseases. Earlier in vivo experiments demonstrated that cardiac hypertrophy induced by AngII is associated with enhanced ASK1/proapoptotic signaling. ASK1 deficiency attenuated cardiac inflammation and fibrosis under diabetic conditions. The main aim of our research is to identify the small molecule inhibitors of ASK1.
Methods: To discover the protein kinase ASK1 inhibitors we have performed screening program, using both in silico and in vitro approaches. AutoDock and DOCK software were used to conduct receptor-ligand flexible docking. The best-scored compounds of different chemical classes were taken for the kinase assay analysis.
Results: *In vitro* observations revealed that derivatives of 2-Thioxo-thiazolidin-4-one exhibited inhibitory activity towards ASK1. The most active compound inhibited ASK1 with IC_{50} = 2 μM. Then, in-depth study of this chemical class was performed using the pre-selected library of 2-thioxo-thiazolidin-4-one derivatives. Ten best-scored compounds were selected for the kinase assay analysis. Compound 2-{5-[5-(3,4-Dichloro-phenyl)-furan-2-ylmethylene]-4-oxo-2-thioxo-thiazolidin-3-yl}-propanoic acid inhibited ASK1 with a K_i of 340 nM. Our preliminary selectivity studies demonstrated that this compound seems to be selective inhibitor of ASK1. In silico analysis of the complexes of ASK1 with compounds indicated, that the peculiarity of the active compound in the comparison to other nine derivatives is its ability to bind simultaneously to the part of kinase domain known as “hinge region” and the phosphate-binding region of the ATP-binding cleft.
Conclusion: Obtained results suggest that the core structure of identified active compound can be used for further optimization and developing more potent and selective inhibitors of ASK1.
Keywords: Apoptosis signal-regulating kinase 1, inhibitor, virtual screening.

Mother and Child Health

The premises for taurine biosynthesis in human placenta

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Aim: Taurine (Tau) is a sulfonic acid that is synthesized from cysteine (Cys) by consequent action of cysteine dioxygenase (CDO) and cysteine sulfinic acid decarboxylase (CSAD). Tau is a multifunctional moiety with its particular role in development of fetal central nervous system, endocrine glands and maintenance of oxidative status. Up-regulation of Cys production in human placenta under hyperhomocysteinemia raises a question concerning the further Cys metabolism. So the main goal of our research was to fill the gap in the study of taurine biosynthesis in human placenta.
Methods: Expression of *CDO, CSAD* at the mRNA level was determined by reverse transcriptase and quantitative polymerase chain reaction in real time. Total amount of taurine in placental samples was determined by using amino acid analyzer. The objects of the study were placentas of the first and the third trimesters of gestation.
Results: Total amount of taurine in term placenta was 0.64mg/g tissue, which consists 29% of the total amino acid content. For the first time the expression of *CDO* and *CSAD* genes was ascertained in human placenta. The amount of CDO-specific mRNA (product length of 380 nucleotides) was $6{,}65*10^6$ number of copies per mg of total RNA in term placenta and $2{,}95*10^5$ number of copies per mg of total RNA in the first trimester one. The level of CSAD mRNA (amplicon of 268 nucleotides) was $1{,}18*10^3$ number of copies per mg of total RNA in the samples from the third and $1{,}66*10^2$ number of copies per mg of total RNA in the samples from the first trimester of gestation. It is suggested that taurine is synthesized more intensively in term placenta than in the first trimester placenta.
Conclusions: On the basis of *CDO* and *CSAD* genes expression in human placenta we suggest that the definite amount of Tau may be synthesized in this organ. It is likely that activity of the synthesis substantially increases during the pregnancy.
Keywords: Taurine biosynthesis, human placenta, *CDO, CSAD.*

Model of one-carbon metabolism in human placenta

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Background: Folate-related one-carbon unit metabolism (FOCM) is a complex metabolic network with the tetrahydrofolate (THF) and methionine (Met) cycles in its core connected with the synthesis of purines and pyrimidines, methylation reactions and synthesis of cysteine (Cys). C677T polymorphism of *MTHFR* gene and the elevated content of homocysteine (Hcy) in human placenta are associated with obstetrical pathologies, particularly preeclampsia while *ex vivo* Hcy increases apoptosis and decreases proliferation.
Objective of our study was to analyze the response of mathematical FOCM model for C677T *MTHFR* heterozygosity, duplicate Hcy content and joint action of both factors.
Methods: Cross-database and literature search for placental-specific expression of FOCM enzymes; Metatool software and COBRAToolbox for construction a stoichiometric model of FOCM on the basis of placental-specific reactions (folate cycle - 9 reactions, methionine cycle - 4, -transsulfuration - 2, glutathione metabolism – 4, taurine synthesis -3, reactions of in/output of metabolites).
Results: The stoichiometric model of FOCM features 7 key metabolites (THF, methylene-THF, formyl-THF, Met, Hcy, Serine and Cys) and 15 elementary flux modes consisting from 3 to 17 reactions with corresponding flux values for each. The simulation of *MTHFR* C677T genotype (65% enzyme activity) induces the least changes in the network, while its combination with double Hcy content induces decrease of metabolic fluxes for purine synthesis and increase for Cys synthesis in parallel with the decrease of flux values for Cys consumption and glutathione (GSH) synthesis. The latter is crucial for cell's red-ox status.
Conclusion: The adverse effect of Hcy on proliferation and apoptosis may be partially explained by the failure of purine synthesis and by oxidative stress due to accumulated Cys autooxidation and decrease of GSH – main antioxidant moiety in the cells.
Keywords: placenta, FOCM, model, Hcy, *MTHFR*

Nanobiotechnology and Cancer Research

Structural-functional characterization of human CHI3L1

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Aim: Chitinase 3-like 1 (CHI3L1, HC gp-39, YKL-40) belongs to glycosylhydrolase protein family 18. Expression of *CHI3L1* was found increased significantly in various tumors in comparison with corresponding normal tissues. CHI3L1 can decrease the doubling time of 293 cells, it allows the anchorage independent growth in soft agar, and stable *CHI3L1* expression makes 293 cells tumorigenic, stimulating the initiation of tumors after xenograft transplantation into the Wistar rat brains. The aim of this work was to perform structural-functional characterization of CHI3L1 and find key motifs responsible for CHI3L1 activity.
Methods: Protein coding region of *CHI3L1* cDNA sequence was amplified from plasmid containing *CHI3L1* insertion and cloned in pcDNA3.1 expression vector at EcoRI and XhoI restriction sites. Site-directed mutagenesis of CHI3L1 heparin-binding region and chitin-binding site was performed by QuikChange or overlap extension strategy. Tyrosine residues were replaced by serine, lysine or arginine were replaced by glutamate. 293 cells were transfected with obtained plasmid constructs, and colony formation assay was used to assess oncogenicity *in vitro*.
Results: CHI3L1 mutants were generated and confirmed by sequencing. Mutations in heparin-binding site of CHI3L1 reduced colony formation efficiency of transfected 293 cells, while mutations in chitin-binding site did not have significant effect.
Conclusions: Our results suggest that heparin-binding site of CHI3L1 is involved in CHI3L1-driven enhancement of oncogenicity of 293 cells. Further characterization of the mechanisms of CHI3L1 activity is necessary for elucidation of the role of CHI3L1 in tumor formation and progression.
Keywords: CHI3L1, heparin-binding site, site-directed mutagenesis

Optimization of enzyme biosensor for fructose determination

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Fructose concentration in human fluids can indicate different diseases connected with fructose metabolism disorders in human organism. For example, high fructose concentration in blood of patients indicates hereditary fructosemia; in urine indicates essential fructosuria; in intestine - fructose malabsorption.

Today various methods are available for fructose determination, but most of them suffer from diverse disadvantages: expensive equipment, time-consuming process, insufficient selectivity etc. Nowadays, much attention is devoted to development of biosensors, modern analytical devices based on the combination of biological component and suitable transducers. The combination of enzymes with electrodes creates enzyme electrochemical biosensors which can provide simple, accurate, high specific, sensitive, fast, convenient and rather cheap determination of substrate. Moreover biosensors can be easy miniaturized what makes them applicable for analysis in situ and *in vivo*.

Aim: The main aim of our work was study and optimization of analytical characteristics of conductometric enzyme biosensor for fructose determination.

Methods: Enzyme electrochemical methods of fructose determination

Results: The new enzyme conductometric biosensor for fructose determination in liquid samples has been developed by immobilizing fructose dehydrogenase using glutaraldehyde on the surface of gold electrodes. Effect of pH of phosphate-acetate buffer solution and different concentrations of mediator in buffer solution (0.5 – 10 mM ferricyanide) on responses of fructose biosensors were investigated. The linear range of fructose determination was from 0.005 to 2 mM. Time of fructose concentration measuring was 1-2 min.

Conclusions: The new fructose biosensor showed good selectivity and high signal reproducibility. The developed fructose biosensors can be applied in medical diagnostics in future.

Keywords: fructose intolerance, fructose dehydrogenase, biosensor.

Structural models of the complete HIV-1 poly(A) region

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Aim: In HIV-1 retrovirus, identical core poly(A) sites are present at both the 5′ and 3′ end of HIV-1 pre-mRNA. The usage of a real 3′ poly(A) site is promoted by upstream sequence elements (USEs) which are uniquely present at this end of HIV-1 transcript. Polyadenylation signal (PAS) is partly occluded by base pairing in the upper part of polyA hairpin which can form at both ends of HIV-1 transcript. To our knowledge there is no structural model of HIV-1 complete 3′ poly(A) region including the core poly(A) site and the USEs. Based on our observation that the minor USEs are located in a G-quadruplex-forming segment, we studied here the secondary structure of the 3′ terminal region of HIV-1 pre-mRNA stretching from the G-rich segment to the end of core downstream element (DSE).
Methods: We have performed secondary structure prediction and phylogenetic analysis of poly(A) region for about 1500 HIV-1 isolates of different subtypes and recombinant forms. Predictions of the secondary structure were performed by mfold program (Zuker 2003).
Results: Folding results are stored in our database CESSHIV-1 (abbreviated from Control Elements Secondary Structures of HIV-1 genome) which is available online at http://www.cesshiv1.org. Based on our phylogenetic findings, we have proposed two common structural models of complete 3′ poly(A) region of HIV-1 pre-mRNA: a domain and an in-line structures.
Conclusions: In proposed structural models of complete 3′ poly(A) region of HIV-1 pre-mRNA both the core downstream element and the upstream sequence element in HIV-1 3′ poly(A) region are exposed in single stranded segments of hairpins or domains and are available for protein factors of polyadenylation machinery. Information on HIV-1 poly(A) region structure may facilitate development of therapeutic strategies targeting polyadenylation process of viral transcript.
Keywords: HIV-1 genome; RNA folding; poly(A) site

Serum cell-free DNA as diagnostic marker in patients with for renal cell carcinoma (RCC)

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Aim: The presence of tumor DNA in cell-free DNA (cfDNA) circulating in the plasma or serum of cancer patients was first demonstrated 30 years ago. Since then, overall plasma DNA concentration in cancer patients and genetic or epigenetic alterations specific to tumor DNA have been investigated in patients diagnosed with different types of cancer. The objective of this study was to evaluate the putative significance of serum cell-free DNA for renal cell carcinoma (RCC).

Methods: In this study blood plasma samples were collected from 28 patients with histologically confirmed RCC and 15 healthy control individuals. Determination of plasma concentration of cfDNA was carried out using quantitative real-time PCR and measuring fluorescence intercalation of SYBR Green I.

Results: We isolated plasma DNA from 2 mL plasma after phased centrifugation/fractionation of blood cells to obtain serum, which does not contain DNA fractions of leukocytes. cfDNA concentration was measured by quantitative PCR assay. Average DNA concentrations for the healthy individuals and RCC patients were 25,4 and 58,6 ng/ml, respectively. Elevated plasma DNA levels were also detected in patients with either stage I or II disease. All plasma DNA samples also were quantitatively assessed by direct SYBR Green I staining in tablet analysis. DNA concentration value in RCC plasma was a median of 254.2 ng/ml, for the control - 57.2 ng/ml ($P < 0.001$). The observed concentrations of circulating DNA differ depending on the analysis methods, but the concentration of DNA in patients is higher than in healthy individuals.

Conclusions: The current results indicated that cell-free DNA represents a serum-based diagnostic and prognostic biomarker for RCC.

Keywords: renal cell carcinoma, cell-free DNA, diagnosis, prognosis.

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Translational Research and Drug Development

PH domain of Bcr-Abl interacts with PLCε in the nucleus

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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 acute lymphoid leukemia suggesting the important role of the DH and PH domains in leukemogenesis. Our resent data demonstrated that Bcr-Abl PH domain binds a number of proteins including phospholipase C**ε** (PLC**ε**). Also it has been shown that p210 Bcr-Abl is localized in perinuclear area. But the fact of interaction between PH domain of Bcr-Abl and PLC**ε** in the living cell should be confirmed as well as the role of this interaction should be explained.

Aim: The aim of this study is to discover the interaction between PH domain of Bcr-Abl and full-length PLCε in the living cell.

Methods: Two expression constructs (mRFP-PLCε and GFP-PH) were used for transfection into Cos-7 cells. The fluorescence of both proteins in living cells was detected by confocal microscopy. Results obtained were confirmed with further Western-blot analysis.

Results: We have found that both proteins are expressed in Cos-7 cells and are co-localized in nucleus, while PH domain alone localizes in perinuclear area.

Conclusion: Our results suggest that PH domain of Bcr-Abl and full-length PLCε co-localize in nucleus. This may indicate the important role of PH domain of Bcr-Abl in intracellular localization of PLCε. Using these data we can predict other proteins localized in perinuclear area that can be important partners in signaling pathway involving p210 Bcr-Abl and PLCε.

Keywords: Leukemia, Bcr-Abl, phospholipase Cε.

UKRAINE – ICB

Mother and Child Health

Autophagy and viability of ovarian tumor cells

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Aim: to study autophagy inhibition to enhance therapeutic effect of enzymotherapy based on arginine (ARG) deprivation for ovarian cancer treatment.
Methods: cell viability test, MDC staining, immunofluorescence, fluorescence microscopy, RNA interference, western blot analysis.
Results: we demonstrated that autophagic response in human ovarian carcinoma SKOV3 cells upon ARG deprivation can be inhibited by chloroquine (CQ), a non-toxic antimalarial drug, proposed as inhibitor of autophagy *in vivo*. CQ decreased SKOV3 cells viability under ARG withdrawal suggesting a prosurvival role of autophagy. We observed that resistant to taxol SKOV3 cells are sensitive to this drug upon ARG deprivation. Analysis of cell viability demonstrated that CQ and taxol negatively affected SKOV3 cell viability even after ARG resupplementation. We also showed that transcriptional silencing of autophagic protein Beclin 1 led to a decrease in cell survival under ARG deprivation and to the decrease in their proliferative potential upon ARG resupplementation.
Conclusions: we demonstrated that autophagy has an important prosurvival role in the response of cultured SKOV3 cells to ARG deprivation. We propose that ARG deprivation-based combinational treatment with CQ and taxol may produce a stronger anticancer effect as a second line therapy for difficult to cure ovarian cancers.
Keywords: arginine deprivation, autophagy, ovarian cancer, chloroquine, taxol.

Nanobiotechnology and Cancer Research

Effect of arginine deprivation on leukemic cells

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Aim: to elucidate the effect of combinational arginine deprivation-based enzymotherapy (ADBE) with exogenous nitric oxide donor, sodium nitroprusside (SNP), on proliferation and viability of human acute lymphoblastic leukemia (ALL) cells and normal peripheral blood lymphocytes (PBL) *in vitro.*
Methods: Trypan Blue Test, MTT Assay, Annexin V Staining Assay, Western Blot Analysis, Separation and Cultivation of Blood Cells.
Results: Several human ALL cell lines and PBL were treated with recombinant human arginase (rhARG) for 24, 48 and 72 hours. ADBE effectively decreased viability of all tested leukemia cell lines but had not significantly effect of PBL. SNP (0.05 mM) statistically enhanced cytotoxicity of ADBE for leukemic cells. PBL were more resistant to this dose of SNP and SNP IC50 concentration for PBL in full and arginine-deficient medium was not significantly different. ADBE effectively induced apoptosis in ALL cells but not in PBL cells. NO donor sped up the apoptotic process. Arginine anabolic precursor, citrulline (0.4 mM), supported proliferation of two leukemia cell lines (CEM-T4 and Namalva) but not Jurkat cells and significantly decreased cytotoxicity of SNP. Simultaneously, ADBE with or without citrulline supplementation induced expression of arginine biosynthetic enzymes argininosuccinate synthase (ASS) in ALL cells.
Conclusions: The obtained results suggest that ADBE may have clinical potential as an inhibitor of leukemic cells proliferation. We demonstrated for the first time that exogenous NO-donor can be potentially used as a secondary agent under ADBE *in vivo* to compensate for NO limitation as an arginine catabolite.
Keywords: arginine deprivation-based enzymotherapy (ADBE), sodium nitroprusside (SNP), leukemic cells, normal lymphocytes (PBL).

Nanocomposites carrying Dx and NSE as Doxil alternative

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Aim: Doxil is a clinically used PEGylated liposome with encapsulated doxorubicin (Dx). It possesses much lower cardiotoxicity comparing to free Dx, however, it is 100 times more expensive. A novel nanoscaled PEGylated polymeric drug carrier was synthesized and functionalized by the biologically active lipid N-stearoylethanolamine (NSE). Here we evaluated the efficiency of such carrier (Dx-NSE-NC) in Dx delivery to tumor cells. The protective role of NSE at the action of that carrier with encapsulated Dx *in vivo* was also predicted.

Methods: Fluorescent microscopy, flow cytometry, Western-blot analysis of proapoptotic proteins, *in vivo* toxicity studies of Dx-NSE-NC, *in vivo* studies of treatment efficiency of Dx-NSE-NC towards L1210 murine leukemia.

Results: Functionalizing by NSE of Dx-NC increased its antineoplastic potential by 40% compared to non-functionalized Dx-NC. NSE functionalizing was even more effective than PEGylation of Dx-NC. Such functionalizing of Dx-NC led to both acceleration of Dx penetration to the nucleus of human tumor HeLa cells and more rapid accumulation of Dx in these cells. Dx-NC-NSE also accelerated apoptosis. It caused early activation of caspase-3 and -7, and cleavage of their substrates PARP-1, DFF45 in 6 h, while non-functionalized Dx-NC and Dx caused such changes only in 9 h. 90% of mice receiving lethal dose of Dx (20 mg/kg) as Dx-NSE-NC, remained alive, while all mice receiving the same dose of Dx and Dx-NC, died. Dx-NC-NSE efficiently inhibited growth of murine L1210 leukemia *in vivo* in cumulative dose 1 mg/kg that is 10 times less than dose of free Dx (10 mg/kg) needed to achieve the same treatment effect.

Conclusions: N-stearoylethanolamine functionalizing of novel PEGylated polymeric nanocarrier with encapsulated doxorubicin significantly enhanced the anticancer action of this drug, and provided this carrier with protective effect towards normal cells *in vivo*. Potentials of novel nanocarrier of doxorubicin as a cheaper alternative of Doxil are considered.

Keywords: polymeric nanocarriers, doxorubicin, N-stearoylethanolamine, treatment, apoptosis, murine L1210 leukemia.

Amino acid depletion: differences in 2D and 3D cultures

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Aim: To compare the effects of arginine, leucine, lysine or methionine starvation on human epithelial cancer cells growth, viability, proliferative potential and apoptosis induction when grown in monolayer versus spheroid culture.
Methods: Monolayer cells were maintained under regular culture conditions. Spheroids were obtained using a standard liquid overlay technique. Amino acid deprivation was achieved by incubating cells in the formulated media lacking arginine, leucine, lysine or methionine. Cell growth, viability and proliferative potential were assessed by Trypan blue exclusion test and MTT analysis. Apoptosis induction was analyzed by Western-blot-mediated detection of fragmented PARP and cleaved caspases.
Results & Conclusions: We revealed that in monolayer culture the effects of starvation for individual amino acids differed, with methionine and arginine having the most severe impact on cancer cell viability and proliferative potential. At the same time, we are first to show that cancer cells spheroids preserve growth potential even after prolonged amino acid starvation, despite the signs of mitochondrial and receptor-mediated caspase-dependent apoptosis induction. This suggests that in 3D culture human cancer cells are much more resistant to individual amino acid starvation relative to monolayer cells. The differences in the cell capacity to preserve growth potential upon individual amino acid starvation between monolayers and spheroids of one cell line might depend on the disparity in hypoxia-mediated signaling pathways or in regulatory networks that control protein translation during amino acid limitation between these types of cell cultures.
Keywords: human epithelial cancer cells, monolayer culture, spheroid culture, amino acid starvation, apoptosis.

Overcoming drug resistance by new delivery platform

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Aim: Drug resistance is widely spread in pathogenic microorganisms and tumor cells under chemotherapy. Development of effective remedies for drug transportation to various target cells is of great significance. New polymeric platform was tested for Doxorubicin (Dx) delivery to drug-resistant human tumor cells.
Methods: Intracellular accumulation of Dx, FACS analysis, cell cycle, Western-blot analysis, DNA comet assay.
Results: Application of the developed nanocomposite (NC) significantly enhanced the intracellular accumulation of Dx. Such treatment resulted in about 10-fold higher Dx levels in human lung carcinoma SW1573 cells, comparing with the action of free Dx. 6-fold increase in the intracellular accumulation of the NC-bound Dx was observed in the Pgp-overexpressing drug-resistant 2R160 subline of these cells. We found that 3 h treatment of tumor cells with the NC-Dx much more effectively enhanced DNA comet tail moment of treated cells comparing with the action of free Dx. The application of such drug delivery platform led to more pronounced stimulation of cell stress pathways such as up-regulation of p53 and phosphorylation of mitogen-activated protein kinase p38. These changes were accompanied by a distinct G2/M arrest of cells surviving after 24 h NC-Dx treatment.
Conclusions: Dx delivery by means of new polymeric platform decreases up to 10 times drug concentration necessary to achieve similar antineoplastic action of this drug in the inhibition of cell proliferation and G2/M arrest. Such way of Dx delivery permitted overcoming resistance of various human tumor cells to the action of this anticancer drug. Perspectives of application of new drug delivery system in cancer chemotherapy are considered.
Keywords: chemotherapy, doxorubicin, cancer, nanocarrier, drug accumulation.

Tumor cells phagocytosis under arginine starvation

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Arginine (ARG) deprivation-based enzymotherapy is a novel anticancer strategy for treatment of haematological tumors. It is also known that ARG (as a component of Arg-Gly-Asp sequences) is essential for proper function of surface proteins involved in cell recognition/clearance by macrophages. We showed that ARG withdrawal inhibited proliferation/viability and induced apoptosis of human leukemic cells.
Aim: to evaluate possible effect of ARG starvation on recognition/clearance of dying leukemic cells by immune-competent cells.
Methods: MMT, *in situ* apoptosis detection and phagocytosis assays, lectin cytochemistry. Jurkat cells were used as experimental model.
Results: We tested 2 distinct phagocytosis assays involving human monocytes-derived macrophages (MoMa) and THP-1-differentiated macrophages with Arg-deprived, Lys-deprived, intact and apoptotic Jurkat cells serving as "prey". It was shown that 24h Arg-deprivated cells were more efficiently phagocyted by macrophages than the intact ones, resembling the phagocytosis of apoptotic (UV-irradiated) Jurkat cells. Lys-deprived cells did not exhibit increased phagocytosis. Combined administration of Arg and nitrite oxide donor sodium nitroprusside (SNP) allows maintaining phagocytic activity of macrophages. Moreover, SNP did not promote survival of tumor cells. Interestingly, Phagocytis Index for Arg-starved cells was higher than Apoptotic Index that was detected by AnnexinV-FITC/PI staining. Apoptosis mediated by Arg-starvation was accompanied by changes in cell surface carbohydrates. It was demonstrated significant reduction of exposition of DGlcNAc-, NeuNAc-and βDGal, NeuNAc-containing glycoconjugates.
Conclusions: We report for the first time that ARG-deprivated tumor cells are efficientlly cleared by macrophages. We propose that combination of ARG starvation with NO donor should support phagocytic activity of macrophages under ARG starvation *in vivo*.
Keywords: arginine deprivation, macrophages, phagocytosis.

Novel nanocarrier enhances doxorubicin action

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Aim: Most anticancer agents cannot effectively discriminate between healthy and malignant cells. Doxorubicin (Dox) is one of the most employed anticancer agents whose clinical application is limited by the acute and chronic cardiotoxicity. Application of novel drug delivery systems is an actual goal in pharmaceutics. Here we investigated the effectiveness of action of Dox transported by novel developed nanosized polymeric carriers whose PEGylated shell should inhibit the access of degrading proteases and nucleases, thus, minimizing drug recognition and elimination by blood cells.
Methods: cell toxicity assay, DNA laddering assay, Western-blot analysis, fluorescence microscopy and experimental tumor models *in vivo.*
Results: The results of in vitro study showed that the application of carriers with immobilized doxorubicin (OC-Dox) reduced proliferation of L1210, Jurkat, MCF-7 and L929 cell lines approximately 10 times more intensively than free Dox. Fragmentation of DNA in cancer cells under the influence of OC-Dox was expressed much better than in cells treated with free Dox used in same concentration. OC-Dox also caused activation of the effector caspase-3 and cleavage of its substrate PARP-1 much faster than free Dox did. Due to such properties, OC-Dox induced apoptotic cell death in much lower concentrations comparing with free Dox. High efficiency of the nanoscale drug delivery system has been confirmed in vivo. OC-Dox possessed a capability of inhibiting growth of NK/Ly lymphoma without causing general toxicity towards the organism.
Conclusions: Application of new polymeric nanocarriers for drug delivery allows a significant reduction of current dose while maintaining antitumor effect, which can provide significant reduction of adverse effects of tumor chemotherapy.

Keywords: nanocarriers, drug delivery, cancer chemotherapy, doxorubicin, apoptosis.

Translational Research and Drug Development

Canavanine: a new look at an old drug

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Arginine analogue of plant origin canavanine (Cav) exhibits antitumor activity *in vitro* and *in vivo*, but has high overall toxicity. Our recent data suggest that malignant cells become selectively more sensitive to low dose Cav treatment upon arginine deprivation in comparison with pseudonormal cells. However, the effect of arginine biosynthetic precursor citrulline (Cit) on Cav toxicity for cancer cells remained to be evaluated.
Aim: to investigate the effect of Cit supplementation on cancer cell sensitivity to Cav treatment under arginine-deprived conditions.
Methods: MTT assay, reverse transcription PCR and Western-blot analyses. Human epithelial colorectal cancer cell lines (HCT-116, HT-29) were utilized as models.
Results: Cit addition to arginine-free medium partly restored growth of tested cell lines. Remarkably, 0.1 mM Cav inhibited proliferation of malignant cells under such circumstances and promoted cell death as efficiently as upon arginine withdrawal without Cit. We revealed that inability of colorectal cancer cells to utilize Cit for arginine resynthesis under Cav treatment resulted from to the decrease in the expression of argininosuccinate synthetase (ASS), the key enzyme of arginine synthesis. Stepwise application of tested impacts (incubation in arginine-free medium for 24 h before Cav supplementation) increased cancer cell sensitivity to Cav relative to simultaneous arginine starvation and Cav treatment. However, supplementation of arginine free medium with Cit 24 h before Cav treatment was sufficient to induce ASS expression and partly protect the cells from Cav toxicity.
Conclusion: Canavanine application in arginine deprivation-based anticancer therapy overcomes one of the significant drawbacks of such treatment *in vivo*, namely, the induction of ASS expression resulting in decreased tumor sensitivity to starvation. The mode of Cav application as a component of combinational therapy has to be further elucidated.

Kywords: arginine deprivation, canavanine, colorectal cancer.

Anticancer effects of novel 4-thiazolidone derivatives

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Aim: Synthetic compounds based on 4-thiazolidone ring are very perspective in cancer chemotherapy. We used three structurally related compounds (Les-28, Les-236, Les-3183) that were synthesized at Danylo Halytskyy Lviv National Medical University. Under Drug Discovery and Development Program at National Cancer Institute (USA) it was shown by that they possess potential antineoplastic activity with highest effect towards human breast cancer cell lines. In our study, we have examined the anticancer action of these compounds more precisely.

Methods: MTS-cell viability assay, soft agar assay, wound healing assay, fluorescent microscopy, targeting experimental tumor *in vivo* and study of histological sections after hematoxylin and eosin staining.

Results: We found that 4-thiazolidone derivatives Les-28, Les-236 and Les-3183 suppressed proliferation of human breast carcinoma cells in vitro: MCF-7 cell line (IC50~0.1 uM) and MDA-MB-231 and 4T1 cell lines (IC50~20 and 25 uM, respectively). Moreover, these compounds reduced substrate-independent cell growth and cell migration in vitro, and also induced apoptosis.

In spite of very low toxicity in vivo (8 injections to each of 6 Balb/c mice causing a cumulative LD50>800 mg/kg), all tested compounds in dose 20 mg/kg caused tumor growth inhibiting effect in model tumor (4T1 mice breast adenocarcinoma). Les-3183 was the most active towards this tumor. Analysis of histological sections revealed necrotic lesions in control tumor, as well as in tumor under treatment with Doxorubicin (1 mg/kg) or Les-3183 (20 mg/kg). However, Les-3183 induced strong immune response and inhibition of tumor neovascularisation.

Conclusions: 4-thiazolidone based compounds under study caused various anticancer effects (inhibiting of cell proliferation and migration, inducing of apoptosis, inhibiting of tumor growth) observed at low general toxicity in vivo. Moreover, one of them stimulated immune response and inhibited neovascularization.

Keywords: 4-thiazolidone, anticancer treatment, experimental tumors.

Overexpression of $(His)_6$-tagged human arginase I in *Saccharomyces cerevisiae* and purification of the enzyme using nickel affinity chromatography

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Aim: Some of actively proliferating cancer cells (*e.g.* many hepatocarcinomas, melanomas, carcinomas, including well-known cervical carcinoma HeLa, as well as some types of leukemia) are strongly dependent on exogenous arginine supply. Arginine deficiency causes antiproliferative effect *in vitro* and *in vivo* and often leads to the death of such cancer cells whereas normal cells move into a quiescent phase. For the treatment of some cancers, it is promising the use of specific enzymes that catalyze degradation of arginine.
Arginase I (EC 3.5.3.1; L-arginine amidinohydrolase) is the main enzyme of the urea cycle that catalyses the hydrolysis of arginine to urea and ornithine.
Methods: The recombinant strain *Saccharomyces cerevisiae* capable of overproducing human liver arginase was constructed. The corresponding gene *HsARG1* was cloned using efficient copper inducible expression system. The active enzyme was accumulated in *S. cerevisiae* cells to the level of approx. 15-30 μmol· min^{-1}·mg^{-1} protein. The highest expression was obtained after 6-8 h of induction.
Results: The $(His)_6$-tagged enzyme was purified from the cell-free extract of the recombinant strain by metal-affinity chromatography on Ni-NTA-agarose or monodisperse Ni-IDA-modified poly(glycidylmethacrylate-co-ethylene dimethylacrylate) microspheres. The specific activity of the enzyme was increased more than 70-fold up to 2000 μmol·min^{-1}·mg^{-1} protein. The yield of the enzyme after Ni-affinity chromatography was approximately 80%. The obtained enzyme preparations are almost homogeneous.
Conclusions: For the first time, $(His)_6$-tagged form of human arginase I was overexpressed in *S. cerevisiae* cells under the control of *CUP1* promotor. It was shown that $(His)_6$-tag bound to the N-terminus does not disturb the arginase enzymatic activity, but allows easy purification procedure by Nickel-chelating chromatography on Ni-NTA-agarose or newly synthesized monodisperse Ni-IDA-modified poly(glycidylmethacrylate-*co*-ethylene dimethylacrylate) (PGMA) microspheres.
Keywords: Arginase, cancer, chromatography.

UKRAINE – PALLADIN

Women's Health and Cardiovascular Diseases

Synthetic peptides γ69-78 and Aα195-205 are specific inhibitors of fibrin polymerization and potential antithrombotic agents

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Aim: localization of a new fibrin functional site using synthesized peptides as inhibitors fibrin polymerization

Methods: Turbidity analysis and transmission electron microscopy were used to study the effect of synthesized peptides on fibrin polymerization, fibrinogen was obtain from human blood plasma by salting out with Na_2SO_4, human fibrin was obtained by dissolving in 20 mM acetic acid.

Results: The peptides imitating the amino acide sequences of the fibrin molecule: γNPDESSKPN(69-78), BβQPDSSVKPY(228-236), BβRPFFPQ(455-460), AαLPSRDRQHLPL(195-205) were synthesized and their effects on the process of fibrin polymerization in fibrinogen+thrombin, fibrin desA, fibrin desAB solution were investigated. Turbidity data showed that synthesized peptides Aα195-205 and γ69-78, but not Bβ228-236 Bβ455-460 retarded fibrin polymerization. IC_{50} value for Aα195-205 and γ69-78 were $2.44 \cdot 10^{-4}$ M and $1.5 \cdot 10^{-5}$ M, respectively. Electron microscopy data show that peptide Aα195-205 inhibits the stage of fibrin protofibril formation and peptide γ69-78 – the step of protofibril lateral association.

Conclusions: The new active sites were localized in the coiled-coil fragment of the fibrin molecule: site γ69-78, and site Aα195-205 which are suggested to be important for the preservation of native structure of fibrin molecule to realize early stages of fibrin polymerization. The fibrin fragment γ69-78 takes part in fibrin protofibril lateral association. The spatial localization of the fragment Aα195-205 in the fibrin(ogen) molecule suggest that this site may be considered as a base for the binding of αC-connector portion of αC-region to the bulk of the fibrin molecule namely: with the coil-coiled region.

Therefore, the synthesized peptides Aα195-205 and γ69-78 may be used for the development of antithrombotic drugs.

Keywords: fibrinogen, fibrin, polymerization, inhibition, synthesized peptides

Mother and Child Health

Vitamin D3 and mineral metabolism in pregnansy associated with pathology and during neonatal period

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Aim: To establish the character of changes in the vitamin D_3 and mineral metabolism in newborn infants related to the maternal D_3 levels under different pathologies that accompany pregnancy.

Methods: 37 pregnant women with preexisting Type 1 diabetes (12.4±7.5 yrs), 30 women with the late toxemia of pregnancy, 35 pregnant women with hypofunction of parathyroid glands and 15 controls with no pathology during pregnancy, well matched for age, were enrolled in the study. Blood serum 25(OH)D_3 levels (ELISA) and markers of mineral metabolism were examined between 18^{th} and 24^{th} gestational weeks in all women and in their newborn infants at 14^{th} and 21^{st} days after delivery.

Results: The blood serum content of 25 (OH) D_3 decreased to 28.7 ± 1.2 nM/L in pregnant women with diabetes vs. 65.0 ± 6.4 nM/L in controls. Diabetes caused impairments of mineral metabolism as it is evident from the reduction of serum calcium (Ca^{2+}) and phosphorus (Pi) to 1.76 ± 0.10 and 1.40 ± 0.10 respectively vs. 2.21 ± 0.02 and 1.85 ± 0.09 mM/L in control. These changes in pregnant women suffering from diabetes led to vitamin D_3 deficits and altered mineral metabolism in newborns. They exhibited reduction of 25(OH)D_3 by 25 % compared with infants from healthy mothers. The levels of Ca^{2+} and Pi were shown to be decreased by 34 and 33% respectively. At the same time, the activity of alkaline phosphatase was upregulated in serum by 79 %. Vitamin D_3 availability and the indices of mineral metabolism were partially normalized in newborns from diabetic mothers that were given D_3 at a dose of 2000 IU/day. Similar changes of 25(OH)D_3, Ca^{2+}, Pi and alkaline phosphatase activity were observed in newborns from women with toxemia and hypoparathyroidism.

Conclusions: Thus, deficiency of vitaminD_3 in pathologies that accompany pregnancy results in the reduction of D_3 content and in impairment of mineral metabolism in newborns. The alterations observed can be eliminated by treatment with adequate doses of vitamin D_3.

Keywords: vitamin D_3, pregnancy, diabetes, toxemia, hypoparathyroidism

Effect of parental smoking conditions on the Tlr2 and Tlr4 gene expression in children's nasal and oral cavity epithelial cells

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Aim: The aim of this study was to investigate the expression level of human Toll-like receptors (TLR), structurally related to Drosophila Toll which play a fundamental role in pathogen recognition, activation of innate immunity and mediate the production of cytokines necessary for the development of effective immunity, in nasal and oral cavity epithelial cells from children of smoking and non smoking parents.
Methods: The nasal and oral cavity epithelial cells from children of smoking and non-smoking parents were used for RNA extraction. Complementary DNA was synthesized from these RNAs and used for analysis of Toll-like receptors (TLR2 and TLR4) mRNA expression by quantitative polymerase chain reaction. Beta-actin mRNA expression was used as control of RNA quantity.
Results: We showed that expression level of TLR2 and TLR4 transcripts was much higher in nasal cavity epithelial cells as compared to oral cavity cells. Moreover, the expression level of TLR2 mRNA is significantly reduced in both nasal and oral cavity epithelial cells from children of smoking parents as compared to children of non-smoking parents. However, the TLR4 transcript level strongly reduces preferentially in nasal cavity epithelial cells from children of smoking parents.
Conclusions: Thus, our results showed that children of smoking parents showed dramatically reduced Toll-like receptor-2 transcript levels both in nasal and oral cavity epithelial cells and reduced TLR4 transcript predominantly in the nasal cavity cells as compared to children of non-smoking parents, which could be responsible for the suppression of pathogen recognition ability, activation of innate immunity, and production of cytokines necessary for the development of an effective immune response.

Keywords: Tlr2, Tlr4, nasal cavity, epithelial cells, children, perinatal smoking

Nanobiotechnology and Cancer Research

ERN1 signalling system as a new target for anticancer therapy

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Aim: The aim of this study is to investigate the expression level of retinoblastoma related genes contribution to endoplasmic reticulum–nuclei-1 (ERN1)-mediated glioma proliferation for identification possible target sites needed for new anti-cancer therapeutic strategies.

Methods: Expression of retinoblastoma (RB1), RB-like-1 (RBL1) and different retinoblastoma related genes (RBAP48, RBAP46, RNF40, CTIP, KDM5A, JARID1B, EID1, E2F1, and E2F3) was studied in glioma cells with ERN1 loss of function by qPCR under hypoxia and glutamine or glucose deprivation (16 hrs) using qPCR.

Results: The blockade of the ERN1 enzyme function increases the expression levels of retinoblastoma, retinoblastoma-like 1 and most retinoblastoma related genes: EID1, JARID1B, E2F1, E2F3, RBAP48, and CTIP, does not change RNF40 and RBAP46 and decreases KDM5A. Moreover, hypoxia reduces the expression levels of RB1, EID1, and E2F1 in glioma cells with ERN1loss of function only. At the same time, the expression levels of RBL1, E2F3, RBAP46, RBAP48, and CTIP decrease in hypoxia, while JARID1B and RBBP2 increase in both types of cells, but much stronger in cells with suppressed function of ERN1. The expression level of JARID1B and KDM5A mRNA is also enhanced in glutamine deprivation condition, but this effect is amplified by the blockade of the ERN1 enzyme function. The expression levels of RB1, EID1, RBAP48, and E2F3 are decreased in glutamine deprivation condition only in ERN1-deficient glioma cells, but RBL1, CTIP, RBAP46, and E2F1 decrease in both tested cell types with more significant effect in ERN1-deficient cells.

Conclusions: Thus, blockade the ERN1 signalling enzyme contributes significantly to the suppression of glioma growth via overexpression of RBL1 and retinoblastoma related genes EID1, JARID1B, E2F1, E2F3, RBAP48, and CTIP that could be used as new the targets for anticancer therapy.

Keywords: RB1, RBL1, EID1, JARID1B, E2F1, E2F3, RBAP48, CTIP, ERN1, glioma cells

Magnetic nanoparticles for cardiovascular – and neuronanotechnology

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Aim: Synthesized superparamagnetic nanoparticles covered with certain types of polymers and native protein complex with magnetic nanoparticles are a very perspective for usage in nanoneurotechnology. The aim of this work was to study interaction of polymer-covered nanoparticles of magnetite with brain nerve terminals and blood platelets as a potent selective tool for drug delivery, contrast agent in magnetic resonance imaging, selective/local hyperthermia enhancement therapy of brain cancer and manipulation of platelet aggregates in circulatory system.
Methods: Spectrofluorimetry with potential-sensitive and pH-sensitive fluorescent dyes, flow cytometry, radiolabeled assay (L-[14C]glutamate), photon correlation spectroscopy.
Results: Using photon correlation spectroscopy, binding of synthesized nanoparticles of magnetite covered with dextran, hydroxyethyl starch, oxidized hydroxyethyl starch, and chitosan with nerve terminals and platelets was demonstrated. We showed that polymer-covered nanoparticles did not influence the potential of the plasma membrane of nerve terminals and platelets. Acidification of synaptic vesicles of nerve terminals and secretory granules of platelets did not change in the presence of nanoparticles. Also, synthesized nanoparticles did not influence glutamate transport in nerve terminals and platelets. In contrast, native protein complex with magnetic nanoparticles, ferritin, in not indifferent for functional state of nerve terminals and platelets significantly altering glutamate transport.
Conclusions: On the base of the experimental data on binding of synthesized nanoparticles with nerve terminals and platelets, certain types of covering polymers were selected for manipulation with externally applied magnetic field. It was concluded that synthetic nanoparticles of magnetite covered with definite polymer and native protein complex with magnetic nanoparticles are a perspective instrument in nanoneurotechnology for selective drug delivery and for hyperthermia therapy of brain cancer by externally applied magnetic field.
Keywords: polymer-covered magnetic nanoparticles, ferritin, synaptosomes, platelets

Translational Research and Drug Development

Anticoagulant effects of Echis multisquamatis venom fibrinogenase

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The **aim** of our work are purification and characterization of the fibrinogenase from crude venom, study of its effects on human blood plasma and purified fibrinogen coagulability, influence on fibrinolysis and on platelets activation and aggregation.

Methods: Q-sepharose and Heparine-agarose chromatography and Laemmli protein electrophoresis were used. MALDI-TOF spectrometry was done on Voyager DE PRO. Inhibitory assay using chromogenic substrate S2238 was conducted on Multiscan EX. N-terminal analysis was carried out using ABI Procise 494HT Protein Sequencer (for cleavage site determination). Aggregation of washed platelets was studied on aggregometer Solar-AP2110, shape and granulation of platelets on flow cytometer COULTER EPICS XL, fibrin polymerization – using turbidimetry on spectrophotometer SF-2000.

Results: A new serine protease with fibrinogenolytic activity from the venom of *E. multisquamatis* was purified. The target of fibrinogenolysis was BβR42-A43 peptide bond. 50% of truncated desBβ$(1\text{-}42)_2$ form in fibrinogen solution decrease polymerization speed 1.8-fold. Fibrinolysis of clot formed with truncated fibrinogen was 2.1-fold faster then the control one. Fibrinogenase and truncated fibrinogen had no influence on platelets shape, granulation or the rate and level of platelets aggregation. However, interaction of platelets in the presence of desBβ$(1\text{-}42)_2$ fibrinogen is weaker and is followed with disaggregation. The enzyme has no obvious hemorrhagic effects and doesn't affect plasminogen, prothrombin, protein C and factor X.

Conclusions: By its characteristics, *E. multisquamatis* venom fibrinogenase is a prospective fibrinogen-depletive agent.

Keywords: Fibrinogen depletion, fibrinogenase, fibrinogen

Protective effect of precursors of ubiquinone biosynthesis on mitochondria under effect of doxorubicin

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Doxorubicin (D) plays a major role in cancer chemotherapy. Its use has been hampered by adverse effects. Ubiquinone (CoQ) is an important electron and proton carrier and antioxidant. Its biosynthesis may be inhibited under number of pathologies.

Aim: The aim was to study the state of mitochondrial electron transport chain (ETC) components, CoQ content and redox state, superoxide anion radicals and NO production rates, and matrix metalloproteinase-2 and -9 activities in rat liver and heart tissues under treatment with D, CoQ_{10} medical, and complex preparation of modulator and precursors of CoQ biosynthesis.

Methods: Doxorubicin was administered intraperitoneally in dose of 2.2 mg/kg daily for 8 days. Experimental rats in addition to Doxorubicin received *per os* α-tocopherol acetate, 4-hydroxybenzoic acid, and methionine (EPM complex) and CoQ_{10}.

Results: Treatment with EPM complex and CoQ_{10} in addition to Doxorubicin administration exerts a protective effect on liver and heart cells' mitochondria, evidenced by restoration of electron transport in ETC, which is expressed as decreased nitrile complexes formation with Fe-S-proteins and increased ubisemiquinone content. It should be stressed that the protective effects of EPM complex on mitochondrial ETC under Doxorubicin administration is on par with those of CoQ_{10}. Concurrently, matrix metalloproteinase-2 and -9 activities are decreased, which gives evidence of lessened extracellular matrix destruction. CoQ_{10} appeared to be more efficient at this if compared to EPM complex.

Coclusions: The expiremental data obtained may become the basis of development of approaches to correction of adverse effects of Doxorubicin. These data may be used to substantiate the application of these biologically active substances within frameworks of complex treatment of oncological pathologies.

Key words: coenzyme Q, doxorubicin, mitochondria

On inhibition of fibrin polymerization by Bβ121-138 peptide

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Aim: Previously site of lateral association of protofibril (SLA) in Bβ118-138 fragment of fibrin has been identified. Synthetic Bβ121-138 peptide inhibited the fibrin polymerization. For clarifying the inhibition mechanism we investigated the location of peptide binding sites onto fibrin molecule.

Methods: The interaction between Bβ121-138 peptide and mAb I-3c, fibrinogen (Fg), fibrin desA (f_b) and desAB (f_0) was investigated by SPR method using Plasmon-6 (produced by Institute of Semiconductor Physics, NASU).

Results: It was found that Bβ121-138 bound to mAbs I-3c, immobilized to the chip, with K_D = 1,6 mM. The control peptid AαP195-L205 did not react with mAb I-3c. The binding of Bβ121-138 peptide with Fg, f_b and f_0 was investigated using Fg-specific mAb II-4d immobilized to the chip. Fg immobilized to mAb II-4d was step-by-step transformed to f_b, then into f_0 using batroxobin and thrombin, respectively, and binding of the peptide to each of these units was measured. Bβ121-138 peptide bound with the same level to Fg and both forms of fibrin. Comparison of peptide binding to f_b and desA X fragment of Fg, deprived of αC-regions, showed that value of peptide binding with X fragment was 5.8 times grater than with f_b.

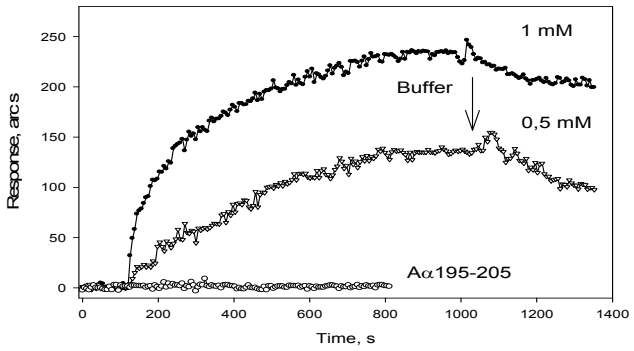

Fig. Binding of Bβ121-138 peptide with mAb I-3c immobilized to the chip

Conclusions: 1) The conformation of Bβ121-138 peptide in solution is identical to that in Bβ-chain of fibrin molecule. **2)** The binding site(s) of Bβ121-138 peptide, probably, are located within a coiled-coil region of molecule. **3)** The ability of the peptide inhibits the fibrin polymerization and binds to the coiled-coil region of molecule indicate its important role in the process of polymerization at the stage of lateral association of protofibrils.

Keywords: fibrinogen, lateral association, Bβ121-138 peptide, SPR

RECOOP Visegrad Scholarship Program

General Information:

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

Visegrad Scholarship Program (VSP)

The International Visegrad Fund offers Master's and Post-Master's scholarships awarded to selected scholars for periods of 1 or 2 semesters (with the exception of Master's scholarships within the In-Coming scheme where 1– to 4-semester scholarships can be awarded). Given the annual budget of €1,448,000, the Fund will award about 400 semesters in total in the academic year 2011/2012 (more information).

Applicants whose current (i.e. at the time of applying) university or employer is further than 1,500 km from the selected host university/institute are eligible for a one-time travel grant (see the Instructions).

Citizens of the following countries can apply:

Albania (AL), Armenia (AM), Azerbaijan (AZ), Belarus (BY), Bosnia and Herzegovina (BA), Croatia (HR), the Czech Republic (CZ), Georgia (GE), Hungary (HU), Macedonia (MK), Moldova (MD), Montenegro (ME), Poland (PL), the Russian Federation (RU), Serbia (RS), Slovakia (SK) and Ukraine (UA). The same rules are applicable to Kosovar scholars.

The scholarship program is not applicable to, nor does it cover full studies within joint programs (i.e. double– or multiple-degree). The scholarship does not cover studies/research at other institutions or in other countries than those specified in the application form. The scholars are expected to work on their study/research projects at the host university/institution for the entire scholarship period. All applicants must have finished at least 4 semesters of university at the time of applying.

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Scholarship Program for Belarusian Students

Scholarship Program for Ukrainian Students

Visegrad Scholarships at OSA Archivum (separate program)

If selected each scholar receives the scholarship funding at the beginning of each five-month period (semester) upon a written confirmation from the host university/institution

Deadline for all scholarship applications is 31 January. Results are announced by mid-May.