PLENARY LECTURES

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CELLULAR PLASTICITY AS A DRIVING FORCE IN CANCER PROGRESSION: THE REGULATORY ROLE OF ADAPTOR PROTEIN RuK/CIN85

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Introduction. Tumor progression is a complex process consisting of several stages including initiation, primary tumor growth, invasion, dissemination and metastasis, which differ by gene expression patterns, level of cell differentiation, proliferation rate, cell motility etc. Metastatic process requires epithelial cells to lose their polarity and intercellular junctions, but to acquire mesenchymal properties, such as cytoskeleton reorganization and increased motility. Such reversible process is called EMT (epithelial-to-mesenchymal transition). It enhances tumor cells motility and dissemination, and also allows cancer cells to acquire stem-like properties and chemoresistance. EMT in cancer cells is triggered through up-regulation of EMT-related transcription factors (TFs), called also EMT master regulators, in response to hypoxia, TGFβ, Notch, Wnt signaling. These TFs, including Snail1, Slug, Twist, ZEB1/2, induce expression of mesenchymal markers (N-cadherin, vimentin, fibronectin) and repress expression of epithelial markers (E-cadherin, claudin, occludin). Study of circulating tumor cells (CTCs) demonstrated several modes of cancer cell migration and metastasis. Mesenchymal type of migration implies spindle-like shape of cancer cells, as well as decomposition of extracellular matrix through increased expression levels and activity of extracellular proteases (MMPs, catepsins, serine proteases). In contrast, amoeboid migration of tumor cells is characterized by rounded shape of migrating cells, cortical actin ring and bleb-like membrane protrusions formation, independency of pericellular proteolysis and increased RhoA-ROCK-dependent signaling. This morphofunctional interconversion is maintained at balanced equilibrium that may be defined as reversal epithelial-mesenchymal-amoeboid transitions. EMT process in tumor cells is strongly linked to dedifferentiation and acquisition of stem-like phenotype. Cancer stem cells (CSCs) are known as small self-renewing subpopulation of tumor cells enable to initiate tumor growth. They are characterized by activation of embryonic signaling pathways (Wnt, Notch, Hedgehog), expression of specific markers (e.g. markers of breast CSCs: $CD44^{+}/CD24^{low/-}$, CD133, ALDH) and increased radio- and chemoresistance. Epithelial-mesenchymal plasticity includes profound changes in cellular signaling. So, identification and targeting the signaling regulators is of high importance strategy to combat the spread of cancer cells. One of such potential regulators is adaptor protein Ruk/CIN85, consisting of three SH3 domains, proline-rich motifs, and coiled-coil domain. It was described as a component of EGFR (and other RTKs) endocytosis complex, and also was found in multimolecular complexes regulating cell proliferation, motility, adhesion and survival. High amounts of this adaptor were found in tumors of different tissue origins and metastatic loci. All these features determine the need of further investigations on the role of Ruk/CIN85 in the control of epithelial-mesenchymal plasticity. Thus, the main aim of present study was to elucidate the role of Ruk/CIN85 in acquisition and maintenance of cancer cells plasticity.

Methods. In order to study the role of adaptor protein Ruk/CIN85 in the control of cancer cells plasticity we used mouse breast adenocarcinoma 4T1

cells with different levels of Ruk/CIN85 expression. Cancer cells invasiveness was studied using Boyden chamber assay, metastatic potential was estimated by experimental and spontaneous metastasis *in vivo* models. The expression levels and/or content of specific EMT, CSCs and reprogramming markers were evaluated by RT-qPCR, Western-blotting and/or immunofluorescent microscopy. Statistical analysis was carried out using ANOVA with Newman-Keuls correction.

Results. Our study demonstrated increased invasiveness and metastatic potential of Ruk/CIN85-overexpressing 4T1 cells and suppression of these abilities in 4T1 cells with Ruk/CIN85 knockdown. Analysis of EMT, CSCs and reprogramming markers showed mixed mesenchymal-amoeboid state with pronounced stem cells features in 4T1 cells with Ruk/CIN85 up-regulation, while Ruk/CIN85-downregulated cells became more epithelial. The obtained results allow us to suggest that high levels of Ruk/CIN85 are associated with cancer cells plasticity and, consequently, increased invasiveness and metastasis, while low levels of Ruk/CIN85 lock tumor cells in the rigid epithelial state resulting in the loss of plasticity.

Conclusions. Cancer plasticity phenomenon combines such features of tumor cell as EMT, stemness and reprogramming, allowing tumor cells adapt to the microenvironment and successfully metastasize. It this study we revealed the role of adaptor protein Ruk/CIN85 as a key regulator of epithelial-mesenchymal plasticity in 4T1 breast cancer cells.

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MODULATING EFFECT OF CHOLECALCIFEROL ON VITAMIN D ENDO/PARA/AUTOCRINE SYSTEM IN TYPE 1 DIABETES

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Introduction. Type 1 diabetes (T1D) is a complex autoimmune-endocrine disease that is associated with the development of a number of side effects, one of which is secondary osteoporosis. It is known that the T1D development is accompanied by a significant decrease in the vitamin D bioavailability, which can lead to a disruption of bone remodeling and a decrease in bone mineral density. At present, there is no clear evidence whether disturbances in the insular apparatus and hyperglycemia reduce vitamin D bioavailability, or vitamin D deficiency can indirectly promote the development of T1D. Thus, further investigation is needed to estimate the vitamin D status in relation to the key components of vitamin D endo/para/autocrine system in different tissues of Wistar male rats with experimental T1D and to assess the effects of vitamin D_3 treatment.

Review. The substances of vitamin D group include biologically active sekosteroids vitamin D_3 (cholecalciferol) and D_2 (ergocalciferol). Cholecalciferol hydroxylation in the liver by the enzymes of cytochrome P450 family – CYP27A1 (mitochondrial) and CYP2R1 (microsomal) isoforms of vitamin D 25-hydroxilase, results in the formation of 25-hydroxyvitamin (25OHD), which enters the bloodstream. The next step is the formation of vitamin D-hormone (calcitriol) under the influence of CYP27B1 hydroxylase that occurs either in the kidneys (5%) or in a number of peripheral tissues (95%). Vitamin D catabolism is ensured by CYP24A1, which is responsible for the formation of chemically inert end product – calcitroic acid. Genomic effects of vitamin D require the heterodimmerization of VDR with RXR receptors. VDR-RXR dimmer regulates at transcriptional level the expression of about 500 genes that are responsible for the synthesis of regulatory factors involved in cell proliferation and differentiation, survival, apoptosis and vascular growth.

Vitamin D deficiency and disruptions in the vitamin D endo/para/autocrine system can increase the risk of autoimmune diseases, in particular T1D. Growing evidence suggests that vitamin D plays an important role in preventing autoimmune destruction of pancreatic β-cells and can be directly involved in insulin production, and improvement of the peripheral tissue sensitivity to its action. Different cell types, such as cutaneous and intestinal epithelial cells, macrophages, breast and bone cells, can also express CYP27B1. Additionally, VDR was found in a variety of target cells and its ability to mediate a significant therapeutic potential of vitamin D as a natural VDR ligand in many diseases has been reported.

Type 1 diabetes induces severe impairments of liver and kidneys function that can, at least partially, be ascribed to abnormal hormonal activity of calcitriol. A marked T1D-elicited decline in the circulating 25OHD may result from decreased levels and activity of vitamin D 25- and 25OHD 1α-hydroxylases as well as up-regulation of 24-hydroxylase. Therefore, our aim was to investigate how T1D-associated changes in tissue distribution of CYPs and VDR correlate with vitamin D insufficiency/deficiency and whether vitamin D_3 (cholecalciferol) treatment can affect diabetes-related dysfunction of the vitamin D endo/para/autocrine system.

Results. Type 1 diabetes was induced in male Wistar rats by a single intraperitoneal injection of STZ (55 mg/kg of body weight). It was demonstrated that after 6 weeks of diabetes, the serum level of 25OHD was 50% lower compared with the control. Vitamin D deficiency was accompanied by a significant decrease in the mRNA level of CYP27A1 and CYP2R1 isoforms of 25-hydroxylases in the liver (4.2- and 6.3-fold respectively). The changes in liver vitamin D para/autocrine function were assessed by measuring the expression of mRNA and protein syn-

thesis of CYP27B1 and VDR. We showed a 5.3- and 2.0-fold diabetes-induced decrease in the CYP27B1 mRNA and protein levels, respectively. Diabetic animals also demonstrated a 14.3- and 1.5-fold decrease in the level of VDR, mRNA and protein, vs. control, respectively. In contrast, the level of CYP24A1 mRNA, which facilitates vitamin D catabolism, was 6.4-fold higher in diabetic rats compared with the control.

In the kidneys of diabetic animals, we observed an increase in the levels of both mRNA and protein of CYP27B1 (1.28- and 1.25-fold respectively), and VDR (5.2- and 2.0-fold respectively) in comparison with the control. Moreover, diabetic animals exhibited a 5.0-fold increase in the kidney level of CYP24A1 as compared with the control.

The study of the vitamin D para/autocrine system in bone tissue showed a 13.0-fold increase in the level of CYP27B1 mRNA. In addition, we observed a decrease in both VDR mRNA (3.3-fold) and protein (1.8-fold) in the bone tissue of diabetic animals. Diabetes caused a profound drop in the mRNA level of CYP27B1 (3.8-fold) and VDR (9.0-fold) in the bone marrow as compared with the control. These changes were confirmed by immunofluorescence staining of bone marrow cells followed by confocal microscopy.

Cholecalciferol treatment (600 IU/kg of body weight for 30 days) led to a partial correction of the vitamin D status of diabetic animals that was accompanied by the normalizing effects on the synthesis of the key elements of the vitamin D endo/para/autocrine system in the liver, kidneys, bone tissue and bone marrow.

Discussion. Among various side effects of T1D, impairments in the tissues and organs involved in the metabolism of vitamin D and its hormonal activity through VDR pathway are of particular interest. A decrease in the level of mRNA of 25-hydroxylases in the liver of diabetic animals indicates impairment of the first stage of cholecalciferol activation. Moreover, a decrease in the level of CYP27B1 and VDR indicates the impairments of the para/autocrine function of the liver. This hypothesis is confirmed by an increase in CYP24A1 hydroxylase, which indicates the probable prevalence of vitamin D catabolism over its synthesis in diabetes. An increase in the synthesis of CYP27B1 and VDR in the kidneys of diabetic animals may reflect a compensatory effect of vitamin D endocrine system in response to an overall decrease in blood 25OHD level.

Secondary osteoporosis is recognized as one of the side effects associated with T1D. The elevation of the CYP27B1 mRNA and the down-regulation of the VDR may indicate impairments of osteoblastic/osteoclastic balance with the prevalence of osteoclastogenesis and bone resorption. Vitamin D_3 administered as a potential hydroxylation substrate to form vitamin D-hormone contributes to the complete or partial correction of the most parameters studied.

Conclusions. Diabetes-induced vitamin D deficiency is associated with the abnormalities in renal and extrarenal expression of CYP27B1 and VDR. Vitamin D_3 is effective in amelioration of diabetes-associated impairments of the vitamin D endo/para/autocrine system in different tissues.

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BIOLOGICAL ROLE OF NICOTINIC ACETYLCHOLINE RECEPTORS IN MITOCHONDRIA

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Introduction. Nicotinic acetylcholine receptors (nAChRs) are classically regarded as ligand-gated ion channels located in the cell plasma membrane to mediate fast synaptic transmission in muscles and neurons. In addition, they are present in many non-excitable cells to regulate cell survival, proliferation, adhesion and production of cytokines. We have found that α7β2, α3β4, α4β4 nAChR subtypes are located in the outer membrane of mitochondria to regulate the early stage of mitochondrial apoptosis, namely, the opening of mitochondrial pore, through which cytochrome *c* (cyt *c*) is released under the effect of apoptogenic agents like Ca^{2+} or H_2O_2. Cyt *c* release from mitochondria can be attenuated by either nAChRs agonist or antagonist indicating that the nAChR signaling in these intracellular organelles is ion channel-independent and is mediated through conformational changes of the nAChR molecule upon specific ligand binding. The lack of α7, α3 or α7 and β2 nAChR subunits in mitochondria of mutant (knockout) mice was compensated by significant increase of α9 and β4 subunits. Correspondingly, mutant mice mitochondria did not change dramatically their response to Ca^{2+}, but were affected by subtype-specific ligands in different way compared to mitochondria of the wild-type mice. The aim of the present study was to evaluate the role of nAChRs in mitochondria under different physiological conditions.

Methods. Experiments have been performed in C57Bl/6 mice and Wistar rats. Mitochondrial and non-mitochondrial fractions were isolated from the liver, brain, lung or lung carcinoma by differential centrifugation according to standard procedures. The level of nAChR subunits was studied by Sandwich-ELISA using subtype-specific antibodies. The apoptotic resistance of live mitochondria was evaluated based on the level of cyt *c* released under the effect of Ca^{2+} and H_2O_2.

Results. It was found that neuroinflammation caused by either LPS injection or immunization with α7(1-208) resulted in decreased level of α7 nAChRs in the mouse brain mitochondria compared to mitochondria of control mice. In functional assay, mitochondria of LPS-treated or α7(1-208)-immunized mice released more cyt *c* in response to Ca^{2+} and were less sensitive to attenuating effect of α7-specific agonist PNU282987 than mitochondria of control mice. Treating mice with N-stearoylethanolamine (NSE) prevented α7 nAChR decrease in the brain mitochondria making them more resistant to apoptogenic effect of Ca^{2+}. In contrast, increased level of a7 nAChRs was observed in mitochondria purified from Lewis lung carcinoma compared to normal mouse lung, making mitochondria less sensitive to low doses of Ca^{2+}. Finally, the increase of a7-, a3-, α4- and, especially, a9-containing nAChRs was found in the rat liver mitochondria 3-6h after partial hepatectomy resulting in increased mitochondria resistance to 0.1-0.9 μM Ca^{2+} and 0.1-0.5 mM H_2O_2.

Conclusions. These data demonstrate a physiological significance of mitochondrial nAChRs in supporting mitochondria sustainability to apoptogenic influence. The level of α7 nAChRs is critically important upon neuroinflammation, while up-regulation of α9- and β4-containing nAChRs in mitochondria is a physiological response to either compensate the deficiency of other nAChR subtypes or support the cell survival in critical circumstances.

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ABSTRACTS OF THE CONFERENCE

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G-QUADRUPLEX DNA BINDING AND TELOMERASE INHIBITION BY PHEOPHORBIDES

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Introduction. G-quadruplexes (G4) are specific structures formed by some guanine rich DNA sequences, e.g. those at the ends of the telomeres. Their stabilization by small molecules can lead to telomerase inhibition and thus is a promising anticancer strategy. This research was aimed at the study of DNA binding and telomerase inhibition of a series of pheophorbides, compounds of porphyrin family.

Methods. Fluorescent Intercalator Displacement (FID) method was used to study G4 and duplex DNA binding affinity and selectivity of three pheophorbides: natural anionic Pheophorbide-a and its neutral and cationic derivatives. The assay is based on the substitution of Thiazole Orange dye in fluorescent DNA complex by a ligand resulting in concentration-dependent fluorescence decrease that allows the determination of binding constant and stoichiometry. Quadruplex was formed by Tel22 oligonucleotide d[AGGG(TTAGGG)$_3$]. DNA binding constants (K_b) were obtained from the titration data by Scatchard method. Telomerase inhibition activity of compounds was determined by Telomeric Repeat Amplification Protocol (TRAP) *in vitro* assay.

Results. DC_{50} parameters (ligand concentration required to induce 50% fluorescence decrease) were in the range of 19.5-62.7 and 11.4-37.2 μM for G4 and duplex DNA, respectively. K_b values of compounds were (2.1-5.6)×10^6 M^{-1} for G4 and 7×10^4-3.1×10^6 M^{-1} for duplex DNA. The highest selectivity for G4 vs. dsDNA was observed for cationic derivative Cat-Pheo-*a* (selectivity index 1.9). IC_{50} values for telomerase inhibition were in the range of 8-40 μM.

Discussion. Binding constants indicate high G4 affinity of pheophorbides. Cat-Pheo-*a* demonstrates both high affinity for G4 DNA (DC_{50} 19.5 μM, K_b 4.5×10^6 M^{-1}) and good selectivity to quadruplex DNA. This G4 ligand has also the highest inhibition activity towards telomerase (IC_{50} 8 μM). The highest DNA binding efficiency and biological activity of Cat-Pheo-a can be due to the presence of cationic trimethylammonium group able to form ionic bonds with DNA phosphates.

Conclusions. Cat-Pheo-*a* is the most efficient G4 ligand and telomerase inhibitor among the studied compounds.

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COMPUTER MODELING OF DIRECT FACTOR Xa INHIBITORS

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Introduction. The vast mortality proportion in the developed countries is caused by cardiovascular related diseases. Hence, biological investigations of blood coagulation cascade and inhibition of related key enzymes should be taken into consideration. Factor Xa (FXa) is the calcium-binding gamma carboxyglutamyl(Gla)-containing vitamin K-dependent glycoprotein, that is directly involved in inhibition of prothrombin conversion into the active thrombin, which provokes clot formation. Thus, FXa inhibitors could be used as potential active treatment for certain thrombotic disorders. Furthermore, it is important to search for direct inhibition compounds due to their more accurate and precise effect on target.

Methods. Structural compounds from ChEMBL (FXa inhibitors) were used in this research, previously filtered according to ADME requirements: molecular weight range (350-650), QlogP (0-6), rotation bonds (3-9), H-bond acceptor (0-10), H-bond donor (0-5). Of 2.5m Enamine compounds, filters have passed more than 1.75m. The structures were translated into a format suitable for docking.

A Schrodinger program was used to prepare binding site of the FXa from crystal structure (PDB entry 1KSN). Initially, all water molecules within 10 Å from the ligand were removed, missing atoms and chains were added and overall protein' structure was optimized and minimized. Docking was performed after posing four main constraints within S1 and S4.

This resulted in a library of approximately 3000 compounds, this sample went through a visual inspection. Selected series for each model were tested using a modified RVV test (RVVT) and inhibitor activity.

Results. Based on all aforementioned criteria, it was noticed that 59 substances reached 70% inhibitor activity. Further biological screening on purified FXa within selected compounds demonstrated activity ranging 40-60%. Seventeen selected compounds inhibited FXa by 60-100%, among which 2 demonstrated the highest activity.

Conclusions. As a result of our investigation we have obtained a virtually generated library of 1200 compounds, within which several revealed high active inhibitors of FXa could be applied as potential cures.

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THE EFFECT OF N-STEAROYLETHANOLAMINE ON AGE-RELATED AND DIET-INDUCED CHANGES OF FATTY ACID PROFILE OF RAT ADIPOCYTES

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Introduction. Chronic hypernutrition and high fat diet (HFD) rich in saturated fatty acids leads to changes in insulin sensitive tissues, impairment in insulin signaling and followed by dyslipidemia. Phospholipids as one of the main component of cell membranes are involved in mechanisms of insulin signaling and a pool of free fatty acids (FFA) plays an essential role in the development of inflammation. That is why the aim of our study was to investigate the fatty acid (FA) composition of different lipid fractions of adipocytes in different age rats with HFD-induced obesity and insulin resistance (IR) and their changes under N-stearoylethanolamine (NSE) administration.

Methods. The experimental model was induced on 10-month- and 24-month-old rats by HFD and confirmed by the oral glucose tolerance test. NSE was administrated per os for 2 weeks. Adipocytes were isolated from abdominal fat using Type 1 Collagenase solution. Adipocytes lipid extract was separated on the fractions by thin-layer chromatography. FA composition was analyzed by gas-liquid chromatography. Experimental data were processed statistically using Student's *t*-test. The statistical significance was determined for $P < 0.05$.

Results. The investigation of FA composition of PL demonstrated that total content of FA is significantly higher in Control and IR groups of 10-month-old rats compared to the same groups of aged animals and NSE normalized FA content in adipocytes PL of the elder rats. In both age groups after HFD we observed statistically significant growth of saturated fatty acids (SFA) as well as unsaturated ones (UFA). There was no significant difference in the ratio of SFA:UFA between control animals at different ages but HFD induced a considerable decrease of FA saturation in PL of adipocytes of elder animals.

It was also demonstrated that total content of FFA is significantly higher in elder control rats than in younger intact animals. The percentage of SUFA was the same with monounsaturated FA (MUFA) in FFA composition of adipocytes of younger rats whereas MUFA level prevails upon SUFA in elder group. The assay showed that HFD caused a considerable growth of FFA content in adipocytes of 10 month old rats and NSE affect positively on its normalization.

Conclusions. It was demonstrated, that prolonged HFD induced IR and leads to changes in FA profile of adipocytes in rats from two age groups. As far as NSE administration had a positive effect on normalization of FA composition of lipid fractions of adipocytes, we can consider NSE as a prospective agent for the treatment of obesity-induced complications and correction of age-related dyslipidemia.

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REGULATION OF GLYCOLYSIS-RELATED GENES EXPRESSION IN U87 ERN1 KNOCKDOWN GLIOMA CELLS

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Introduction. Glioma cells are enhanced by glycolytic activity irrespective of the supply of oxygen. This leads to an increase of cell proliferation performed through the formation of intermediates. ER (Endoplasmic reticulum) stress as well as hypoxia and ischemia are important factors influencing tumor cells proliferation and survival, responsible for metabolism reprogramming mainly through ERN1(endoplasmic reticulum to nuclei-1) signaling enzyme. Goal of this study was to investigate the expression level of *GPI, GNPDA1, ALDOA, ALDOC, ENO1* and *ENO2* genes in glioma U87 cells upon hypoxia and ischemia, to evaluate their role in glioma cells proliferation through ERN1 mediated signaling.

Methods. Human glioma cell line U87 and its subline with complete suppression of ERN1 enzymatic activities were used. Expression of glycolysis-related genes was measured in glioma cells using qPCR. Hypoxic condition was created in incubator with 3% oxygen and 5% carbon dioxide levels. Culture plates were exposed to this condition for 16 h. Cells were also cultivated in DMEM without glucose or glutamine for glucose and glutamine deprivation.

Results and Discussion. We have demonstrated that the expression of genes encoding GPI, ALDOA, ALDOC, ENO1 and ENO2 enzymes is increased (+30%, +28%, +822%, +25% and +272%, respectively) in glioma cells with totally suppressed enzymatic activity of ERN1 (dnERN1), being more intense for *ALDOC* and *ENO2* genes. It is also increased (+49%, +33%, +16%, +27% and +252%) in glioma U87 cells when only endoribonuclease activity of ERN1 signaling enzyme is suppressed (dnr-ERN1). Tunicamycin-induced ER stress decreases (-22% and -40%) the expression level of *ALDOC* and *ENO2* genes in glioma cells without endoribonuclease activity of ERN1, but does not significantly change the expression level of *GPI, GNPDA1, ALDOA* and *ENO1* mRNAs.

Multidirectional changes have been demonstrated for expression levels of *GPI, GNPDA1, ALDOA, ALDOC, ENO1* and *ENO2* mRNAs both in wild type glioma cells and in cells with totally suppressed enzymatic activity of ERN1 under glutamine and glucose deprivation as well as hypoxia. Furthermore, ERN1 knockdown modifies effects of hypoxia as well as glutamine and glucose deprivations on the expression of most studied genes.

Conclusions. Results of this investigation clearly demonstrate that the level of glycolysis-related genes expression depends on ERN1 enzymatic activity as well as on hypoxic and ischemic conditions and that the expression of *GPI, ALDOC*, and *ENO2* genes can contribute to the suppression of glioma cell proliferation introduced by downregulation of ERN1 signaling enzyme function.

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DEFICIENCY OF ADAPTOR PROTEIN RuK/CIN85 IN LEWIS LUNG CARCINOMA CELLS INHIBITS MALIGNANCY HALLMARKS *IN VITRO* AS WELL AS TUMOR GROWTH AND PULMONARY METASTASIS *IN VIVO*

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Introduction. Lung cancer is a common cause of cancer mortality associated with distant metastases. Progress in metastatic research, however, is constrained by the lack of tumor-bearing animal models that would allow understanding comprehensively the complex network of signaling pathways that drives the multistep process of metastatic cascade. Adaptor/scaffold proteins are key regulators able to effectively process the information through signaling networks. It was shown previously that high levels of adaptor protein Ruk/CIN85 contribute to the conversion of weakly invasive human breast adenocarcinoma MCF-7 cells into a more malignant phenotype. In addition, we found high amounts of this adaptor in aggressive Lewis lung carcinoma cells (LLC cells). In the current study, we set a goal to determine interplay between Ruk/CIN85 down-regulation in LLC cells and their metastatic potential using experimental and spontaneous metastasis models in syngeneic C57BL/6 mice.

Methods. To down-regulate Ruk/CIN85, LLC cells were stably infected with lentivirus encoding Ruk/CIN85-specific shRNA as well as irrelevant virus to obtain control cells. The expression levels of Ruk/CIN85 in LLC cells and primary tumors were assessed by Western-blotting. Cancer cells proliferation was studied by direct cell count and MTT test, cell migration – by scratch test and invasiveness – using Boyden chamber assay. The influence of Ruk/CIN85 down-regulation on the morphology of LLC cells was studied by confocal microscopy. The expression levels of specific EMT markers were evaluated by qRT-PCR. To estimate efficiency of experimental and spontaneous metastasis, C57BL/6 mice were inoculated intravenously or subcutaneously into right hind leg with control and Ruk/CIN85 down-regulated LLC cells. Primary tumors and lungs were processed for histological evaluation according to standard protocol. Statistical analysis was carried out using ANOVA with Newman-Keuls correction.

Results. It was demonstrated that Ruk/CIN85 knockdown in LLC cells caused attenuation of their proliferative rate, decreased motility and invasiveness while increased adhesion properties *in vitro*. Down-regulation of Ruk/CIN85 significantly reduced metastatic potential of LLC cells both in experimental and spontaneous metastasis models *in vivo*. According to changes in cell morphology and qRT-PCR data, the suppression of aggressiveness of Ruk/CIN85 knockdown cells was associated with mesenchymal-to-epithelial transition.

Conclusions. Taken together, the data obtained suggest that adaptor protein Ruk/CIN85 could function as a concentration-dependent switch of mesenchymal-to-epithelial transition in Lewis lung carcinoma cells being thus a promising target for therapeutic intervention.

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EFFECTIVE METHOD FOR CLEANING OF ORE OF ORE-FERROUS FACTORY FROM IONS OF HEAVY METALS BASED ON BACTERIA ASSOCIATION OF THE GENUS *PSEUDOMONAS*

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Introduction. Discharge of sewage, which is pumped out during the work of mining complexes, leads to the contamination of surface water bodies with various toxic chemical pollutants (oil products, heavy metal ions, etc.). Mountain mining leads to an increase in the runoff of mine and mine waters, which carry a significant amount of chloride compounds, sulfuric acid, soluble salts of iron, manganese, copper, etc. Particularly dangerous are the heavy metal ions Pb, Cd, Mo, Ni, Zn, As, which enter the environment with mine waters and run-off from ore-processing plants. The aim of the work is the development of an environmentally safe method for purification of ore-dressing plants effluents from heavy metal ions on the basis of the association of non-pathogenic bacterial strains of the genus *Pseudomonas.*

Methods. The content of heavy metal ions in aqueous solutions before and after microbiological purification was determined by the atomic absorption method on a flame atomic absorption spectrophotometer Saturn in the flame of an air-propane-butane mixture. The effectiveness of the proposed microbiological method is estimated by the degree of extraction from the cations of heavy metals [Pb (II), Cd (II), Zn (II)].

Results. For the first time, cells of non-pathogenic strains of *P. fluorescens* ONU328, *P. maltophilia* ONU329, *P. cepacia* ONU327 were proposed as part of the biofloquial for the effective and ecologically safe treatment of run-off plants. The degree of extraction of Pb (II), Cd (II), Zn (II) from concentrated solutions reached 93.00-99.85% with their residual content in solution (0.03-4.9) mg/dm^3. The use of strains of *P. fluorescens* ONU328, *P. maltophilia* ONU329, *P. cepacia* ONU327 (1:1:1 in volume ratio) in the developed association method provides the greatest efficacy.

Discussion. When processing technogenic solutions with immobilized bacterial cells in the composition of bioflecula, the residual concentration of Pb (II), Cd (II), Zn (II) was within 0.02-0.1 mg/dm^3 that is much lower than their maximum allowable concentration for discharge cleaned solutions into the sewage system.

Conclusions. The non-pathogenic strains of the bacterium of the genus *Pseudomonas* possess a broad biochemical potential of biotechnological purpose – sorption-accumulating relative to ions of heavy metals and destructive for hydrocarbons of oil, which opens wide prospects for their use in biotechnology of cleaning the environment from pollutants of various nature.

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ANALYSIS OF THE EXPRESSION AND PRODUCTS ACCUMULATION OF CD-MARKER GENES IN CULTURES OF LUNG AND SKIN RATS FIBROBLASTS IN ONTOGENESIS

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Introduction. Products of CD genes are not only indicators of cell membership to a particular population, but also play an important role in the adhesion cells process and their perception of various signals. The purpose of our work was to determine the dynamics of the expression and products accumulation intensity of specific CD genes in the lung and skin of rat fibroblast cultures in ontogenesis.

Methods. The tissues were ground in DMEM medium containing 1% trypsin. After 30 min incubation at 37 °C, the cells were harvested and sown in ventilated culture flasks in a DMEM medium containing 10% FBS and cultured them (37 °C and a humidity of 95% in the presence of 5% CO_2). Cell attachment and cell culture density were monitored using an inverted microscope, using 3rd passage fibroblasts, the gene expression analysis was performed on DNA – microchips (Arrayit) and Affymetrix 428 Scanner. The total RNA from the cells was isolated on spin columns with a set of RNeasy Mini Kit (Qiagen). Synthesis of cDNA by reverse transcription was performed using QIAGEN OneStep RT-PCR Kit (Qiagen). The amplification was carried out using a BIO-RAD iCycler. The final amount of the produced protein product was measured immunochemically on antibody-conjugated ELISA-microchips using the Antibody Array Assay Kit (Full Moon BioSystems, Inc.) reagent kits.

Results and Discussion. An analysis of the expression of CD-marker genes has shown that the cells we are investigating have a set of molecular data that is characteristic of mature fibroblasts. The results of measuring genes expression suggest that in all the age groups of lungs and skin fibroblasts there is practically no expression of markers, which are characteristic exclusively of mesenchymal stem cells (MSC). At the same time, markers common to mature fibroblasts and MSC are expressed in cells of both types of tissues in all age groups, indicating the "purity" of the fibroblasts culture. CD-marker genes expression intensity indicators in lung fibroblasts with age vary insignificantly, as opposed to skin cells, where this index in the case of all genes has a maximum in cells of 1-month-old animals, and in the future ontogenesis tends to decrease. The products accumulation of the CD-markers genes is much more intense in the skin fibroblasts than in the lung cells.

Conclusions. The obtained results indicate that, despite the phenotypic homogeneity of skin and lungs fibroblasts, the composition and number of surface cell markers in the course of ontogenesis vary unevenly.

Acknowledgments. I am heartily thankful to my supervisor, Prof., Dr.Sc. Ye. Persky for his guidance during the development of this experimental work.

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EXPRESSION OF PROTEASE GENES IN IRE1 KNOCKDOWN U87 GLIOMA CELLS UPON GLUTAMINE DEPRIVATION

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Introduction. Proteases are an important part of the complex regulatory cascades in cells and play an extremely important role in the dynamic mechanisms of metabolism regulation in different pathological conditions. Living systems maintain a balance between proteases and their inhibitors and disturbing of this equilibrium leads to the development of many diseases, including malignant tumors. In this regard, the study of the role of key proteases and processes that they control is important for understanding the molecular mechanisms of cancer development. IRE1, the most evolutionarily conserved signaling pathway of the endoplasmic reticulum stress, is highly implicated in sustaining the proliferation of glioma cells and subsequent tumor growth, which is decreased by the inhibition of IRE1. Glutamine is an important factor of glioma development and a more aggressive behaviour. To explore the effect of glutamine deprivation on gene expressions in glioma cells in relation to the functional activity of IRE1 signaling, we studied the expression level of ubiquitin specific peptidase (USP) and cathepsin (CTS) genes, during glutamine deprivation in wild type U87 glioma cells (control cells) and cells with inhibited IRE1.

Methods. The following methods were used in this work: RNA extraction, electrophoresis in agarose and polyacrylamide gels, reverse transcription method, real-time qPCR and statistical analysis of results in Excel programs.

Results and Discussion. IIt was shown that the exposure of control glioma cells (transfected by empty vector) upon glutamine deprivation led to suppression of *USP1* (-13%), *CTSC* (-26%) and *CTSK* (-15%) gene expressions and upregulation of *CTSD* (+64%) and *CTSO* (+18%) mRNA. At the same time, glutamine deprivation did not significantly change the expression level of *USP4, USP10* and *USP14* genes in control glioma cells. Inhibition of IRE1 signaling enzyme function in U87 glioma cells increases the effect of glutamine deprivation on the expression of *USP1* gene (-32%), decreases *CTSD* gene expression (+38%) and introduces sensitivity of *USP4* and *USP14* genes to this condition.

Therefore, the inhibition of IRE1 signaling enzyme in U87 glioma cells modifies the effect of glutamine deficiency on the expression of most studied genes encoding cathepsins and ubiquitin specific peptidases: inducing the effect of glutamine deficiency on the *USP4* and *USP14* genes expression, decreasing – on the expression of *CTSD* gene, and amplifying – on the *USP1* gene expression.

Conclusions. Glutamine deprivation affects the expression level of most studied genes encoding cathepsins and ubiquitin specific peptidases in gene specific manner in relation to the functional activity of IRE1 signaling enzyme, a central mediator of endoplasmic reticulum stress, which control cell proliferation and tumor growth, and these changes in gene expressions possibly contribute to suppression of glioma cell proliferation.

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P60-S6K1 mRNA TRANSCRIPT EXPRESSION PROFILE IN A PANEL OF CELL LINES AND BREAST CANCER TISSUE SAMPLES

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Introduction. The *RPS6KB1* (S6K1 (ribosomal protein S6 kinase 1) gene is frequently amplified in breast cancer that is associated with shorter survival of patients suggesting that S6K1 protein overexpression (p85- and p70-S6K1 isoforms) plays a significant part in breast cancer biology. Unlike the major p85- and p70-S6K1 isoforms, expression and a role of the p60-S6K1 isoform in breast cancer has been poorly studied to date. The aim of the study was to analyze the expression profile of p60-S6K1 mRNA transcript in different cell types and breast tumors of various clinical subtypes.

Methods. RNA isolation, RT-PCR, DNA gel electrophoresis, DNA sequencing.

Results. Initially, we focused on confirmation of the existence of the p60-S6K1 mRNA transcript by PCR. PCR analysis of cDNA from HEK-293 and subsequent DNA sequencing verified that the sequence of the PCR product corresponded to the p60-S6K1 transcript-specific nucleotide sequence. Expression levels of the p60-S6K1 transcript were estimated using PCR analysis of cDNA from 8 different cell lines (MCF-7, HEK-293, HeLa, HepG2, U-87, U-373, U-937, Jurkat), 20 breast cancer tissue samples grouped according to the clinical subtype (Luminal A (7), Luminal B (7), HER^+ (6) and 6 normal breast tissue samples adjacent to breast tumors. Data showed the presence of heterogeneity of p60-S6K1 mRNA expression between different cell lines and also heterogeneity between 3 clinical breast cancer subtypes. What is more, the expression levels of the given transcript were elevated in the samples of Luminal B and HER^+ subtypes, which are associated with poor prognosis, compared to that of Luminal A subtype associated with good prognosis. In addition, there was no correlation observed between expression of the p60-S6K1 transcript and total S6K1 RNA transcripts in all tested cell lines and tissue samples.

Conclusions. Additional evidence of the existence of the p60-S6K1 mRNA transcript, which supposedly encodes the p60-S6K1 isoform, was received. The given transcript exhibits a differential expression pattern in a number of cell lines, as well as in breast cancer tissue samples of different clinical subtypes. The increased expression levels of the p60-S6K1 transcript seem to correlate with poor prognosis in patients with breast cancer.

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CENTRAL SEROTONIN AND TRYPTOPHAN LEVELS IN RATS WITH DIET-INDUCED OBESITY AT THE DIFFERENT TIME OF MELATONIN ADMINISTRATION

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Introduction. Obesity is often associated with a decreased level of brain serotonin. It was found on serotonergic neurons receptors for melatonin – a promising agent for the treatment of obesity [Ríos-Lugo M. J., 2015]. The aim was to detect changes in brain serotonin and tryptophan content on the obesity development under various (morning, evening and continuous) modes of melatonin administration.

Methods. Male rats were divided into 6 groups: 1) control – received a standard diet (C); 2) high caloric diet (HCD) group; 3) standard diet and melatonin treated group either 1 h after lights-on (M ZT01) or 4) 1 h before lights-off (M ZT11); 5) HCD and melatonin 1 h after lights-on (HCD ZT01) or 6) 1 h before lights-off (HCD ZT11). Melatonin was administered daily by gavage (M ZT01, M ZT11, HCD ZT01, HCD ZT11) or in drinking water (M W, HCD W) for 7 weeks (30 mg/kg). Both the tryptophan and serotonin content were analyzed using ion exchange chromatographic method (KM-sepharose). Hypothalamus serotoninergic neurons were marked by histochemistry method of formaldehyde-induced fluorescence.

Results. The brain serotonin and tryptophan levels in the HCD were decreased by 30 and 15%, respectively, while the serum serotonin and tryptophan levels were increased by 40 and 20%. Melatonin administration increased the brain serotonin and tryptophan level to control values (in HCD W group it was closer). Also, the quantity of serotonin-positive hypothalamic arcuate nucleus neurons after melatonin use was higher by 40% in HCD ZT01, by 63% in HCD ZT11 and by 68% in HCD W compared with HCD group. In addition the number of serotonin vesicles was calculated in each cell: the amount of vesicles in HCD group has decreased by 57%. The melatonin treatment improves the value of this parameter: its amount grew up compared with HCD – HCD ZT01 by 30% (although, its value significantly differs compared to C group), in HCD ZT11 by 47% and in HCD W by 51%. The serum serotonin level was lower by 25% (prominent status, differs compared to C group), 35% and 43% in HCD ZT01, HCD ZT11 and HCD W group, respectively; but tryptophan content was higher in all groups, which received melatonin, compared with HCD and C (HCD ZT01 – by 38%, HCD ZT11– by 35%, HCD W – by 31%).

Conclusions. Thus, the administration of melatonin (the best modes are continuous in water) can improve the state of hypothalamus serotoninergic neurons in terms of the obesity development.

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THE CYTOTOXICITY OF CADMIUM IONS SMALL DOSES IN LONG-TERM CULTURE OF BONE MARROW CELLS

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Introduction. It is known that cadmium ions have the property to accumulate in cells, leading to disturbances in their metabolism. The effects associated with the long-term influence caused by small doses of cadmium ions have not been studied at all. The purpose of this work was to assess the cytotoxicity effects and degree of damage to bone marrow DNA in rats during prolonged cultivation in the medium containing small doses of cadmium ions – 0.1-10 μM/liter of culture medium. The extent of cell adhesion and their morphology, culture density, cell membrane integrity, and the number of apoptotic cells were analyzed. The extent of DNA damage was assessed by the number of micronuclei, fragmentation of nuclear DNA and single-strand DNA concentration in cells.

Methods. Studies were carried out on a monolayer of bone marrow cell culture from the femur of a three-month-old Wistar rats. The cells were cultured in a storage medium with cadmium ions in concentrations of: 0.1; 0.5; 1.0; 10 μM/l of culture medium. Studies were conducted every 48 hours for 30 days, before replacing the medium with fresh, containing Cd^{2+}. The number of cells in the early and late stages of apoptosis was determined by flow cytofluorometry (Millipore Guava Nexin Kit). Detection of micronuclei was carried out by fluorescence microscopy. To assess the extent of DNA fragmentation, comet analysis was performed, the DNA tracks were visualized with fluorescence microscopy and analyzed using CASPlab software. The single-strand DNA concentration was determined by Molecular Probes Qubit ssDNA Assay Kit.

Results and Discussion. It is shown that the degree of damage to DNA cells depends on the exposure time and the concentration of cadmium. Exposure to cadmium for 30 days at a concentration of 0.1 and 0.5 μM/l leads to an increase in the number of cells in the early apoptosis stage, which is reversible and does not affect the fragmentation of nuclear DNA. Exposure to cadmium at a concentration of 1.0 and 10.0 μM leads to a significant increase in the number of cells in the irreversible stage of late apoptosis, the fragmentation of nuclear DNA and ssDNA concentration by 30 days of observation.

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THE COMPOSITION OF ISOLATED VOLUTIN GRANULES OF *SACCHAROMYCES CEREVISIAE* UCM Y-517

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Introduction. Volutin polyphosphate granules are intracellular complexes of the linear polymers of condensed inorganic phosphates, which are linked by high-energy phosphoanhydride bonds, with other compounds. The studies of the composition of isolated volutin granules are sporadic and mainly performed on bacteria with only one work on yeasts. The aim of this work was a detailed study of the composition of isolated volutin granules of *Saccharomyces cerevisiae* UCM Y-517.

Methods. Volutin granules were isolated by method of Eixler et al. (2005) and studied by energy-dispersive X-ray spectroscopy, protein and polyphosphate electrophoresis, gas chromatography.

Results. The high phosphorus concentration in isolated volutin granules was confirmed. This phosphorus has mainly been presented by high molecular polyphosphates (< 200 phosphate residues). The metal composition showed the highest signal from K, a lower one from Mg and Ca, as well as traces from Na and Fe. Detected trace of sulfur and sufficiently high values of carbon and oxygen could indicate the presence in isolated volutin granules of proteins and other organic compounds. Volutin contains two protein fractions with molecular masses of 5-15 and 50-100 kDa. High hydrophobicity (87%) could suggest that isolated volutin granules have lipids. There were detected two saturated fatty acids that had 16 and 18 carbon atoms.

Discussion. Literature data on the chain length of granule polyphosphates were contradictory. Our results have demonstrated their long-chained nature. Proteins of yeast volutin are known to be presented by only one fraction (10-20 kDa). However, we showed the second heavier fraction. Here we first report the presence of lipids in yeast volutin. Metal content in volutin directly depends on nutrient medium, with K, Mg, Ca being most abundant, that is confirmed by our data.

Conclusions. Thus, volutin granule composition of yeast *S. cerevisiae* involves high molecular polyphosphates, metals K, Mg, Ca, Na and Fe, proteins of two fractions with molecular masses of 5-15 and 50-100 kDa, two saturated fatty acids with chain length of 16 and 18 carbon atoms.

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THE EFFECT OF VANADIUM CITRATE ON THE BIOCHEMICAL PARAMETERS OF THE BLOOD PLASMA OF PREGNANT FEMALE RATS

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Introduction. Pregnancy is accompanied by significant changes in biochemical parameters, which causes metabolism intensification, accelerated excretion of substances and microelements. It is known about the normalizing effect of some vanadium compounds on metabolic processes in the body during pregnancy.

The purpose of our research was to find out the effect of the organic compound of vanadium citrate on the activity of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), urea and creatinine in pregnant rats.

Methods. The research was conducted on female white laboratory rats with the body weight of 140-160 g, which were divided into five groups: I group – control, II, III, IV, V – experimental. I group – non-pregnant females, II – pregnant females (consumed pure water), III, IV, V – pregnant females, which during the period of mating and pregnancy received vanadium citrate solution in concentrations of 0.03, 0.125 and 0.5 μg V/ml of water. The study material was blood plasma of rats, in which the activity of AST, ALT, AP, urea and creatinine (on the biochemical analyzer Humalyzer 2000, Germany) was determined.

Results. The authors have concluded that the activity of AST, ALT and creatinine level decreased, while the activity of AP and urea level increased as compared to the control.

Under the conditions of watering vanadium, the rats of III, IV and V experimental groups experienced the increase in the activity of AST, ALT, urea and creatinine levels, while the AP activity decreased (unless V group) as compared to pregnant females of II group, which didn't consume vanadium citrate.

Discussion. Investigating the activity of AST and ALT in pregnant rats enables to detect possible heart and liver complications. The decrease in AST activity may be caused by a lowered level of vitamin B_6 in pregnant animals. The increase in AP activity at the end of pregnancy might be due to the restructuring of life-support systems, include bone tissue growth and placenta development. The increase in the urea level in blood plasma of pregnant females is due to renal function changes caused by the increase in the urine formation and its excretion, whereas the decrease in creatinine level is caused by its excessive excretion with the urine.

It is known that vanadium has a hepatoprotective effect, which causes stabilization of AST and ALT activity, normalization of creatinine level.

Conclusions. Rats that received vanadium citrate showed the approximation of the activity of AST, ALT, AP, creatinine level to the control group, but the urea level increased as compared to II group, which indicates the normalizing effect of this compound on some biochemical parameters in pregnant females.

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UDC 581.19

THE ROLE OF BRASSINOSTEROIDS IN REGULATION OF PHOSPHOLIPID SIGNALING IN PLANT CELLS

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Introduction. Brassinosteroids are plant steroid hormones that play key role in the regulation of plant growth and stress tolerance. The aim of this work was to investigate the brassinosteroid-induced dynamics of phospholipid signalling in normal growth conditions and under salt stress, and to assess the roles of different lipid signalling pathways in the production of lipid second messengers.

Methods. Levels of fluorescently-labelled lipid products of BODIPY-phosphatidylcholine hydrolysis were analyzed in seedlings of *Brassica napus*, wild-type and AtCAX1-overexpressing *Nicotiana tabacum*. Plants were grown in solution containing salt (NaCl) or brassinosteroids (24-epibrassinolide) during 7 days and then separated roots were additionally treated for 2 hours by brassinosteroids or salt, respectively. Another set of plants was pretreated by N-ethylmaleimide, an inhibitor of phosphatidic acid phosphatase, or R59022, an inhibitor of diacylglycerol kinase, and then subjected to brassinosteroids for 2 h.

Results. Salt stress and brassinosteroids induced dramatic elevation of second messenger phosphatidic acid (PA) and diacylglycerol (DAG) levels in *B. napus* plants. Moreover, DAG and PA were shown to accumulate on a higher level when plants were initially grown in brassinosteroid-containing solution and then treated by salt. Accumulation of DAG and PA in response to brassinosteroids was reduced in plants subjected to R59022, but not NEM.

Discussion. DAG accumulation in response to brassinosteroids is mediated by non-specific phospholipase C hydrolyzing phosphatidylcholine, in spite of phosphatidic acid phosphatase that dephosphorylates PA. PA accumulation induced by brassinosteroids is mediated by further activation of diacylglycerol kinase phosphorylating DAG, the product of non-specific phospholipase C.

Conclusions. The results suggest that brassinosteroid signalling and brassinosteroid-induced plant adaptation to salt stress are mediated by DAG and PA as lipid second messengers.

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EXPRESSION PROFILE OF FATTY ACIDS IN THE LIVER AND GUERIN'S CARCINOMA OF RATS UNDER CONDITIONS OF Ω-3 POLYUNSATURATED FATTY ACIDS ADMINISTRATION

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Introduction. Omega-3 (ω-3) fatty acids are essential nutrients, because they cannot be synthesized endogenously. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (ω-3) decrease inflammation by competing with arachidonic acid (AA) (ω-6) as enzymatic substrates and serving as precursors of resolvins and protectins. A high ω-6 intake relative to ω-3 fatty acids may contribute to cancer risk, because a low ω-3/ω-6 ratio in tissue can create a proinflammatory milieu and thereby promote tumor formation and progression. The aim of this study was to evaluate the modification of fatty acid profiles in the liver and Guerin's carcinoma microsomal fraction in rats under conditions of ω-3 polyunsaturated fatty acids (PUFAs) administration.

Methods. The experiments were performed on white female rats. Guerin's carcinoma was used as a cancer model. 0.5 ml of 30% carcinoma cell suspension in normal saline was injected subcutaneously into thigh of a hind limb. The animals were administered ω-3 PUFAs for 4 weeks prior to the carcinoma implantation and then for the entire duration of tumor growth. A daily dose was 120 mg of ω-3 PUFAs per kg of body mass. The content of fatty acids in the microsomal fraction was analyzed by HRGC 5300 gas chromatography in glass column with Chromosorb W/HP sorbent in 10% Silar 5CP liquid phase.

Results. In the liver microsomal fraction of the tumor-bearing rats during the intensive growth of the tumor (14 days, which corresponds to the logarithmic phase of oncogenesis) the levels of AA and linoleic acid (LA) (ω-6) were higher, whereas the levels of DHA were significantly lower as compared to controls. The ω-3 PUFAs administration prior to and post-implantation of tumor leads to increasing DHA and EPA, decreased level of AA in comparison to tumor-bearing rats that had not received the lipophilic nutrients. We found increased levels of DHA, EPA, LA and AA in microsomal fraction of Guerin's carcinoma in animals of the group that was administered ω-3 PUFAs both before and after implantation of the carcinoma during logarithmic phases of carcinogenesis.

Discussion. The high level of DHA and EPA in the liver microsomal fraction of rats can be associated with the ability of ω-3 PUFAs to be incorporated into membrane phospholipids and replace the content of ω-6 PUFAs in their composition while the ratio of ω-6/ω-3 PUFAs decreases from 4/1 to 2/1. The high level of LA and AA in tumor microsomal fraction of rats may indicate a decrease in their metabolism as a result of competition with ω-3 PUFAs for metabolic enzymes. However, the ratio of ω-6/ω-3 PUFAs in tumor tissue decreases from 5/1 to 2/1.

Conclusions. The ratio of ω-6/ω-3 PUFAs plays an important role in the metabolism of PUFAs of the liver and tumor and may be seen as a potential marker for prognosis of tumorigenesis.

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THE MYELIN BASIC PROTEIN AND S100B LEVEL IN THE DIFFERENT BRAIN AREAS OF RATS AFFECTED BY PITUITRIN AND IZADRIN

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Introduction. The S100b is produced mainly by astrocytes in the brain and depends on the concentration provides trophic or toxic effect on the neurons and glial cells. Strong stress and ischemia induce redistribution of calcium-binding protein S100b and elevation of its level. Myelin basic protein (MBP) is the main protein component of myelin. The MBP level is in direct proportion to the extent of myelin degradation. Glia-specific proteins are used as markers of glia state, especially during brain damage. The aim of our study was to investigate the distribution of MBP and S100b in different brain parts in rats treated by pituitrin and izadrin.

Methods. The Wistar 6 month rats were divided into two groups ($n = 6$): 1 – control rats maintained under standard condition; 2 – rats with the pituitrin-izadrin induced myocardial attack (PIIMA). The animals were decapitated under anesthesia (thiopental, 60 μg/kg) and different brain parts were isolated. The levels of S100b and MBP in obtained protein fractions were measured with competitive ELISA.

Results. Under the pituitrin-izadrin effect, there was a significant decrease in the S100b concentration in the cerebellum in comparison to control; 2.24±0.13 to 1.82±0.16 μg/100 mg tissue. No statistically significant decrease in the level of this protein was detected in the other parts of the brain. The reduction of myelin basic protein level in the cerebellum, thalamus and hippocampus of rats under the pituitrin-izadrin effect suggested nerve fibers demyelination and reduced functional activity of oligodendroglia. According to the obtained data, the concentration of MBP in the control group was in cerebellum – 4.52±0.36 μg/100 mg of tissue, thalamus – 3.08±0.43 μg/100 mg of tissue, and hippocampus – 3.13±0.31 μg/100 mg of tissue, while the following results were acquired from the pituitrin-izadrin treated animals: cerebellum – 3.03±0.39 μg/100 mg of tissue, thalamus – 1.50±0.07 μg/100 mg of tissue, and hippocampus – 2.25±0.15 μg/100 mg of tissue.

Conclusions. Obtained data allow suggesting that pituitrin-izadrin induced myocardial attack can provoke the complication of brain function by induction of demyelination and astrocyte dysfunction.

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EFFECTS OF CERIUM (IV) OXIDE NANOPARTICLES ON RAW 264.7 CELLS ACTIVITY AND RANKL-STIMULATED OSTEOCLASTOGENESIS

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Introduction. Osteoclastogenesis is a complex process that plays a critical role in bone remodeling. Based on detailed knowledge of the molecular mechanisms involved in osteoclastogenesis, new pharmacological agents (including nanoparticles) that selectively influence the differentiation or the activity of bone cells were developed during the last decade. The purpose of our research was to study the molecular mechanisms of influence of 5 μM citrate-stabilized CeO_2 nanoparticles (CNPs) on the RAW 264.7 cells, its proliferative activity and level of multinuclear cells formation during RANKL-stimulated osteoclastogenesis.

Methods. The murine macrophage cell line RAW 264.7 was cultured with CNPs (2-4 nm) in DMEM (4.5 g/l glucose). Cell proliferative activity and apoptosis were assessed and visualized with IncuCyte ZOOMinstrument. Bovine bone slices were stained with *TRAP* and Hoechst 33258 for TRAP-positive multinuclear cells detection. The levels of TNF-α, CCL2, COX2, IL-6, Rel A mRNA expression were examined by RT-PCR analysis.

Results and Discussion. Exposure of RAW 264.7 cells to CNPs (5 μM) during 70 hours decreased cell proliferation and apoptosis by 20 and 12%, respectively compared with control ($P < 0.05$). MTT test has shown a mild cytostatic effect of CNPs on RAW 264.7 cells. On the other hand, a significant 26% increase was revealed in the number of multinuclear cells in bone slices under the effect of CNPs ($P < 0.05$). CNPs led to upregulation of TNF-α and Rel A (4.1- and 1.6-fold respectively) and downregulation of IL-6, CCL2, COX2 and GLUT 1 (1.9-, 1.8-, 1.3- and 1.6-fold, respectively) mRNA expression after 24 hours of RANKL-stimulated osteoclastogenesis compared with control ($P < 0.05$).

Conclusions. Our results demonstrate that CNPs caused a slight cytostatic effect on RAW 264.7 cells and enhanced the fusion of macrophages during RANKL-stimulated osteoclastogenesis. The findings suggest a significant CNPs-induced activation of TNF-α with the lowering effect on the levels of other inflammation factors, as well as GLUT 1 transporters.

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EFFECT OF GLUTAMINE DEPRIVATION ON THE EXPRESSION OF *DEK, TPD52, BRCA1, ADGRE5, LIF, GNPDA1,* AND *COL6A1* GENES IN IRE1 KNOCKDOWN U87 GLIOMA CELLS

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Introduction. Glutamine is an important factor of glioma development and a more aggressive behaviour. The aim of this study was investigation of the effect of glutamine deprivation on the expression of genes encoding the key proliferation associated factors on a relation to inhibition of IRE1 in U87 glioma cells.

Methods. The expression of *DEK, TPD52, BRCA1, ADGRE5, LIF, GNPDA1,* and *COL6A1* genes in U87 glioma cells transfected by empty vector pcDNA3.1 (control) and cells without IRE1 signaling enzyme function (transfected by dnIRE1) was studied by qPCR. The data were analyzed by 2-tailed Student's *t*-test.

Results. It was shown that glutamine deprivation down-regulated the expression of *DEK, BRCA1, LIF,* and *COL6A1* genes in control glioma cells (transfected by empty vector), up-regulated *ADGRE5* gene expression, and did not significantly change the expression of *TPD52* and *GNPDA1* genes. Inhibition of IRE1 signaling enzyme activity modified the effect of glutamine deprivation on the expression of *TPD52, BRCA1, LIF, DEK, ADGRE5,* and *COL6A1* genes: induces the effect of glutamine deprivation on *TPD52* and *GNPDA1* genes, reduced – on *COL6A1* gene, and enhanced – on *ADGRE5, DEK,* and *BRCA1* genes in U87 glioma cells.

Discussion. Therefore, this study has demonstrated that glutamine deprivation affects the expression of the majority of the studied genes encoding important proliferation related factors preferentially in the IRE1-dependent manner and that these changes potentially contribute to suppression of glioma cell proliferation upon glutamine withdrawal.

Conclusions. Our results demonstrate, that glutamine deprivation affect the expression level of most studied proliferation associated genes in U87 glioma cells in relation to the functional activity of IRE1 signaling enzyme, which is responsible for control of cell proliferation and glioma growth.

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THE EFFECT OF MANGANESE, NICKEL AND LEAD IONS ON LDH ACTIVITY OF THE *PROCAMBARUS VIRGINALIS* (LYKO, 2017)

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Introduction. The marbled crayfish *Procambarus virginalis* (Lyko, 2017) (Decapoda) – new alien species, that got into the reservoirs of the Dnipropetrovsk region in 2015, it was necessary to study the possibilities of its adaptation to environmental factors of reservoirs for further prediction of its distribution or even acclimatization under conditions of toxicological contamination of the ponds of Ukraine. The disturbance of vital functions of hydrobionts living in changed conditions is precisely at the biochemical level. As an indicator of the stress, LDH activity is used to biomark the physiological state of animals, the potential pollution of the reservoirs. The working hypothesis of the study was based on determining the reaction of marble crayfish to the influence of the simulated concentrations of nickel, manganese and lead ions under controlled conditions.

Methods. Crayfish were divided into 4 groups (n = 15): 1st – control; 2nd – with $Ni(NO_3)_{2x}6H_2O$; 3rd – with $Pb(NO_3)_2$; 4th – with $MnSo_{4x}5H_2O$ (Sigma, USA). Water in aquariums was completely changed twice a week and toxicants were added at the rate of concentrations of metal ions: Ni^{2+} – 0.04 mg/l (4 MAC), Pb^{2+} – 0.15 mg/l (1.5 MAC), Mn^{2+} – 0.02 mg/l (2 MAC). Concentrations of heavy metals were determined by their content in water of Zaporizhzhya reservoir as control, the main recipient reservoir for this new species. The experiment lasted 21 days. The activity of lactate dehydrogenase (LDH) was determined using standard commercial sets LDH (Filisit Diagnostika, Ukraine) on a spectrophotometer SP-26 at a wavelength of 340 nm. There liability of the difference between data samples was determined using one-factor ANOVA ($P < 0.05$).

Results. On the 21st day of the experiment in the lead aquarium 26.7% of crayfish were dead. In the nickel experiment, crustacean mortality was the highest and reached 60.0%. It was found that the activity of LDH increased by 29.6 and 32.3%, during the effect of Mn^{2+} and Ni^{2+} on the tissues of marmorkrebs. In the control group, LDH activity was 48.04±4.03 NADH/mg. The influence of Mn^{2+} increased the activity of lactate dehydrogenase up to 67.23±5.69 NADH/mg, and the influence of Ni^{2+} increased it up to 69.84±2.1 NADH/mg. The influence of Pb^{2+} also showed improbable changes by 15.6% in the activity of the enzyme. The activity of LDH under the influence of lead was at the level of 56.23±4.46 NADH/mg.

Conclusions. Functional activity of LDH increases with poisoning marbled crayfish with heavy metals, when inhibiting tissue respiration. The growth of LDH activity leads to increased utilization of lactate by various tissues, with the urgent need with tissue damage by toxic factors. The rate of utilization of lactate by different tissues indicates the biochemical plasticity of *P. virginalis* to adverse conditions of existence. These processes contribute to the survival of resistant species of hydrobionts under toxicologically hard conditions.

UDC 577.125

HEPATOPROTECTIVE EFFECTS OF Ω-3 POLYUNSATURATED FATTY ACIDS ON RATS WITH TRANSPLANTED GUERIN'S CARCINOMA

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Introduction. Aminotransaminases are expressed in several cellular compartments by malignant or nonmalignant cells. Alanine aminotransaminase (ALT) is only existent in the hepatocellular cytoplasm and mitochondria; however, aspartate aminotransaminase (AST) is widely spread in several organs, including heart, kidney, brain, skeletal muscle, and liver. The relationship between different levels of these enzymes and patient prognosis are stated in several types of cancer. Omega-3 (ω-3) polyunsaturated fatty acids (PUFAs) play protective roles in the liver, cardiovascular and kidney disease and they have been widely used in clinical preoperative total parenteral nutrition. The aim of this study was to evaluate AST/ALT (De Ritis) ratio and γ-glutamyl transferase (GGT) activity in rat blood serum under conditions of carcinogenesis and ω-3 polyunsaturated fatty acids (PUFAs) administration.

Methods. Female albino rats weighing 130-150 g were used in this study. Animals were subdivided into three groups: I – intact animals (control); II – rats with transplanted Guerin's carcinoma; III – animals that were administered ω-3 PUFAs prior- and post-Guerin's carcinoma injection. ω-3 PUFAs were administered as Vitrum Cardio Omega-3 (Unipharm Inc., USA), derived from fish oil. Rats were decapitated on the 14th day after implantation of Guerin's carcinoma.

Results. The activity of ALT, AST, and GGT increased in the blood serum of the tumor-bearing rats during the intensive growth of the tumor (14 days, which corresponds to the logarithmic phase of oncogenesis) and the De Ritis ratio decreased as compared with the control group. ω-3 PUFAs – eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in dose 120 mg / kg body weight have no influence on the enzyme activity of serum AST, but decreases activity of serum ALT, and GGT as compared with the tumor-bearing rats.

Discussion. The increasing of ALT, AST and GGT in serum of tumor-bearing rats may be due to spill out of these enzymes from the liver cytosol into the bloodstream and/or liver dysfunction and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane. Releasing of AST, ALT, and GGT from the liver cells can occur as secondary changes to cellular necrosis. The protective effect of ω-3 PUFAs on liver tissue was confirmed by the attenuation of the activities of serum ALT, AST, GGT and in addition to the normalization of De Ritis ratio. The mode of action of ω-3 PUFAs can be intercepted pharmacologically at different levels with agents that scavenge free reactive oxygen, block their generation, or enhance endogenous antioxidant capabilities.

Conclusions. The present results indicated that administration of ω-3 PUFAs had a protective role against hepatotoxicity in rats with transplanted Guerin's carcinoma during intensive tumor growth.

UDC 577. 112

THE NEURAL CELL ADHESION MOLECULES IN THE RAT BRAIN AFTER WATER-IMMOBILIZATION STRESS AND AFTER THE RECOVERY PERIOD

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Introduction. Stress is one of the main factors of the disturbance of the functioning of the central nervous system. However, the stressfulness of modern society increases over time. The most important intercellular compounds are adhesive proteins. The system of intercellular adhesion provides main mechanisms for the development of nerve cells, including their migration, formation of the axons, synapses and neuron-glial networks, which totally affect neuronal function. The purpose of this work was to investigate changes of the ratio of the soluble and membrane forms contents of neural cell adhesion molecules (NCAM) under water-immobilization stress and after a period of physiological recovery.

Methods. The experiment was conducted on 18 Wistar rats divided into 3 groups ($n = 6$). 1 – control; 2 – water-immobilization stress (WIS); 3 – rats that had a recovery period of 14 days. The animals were withdrawn from the experiment under a weak anesthetic according to the ethical rules of handling of laboratory animals. Different areas of the brain were isolated, from which the cytosolic and membrane fractions of proteins were obtained by differential ultracentrifugation. The quantity of NCAM was measured by competitive ELISA using monospecific antibody (Abcam, UK). Statistical processing of the results was performed with one-factor ANOVA dispersion analysis.

Results. The impact of WIS for 3 days led to different change of the content of both soluble and membrane NCAM in different studied brain areas. The level of NCAM in the hippocampus and cerebellum did not change at the moment of stress procedure compared with the control group. However, it was decreased to 32% in hippocampus at the distant time after stress. More critical decrease to 53% level of soluble NCAM was detected in the thalamus of stressed animals just after the stress procedure. The physiological recovery of rats after stress for 14 days did not result in the absolute restoration of NCAM pool.

Discussion. Soluble NCAMs regulate more quick mechanism of adaptation of neural cells after stress to have impact to the strength of adhesive contact between them. A short-term stress leads to the decreased level of membrane NCAM at the distant time that can affect the synaptic plasticity and can be a trigger for the neural community disturbance.

Conclusions. The impact of water-immobilization stress on the NCAM level depends on time after stress and can provoke neurodegeneration at the long remote period.

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DOSE-DEPENDENT ACTION OF POLYHEXAMETHYLENE GUANIDINE HYDROCHLORIDE ON RELEASE OF L-[^{14}C]GLUTAMATE FROM RAT BRAIN NERVE TERMINALS

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Introduction. Polyhexamethylene guanidine hydrochloride (PHMG-Cl) is a polycationic compound that exerts surface-active effect on different biological membranes. Now it is intensively studied for safety as disinfectant. PHMG-Cl is considered to act predominantly on bacterial and fungal membranes, because of negative charge on their cells surfaces. However, there was an evidence of its harmful effect on human health, e.g. bronchiolar disorders. This research is devoted to assessing the influence of PHMG-Cl on glutamate transport in the rat brain nerve terminals (synaptosomes).

Methods. Synaptosome preparations in the standard salt solution were diluted up to the end concentration of 0.5 mg of protein/ml per sample; after pre-incubation at 37 °C for 10 min the synaptosomes were loaded with radiolabeled L-[^{14}C] glutamate (238 mCi/mmol, 1 nmol/mg of protein) and incubated at 37 °C for 10 min. Water solution of PHMG-Cl was added to synaptosomal suspension separately at the end concentrations of 1, 5, 10, 25, 50 and 500 mg/ml. After 8 min incubation, samples were rapidly sedimented in a microcentrifuge (20 s at 10,000 g). L-[^{14}C]glutamate release was measured using liquid scintillation counting with scintillation cocktail ACS (1.5 ml).

Results. Extracellular level of L-[^{14}C]glutamate in the synaptosomal suspension increases in a dose dependent way with the increase of end concentration of PHMG-Cl. At the final concentration of 500 mg/ml PHMG-Cl caused complete release of the preloaded glutamate.

Discussion. We can suggest that the effect found may be caused by membrane disruption, inducing pore formation. Or, despite positive charge on synaptosomal surface, PHMG-Cl penetrates into cell and breaks down proteins functioning directly or veiledly, thereby producing L-[^{14}C]glutamate leakage.

Conclusions. Starting from concentration of 1 mg/ml PHMG-Cl per 0.5 mg of protein, it might be potentially toxic for the mammalian brain tissues, inducing overbalance in extracellular level of neurotransmitter.

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EFFECTS OF INTERMITTENT FASTING ON RESPIRATORY CAPACITY OF MITOCHONDRIA AND ACTIVITY OF ACONITASE IN CEREBRAL CORTEX OF C57BL/6 MICE

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Introduction. Recently several studies have found that intermittent fasting may improve health. We have assumed that positive effects of intermittent fasting on healthspan can be mediated by mitochondrial electron transport chain (ETC). Therefore, the aim of our work was to investigate the effects of intermittent fasting on respiratory capacity and activity of aconitase, a marker of oxidative stress, in isolated mitochondria of mouse cerebral cortex.

Methods. The experiment was conducted on 12- and 18-month-old mice. The control group had unlimited access to food (*ad libitum*). Mice subjected to intermittent fasting (IF) received food every other day.

Results. *Ad libitum* 18-month-old males had 1.6-fold lower NADH-linked oxygen consumption as compared to 12-month-old males. Such difference was not observed between females of different ages on both regimes and between IF males. Those belonging to IF subgroup of 18-month-old males showed 2-fold higher NADH-linked oxygen consumption than those ad libitum fed. The activity of cytochrome-*c*-oxidase was not affected by age, sex, and feeding regimen. The respiratory control ratio (RCR) was 1.5-fold lower in 18-month-old *ad libitum* males as compared to 12-month-old males on the same feeding regimen. However, RCR did not drop with age in IF males. Age did not influence RCR in *ad libitum* fed females, while 18-month-old IF females had 2-fold lower RCR than the 12-month-old ones. Fasted 18-month-old males had 2.2-fold higher RCR as compared with *ad libitum* one. Activity of aconitase in 18-month-old males was 1.2-fold lower as compared with 12-month-old ones. Age did not influence aconitase activity in females.

Conclusions. Intermittent fasting prevents age-related decline in mitochondrial respiratory capacity in males while does not have positive effects on mitochondrial respiration in females. Also, intermittent fasting does not have an effect on aconitase activity.

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BIOCHEMICAL MECHANISMS OF FIBRIN CLOT FORMATION AND SUBSEQUENT LYSIS REGULATION BY PLATELETS

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Introduction. Platelets as key regulators of hemostasis and thrombosis are responsible for dynamic balance between coagulation and fibrinolysis pathways. They form an interface on which important events – thrombin generation, fibrin formation and degradation of blood clot – are initiated, progressed and terminated. Besides, platelets also mediate coagulation and fibrinolysis by supplying a number of coagulation and fibrinolytic proteins as well as their inhibitors. The aim of this study was to investigate the biochemical mechanisms underlying the net effect of platelets on the processes of fibrin clot formation and subsequent lysis.

Methods. Coagulation and lysis of freshly obtained platelet rich plasma (PRP) were monitored using clot waveform analysis assay, by absorbance measurements at 405 nm. Activation of protein C and thrombin generation was estimated with specific chromogenic substrate assay. Flow cytometry analysis was performed to evaluate platelets activation.

Results and Discussion. To investigate platelets impact on the intrinsic coagulation pathway, coagulation of PRP was initiated by calcium chloride (final concentration 8 mM), and the initiation of coagulation by 8 mM calcium chloride with 0.5 nM thrombin was then performed to establish the influence of platelets on terminal reactions of coagulation cascade. Under these conditions, platelets stimulated coagulation in direct proportion to the cell number. Nevertheless, for most of the PRP samples tested, the increase in maximum absorbance was accompanied by its subsequent decrease, which indicates the conversion of the polymerization of fibrin into its spontaneous lysis, even without rt-PA addition. To prove the contribution of the plasminogen/plasmin system to spontaneous degradation of PRP-derived clot, PRP was preincubated with 6-aminohexanoate (AHA), a blocker of lysine-binding sites in plasminogen. Addition of 5 and 10 mmol/l of AHA to PRP prior to clotting caused complete inhibition of spontaneous dissolution of the clot. The inhibition of fibrinolysis after the addition of anti-PC antibodies in PRP (final concentration 18 μg/ml) and the activation of protein C (confirmed by specific chromogenic substrate assay) on platelets suggests certain role of protein C pathway in platelets regulation of hemostasis. Additionally, platelets accelerated thrombin production by prothrombin complex. These results were supported by flow cytometry analysis, which showed that under thrombin activation, a population of platelets with high level of PS and PI signal was formed, that provide them procoagulant properties due to the binding and activation of coagulation cascade proteins. The impact of prothrombin complex on plasminogen binding and activation can be another possible phase on which coagulation and subsequent lysis can be regulated by platelets.

Conclusions. The obtained data gives the evidence that platelets can selectively regulate coagulation and fibrinolysis, thereby adapting the local hemostatic balance, the size and lifetime of the fibrin clot to formation of physiological hemostatic plug or thrombus. Modulation of plasminogen binding and activation, as well as the activation of protein C on platelets surface can be one of the possible mechanisms of such regulation.

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THE EXPRESSION OF COX AND NDUF GENES IN U87 GLIOMA CELLS WITH IRE1 KNOCKDOWN

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Introduction. Tumor growth is tightly associated with the endoplasmic reticulum stress response-signaling pathway and hypoxia. Multiple studies were shown that inhibition of IRE1, a central mediator of the unfolded protein response, results in a significant anti-proliferative effect in glioma growth through down-regulation of angiogenesis and proliferation processes. Mitochondrial enzymes and factors play a vital role in the regulation of cell metabolism and bioenergetics, and most of these proteins take part in the functional reprogramming of mitochondria in cancer as well as in other diseases. The aim of our study was to examine the effect of inhibition of IRE1 and hypoxia on the expression of nuclear genes encoding mitochondrial enzymes of respiratory chain in glioma cells for evaluation of their possible significance in the IRE1-mediated inhibition of glioma growth.

Methods. We used U87 glioma cells and their subline without IRE1 signaling enzyme function, transfected by dnIRE1. The expression level of COX6B1 (cytochrome *c* oxidase subunit 6b1), COX7A2 (cytochrome *c* oxidase subunit VIIa polypeptide 2), COX8A (cytochrome *c* oxidase subunit 8A) and NDUFB5 (NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 5) mRNAs as well as ACTB mRNA were measured in U87 glioma cells by quantitative polymerase chain reaction.

Results and Discussion. Inhibition of IRE1 signaling enzyme function down-regulates the expression of *COX6B1*, *COX7A2*, *COX8A* and *NDUFB5* genes in U87 glioma cells in comparison with the control cells. It was also shown that hypoxia slightly suppresses the expression of NDUFB5 gene, but IRE1 knockdown eliminates hypoxic regulation of this gene. At the same time, the expression of *COX6B1*, *COX7A2*, and *COX8A* genes is resistant to hypoxia condition in both types of glioma cells.

Conclusions. Results of our investigation demonstrate that expression of all studied genes is responsible to IRE1-mediated endoplasmic reticulum stress signaling, because inhibition of IRE1 leads to significant changes in their expression in U87 glioma cells in a gene specific manner. In addition, hypoxia does not affect the expression of most of these genes. Therefore, changes in the expression level of nuclear genes encoding COX6B1, COX7A2, COX8A and NDUFB5 proteins may reflect IRE1-mediated metabolic reprogramming of mitochondria and correlate with suppression of glioma cell proliferation upon inhibition of the IRE1 enzyme function.

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THE INFLUENCE OF MONOSODIUM GLUTAMATE ON REACTIVE OXYGEN SPECIES PRODUCTION IN RATS

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Introduction. One of the most common food additives in Ukraine and in Europe is monosodium glutamate (MSG, $C_5H_8NO_4NaH_2O$). Encoded E621, it is a food additive from a group of flavor enhancers, used in a wide range of foods, such as soups, sauces, mixed condiments, chips, meat products, and puddings. Despite its widespread use and generally considered safety, some questions regarding the impact of MSG on general health have arisen. There are studies that MSG intake at a dose of 3 g per day is dangerous to human health. The aim of our study was to investigate the effect of monosodium glutamate administration at "safe" (allowed) dose on the content of reactive oxygen species (ROS) in leukocyte blood suspension of rats.

Methods. Experimental studies were conducted on 16 nonlinear, white male rats weighing 180-200 g. MSG was purchased from Sigma-Aldrich (USA). Laboratory animals were divided into 2 groups. The first group was administered MSG at a dose of 30 mg/kg body weight (corresponds dose 2 g per day in humans) for 30 days. The control group of animals was given normal saline. Analysis of cell samples to determine ROS was evaluated by the flow laser cytometry method on flow cytometer Epics XL (Beckman Coulter, USA), using 2.7-dichlorodihydrofluorescein diacetate. The value of the studied parameter was expressed as a percentage (the ratio of the number of leukocytes with increased intracellular content of ROS (ROS+ cells) to the total number of cells).

Results. We have established that MSG administration at a "safe" dose can induce oxidative stress. Content of ROS increased in leukocyte blood suspension by 40.3% vs control group ($P < 0.05$).

Discussion. Excess generation of ROS in cells is known to damage DNA, lipids, and proteins resulting in several biological effects, ranging from alterations in signal transduction, gene expression, mutagenesis, and apoptosis. Moreover, the activation of lipid peroxidation processes (direct effect of increased generation of ROS) is an important biochemical mechanism for the development of endogenous intoxication. Excessive lipoproxidation is accompanied by the accumulation of peroxide oxidation products and the depletion of antioxidant system reserves, which causes hyperenzymemia and accumulation of toxic substances.

Conclusions. Thus, our results indicate that administration of MSG at a dose of 30 mg/kg body weight was associated with development of excessive ROS production in leukocytes of rats. Therefore it is advisable not only to investigate the established dangerous doses of E621, but also to study the molecular mechanisms of the "safe" (allowed) doses of MSG effect on a living organism.

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THE ROLE OF 2′- AND 3′-HYDROXYL GROUPS OF A76 tRNATyr AT THE FIRST STEPS OF TRANSLATION QUALITY CONTROL

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Introduction. Translational control is an essential step for the quality and fidelity of protein biosynthesis. Several checkpoints exist to avoid the mistakes of initiation and elongation steps. Aminoacyl-tRNA-synthetases (aaRSs), enzymes which activate amino acids and attach them to cognate tRNAs, can possess editing domains in their structure, therefore protecting cells from including non-proteinogenic amino acids into proteins. Some aaRSs, for example, tyrosyl-tRNA-synthetase (TyrRS), do not have such domains; additionally, TyrRS demonstrate the weakest specificity in recognition of D- and L-Tyr enantiomers. Besides, supplementary trans-editing enzyme – D-aminoacyl-tRNA-deacylase (DTD) – can remove the mistakes of aaRSs. It hydrolyses the ester linkage between D-amino acids and tRNAs. Previously, we have successfully cloned, expressed and purified DTD from Thermus thermophilus. In this work we have analyzed and identified the role of 2′- and 3′-OH groups of A76 tRNATyr as the primary sites for aminoacylation by TyrRS and deacylation by DTD during translation initiation step.

Methods. To address this issue, we applied two biochemical assays with [32P]-labelled tRNATyr substrates: wild type A76 tRNATyr and its 2′- and 3′-deoxyA76 derivatives. We determined the catalytic parameters of these reactions and analyzed data in Origin 9.0.

Results and Discussion. We identified the primary site of D-Tyr attachment to tRNATyr – its 2′-OH group of terminal ribose in A76. L-Tyr bounds similarly to 2′- and 3′-OH groups. DTD catalyzes deacylation specifically from the 3′-OH group and 2′-OH only assists in this hydrolysis.

Conclusions. Our research resulted in the molecular mechanisms of cooperations between tRNATyr, TyrRS, and DTD, representing the studies of D-Tyr involvement in translation process in thermophilic bacteria *T. thermophilus.*

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FEATURES OF FATTY STRENGTH PROFILE OF STRAIN *BREVIBACILLUS CENTROSPORUS* F14 – DESTRUCTOR OF PHENOLIC COMPOUNDS

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Introduction. Close-bodied microorganisms are clearly delineated by the presence and percentage content of their cellular lipids of fatty acids. Particular attention is paid to the search for new and identification of non-pathogenic microorganisms intended for the purification of effluents from the production of pharmaceuticals, medical institutions with the predominant content of highly toxic phenolic compounds in them. The aim of the work is to establish the characteristics of the fatty acid profile of the non-pathogenic strain *Brevibacillus centrosporus* F14, a destructor of phenolic compounds isolated from sewage from Ukrainian pharmaceutical products.

Methods. Fatty acid analysis of the strain *Brevibacillus* sp. F14 was performed by gas chromatography using the Sherlock MIDI microorganism identification system.

Results. The saturated fatty acids of the branched structure prevailed in the spectrum of the strain from the fatty acids (in the sum of 82.0%): C14:0 iso (14.9%), C15:0 iso (14.8%), C15:0 anteiso (34.9%), C16:0 iso (11.1%), C17:0 iso (1.4%), C17:0 anteiso (4.9%). A feature of the fatty acid profile is *Br. centrosporus* F14 is the presence on the chromatogram of the strain-destructor of cyclic aromatic xenobiotics of biomarker fatty acids: 16: 1 w7c alcohol (7.7%) and 16:0 N alcohol (0.6%). The unsaturated long chain fatty acids found in minor amounts of biomain fatty acids also include: 16:1 w11c (3.0%), 17:1 iso w10c (1.3%), 18:3 w6c (0.4%). The total amount of saturated fatty acids of the normal structure was 3.1% 12: 0 (0.7%), 14:0 (0.4%) and 16:0 (2.0%).

Discussion. The presence of fermented fatty acids C15:0 and C17:0 in the fatty acid profile of the strain *Br. centrosporus* F14 in the form of iso and anteiso made it possible to calculate the biomarker ratios [C15:0 anteiso/C15:0 iso] and [C17:0 anteiso/C17: 0 iso], which were 2.4 and 3.5, respectively, and 2-2.5 times higher than those for the previously identified soil oil-oxidizing strain *Bacillus megaterium* ONU-542. Difference of fatty acid profile of strain *Br. centrosporus* F14 from the fatty acid profile of the strain of *Bacillus subtilis* ONU-551, also having a destructive potential with respect to phenol, is that the maximum content of the sum of the peak area on the chromatogram of strain *Br. centrosporus* F14 accounted for fatty acid 15:0 anteiso (34.9%), and on the chromatogram of a similar strain-destructor *B. subtilis* ONU-551 – for fatty acid 15:0 iso (34.7%).

Conclusions. According to the fatty acid composition using the RTSBA6 6.21 library of the MIDI Sherlock program, the investigated strain with a high similarity index was identified as *Br. centrosporus* F14. In laboratory conditions, the high phenol-oxidizing capacity of the strain is confirmed, which opens the possibility of its use in biotechnology of environmental purification from aromatic xenobiotics.

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THE HEMOLYTIC EFFECT OF OXIDATIVE STRESS CAUSED BY CHRONIC EXPOSURE TO LOW CONCENTRATIONS OF CADMIUM ON THE BODY

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Introduction. The relationship between the degree of erythrocytes acid hemolysis and the indicators of oxidative stress in the blood of 3-month-old rats, which were receiving Cd^{2+} in doses of 0.1 and 1 μg/kg of body weight through the intragastric tube for 36 days daily, was studied.

Methods. Erythrocytes were obtained from rats' blood after animal decapitation under thiopental narcosis. Blood and 0.15 mol/l NaCl were mixed in ratio 0.02:1, with 5 μl of heparin (5000 U/ml) were added in advance. The mixture was washed 4 times by 3 min centrifugation at 3000 rpm in 10x volume of 0.15 mol/l NaCl at the room temperature. Leukocyte film and supernatant were eliminated by aspiration. 5 μl of obtained erythrocytes were resuspended in 1.0 ml of 0.15 mol/l NaCl and transferred into 1 cm cuvette. Acidic hemolysis of erythrocytes was used in the research. Hemolysis was induced by HCl with concentration in samples of erythrocytes suspension equal 0.002 n. Under such conditions the optical density of the samples in cuvette was 0.2. Curves of erythrocytes hemolysis were registered by samples' optical density changes, measured at the wavelength λ = 670 nm and continuous careful mixing. Registration of a signal was carried out with a frequency of 1 sec. The indicators of hemolysis were the time of structural rearrangement of the erythrocyte membrane before the beginning of the process of their destruction (*t*) and the rate of its destruction (*v*).

Results and Discussion. It was found that on day 36 in the presence of Cd^{2+} the value of *t* significantly decreases, and *v* – increases in the organism of animals. This additional destabilizing effect of Cd^{2+} on the erythrocytes membranes is greater at a dose of 1, than 0.1 μg/kg of the body weight. The indicators of oxidative stress were contents of 8-isoprostane, carbonylated proteins, and activity of SOD and CAT in the serum. Cd^{2+} induces the development of oxidative stress, as evidenced by an increase of the content of 8-isoprostane, the level of proteins carbonylation, a slight increase of CAT activity and a tendency to increase of SOD activity in serum on the 36^{th} day. As in the case of hemolysis, the degree of expression of these effects is greater at a dose of 1, than 0.1 μg/kg of body weight.

Between the numerical values of 8-isoprostane, carbonylated proteins and the activity of CAT, a negative correlation with *t* and a positive correlation with *v* were found.

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UDC 577. 112

THE ACTIVITY OF GLYCOLYTIC AND ANTIGLYCATIVE ENZYMES IN MOUSE BRAIN UNDER INTERMITTENT FASTING

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Introduction. Metabolic processes in brain occurring under normal food conditions and when food availability is experimentally reduced are usually close as possible. Such effect is not observed for other organs and can be explained by paramount importance of preservation of cognitive functions for survival. However, the effects of intermittent fasting may differ for various age groups. Our goal was to investigate the influence of intermittent fasting on the activity of glycolytic and antiglycative enzymes in order to find out how intermittent fasting influences glucose metabolism and level of carbonyl stress in brain of 12- and 18-month old mice.

Methods. For experiments, the animals were divided into two groups: (i) control – fed ad libitum and (ii) experimental – provided access to food every other day over 6-month period. Water was available ad libitum. The activities of hexokinase, phosphofructokinase, pyruvate kinase, glyoxalase 1 and glyoxalase 2 were measured spectrophotometrically in brain cortex.

Results. Generally, the activity of key glycolytic enzymes was significantly lower or demonstrated tendency to reduction in experimental males and females if compared to respective control values. The effect observed was more pronounced in 18- than 12- month animals. The activity of glyoxalase 1 and 2 was not significantly different in control and experimental animals in both age groups studied.

Discussion. Decrease in the activity of key glycolytic regulatory enzymes in the brain cortex under conditions of limited food availability is suggested to be associated with reduced glucose level, since these enzymes are most sensitive to any changes in glucose level. Despite the fact that glycolytic and antiglycative enzymes are often closely related, the activity of glyoxalases 1 and 2 was virtually the same in all animal groups investigated, suggesting a little effect of intermittent fasting on carbonyl stress markers.

Conclusions. Intermittent fasting affects the brain more strongly than it was supposed, especially in old organisms.

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BIOCHEMICAL PARAMETERS OF BLOOD WITH INCREASING CONCENTRATION OF NITRIC OXIDE

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Introduction. The nitric oxide molecule is the regulator of necessary functions of the organism. It implements intercellular communication and regulation of important physiological functions such as neurotransmission, platelet aggregation, lipid peroxidation processes in the development of inflammatory processes. Information about the effect of NO on the oxidant/antioxidant systems is controversial, therefore this requires detailed study. The purpose of research is to estimate indicators of oxidative stress and markers of organ damage in the blood under conditions of excess of nitric oxide concentration.

Methods. The experiment was conducted on 18 white male rats, who weighed 200-230 g. Animals were randomly divided into three groups (n = 6). Rats of the control group were injected with 1 ml of 0.9% NaCl solution (group I). The increase of NO concentration was caused by a 6- and 12-day intraperitoneal injection of sodium nitroprusside (SNP) at a dose of 1.5 mg/kg (groups II and III). After completion of the simulation we subjected the animals to 18-hour food deprivation with free access to water then anesthetized the rats with a lethal dose of ketamine hydrochloride solution (220 mg/kg) and collected blood.

Results. We determine the intensity of lipid peroxidation processes by the concentration of the secondary lipoperoxidation product – malondialdehyde (MDA) in the serum (Volchegorsky et al., 2002). The content of circulating ceruloplasmin was measured using the modified Revin method (Kamyshnikov, 2002). We studied fibrosis process by concentrations of protein-bound hydroxyproline (Osadchuk et al., 1987). Also we raised activity of pancreatic enzymes: a-amylase and trypsin (Kamyshnikov, 2002).

Discussion. The content of MDA in serum increases by 18% for 6 days and by 92% for 12 days of injected SNP that indicates the development of oxidative stress. At the same time the content of ceruloplasmin increases 1.76 and 1.94 times, that indicates the active work of the antioxidant system. Also, there was a decrease in the total protein content by 12% with a 12-day injection of SNP. The concentration of protein-bound hydroxyproline and activity of pancreatic enzymes increased unreliably.

Conclusion. Increased concentration of NO stimulates the formation of active forms of oxygen and free radicals. But at the same time it has a positive effect on the antioxidant system and activates the synthesis of enzymes that perform the protective function. Therefore internal organs do not undergo a critical effect of oxidative stress when NO synthesis is elevated to a certain extent.

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THE DEVELOPMENT OF NEW METHOD OF THE DETERMINATION OF BACTERIAL TRANSGLUTAMINASE ACTIVITY USING FIBRINOGEN AS A SUBSTRATE

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Introduction. Bacterial transglutaminase (TG) is widely used in food industry, because it is a calcium-independent enzyme. The determination of TG activity is an important issue that is necessary for adequate use. However the control of TG activity on site is complicated. Most of manufactures do not control TG activity during the use that can much affect the efficiency of its application in food industry. That is why the aim of our work was to develop a simple and accurate method of TG activity determination using human fibrinogen as a substrate

Methods. Fibrinogen was purified from human blood plasma. Thrombin (50 NIH/mL was purchased from Sigma (USA). For preparation of polymeric fibrin 0.3 mg/ml of fibrinogen was mixed with 0.5 NIH/ml of thrombin. Samples were incubated at 37 °C during 30 minutes in the presence of TG or equivalent volume of buffer. Cross-linking was detected using SDS-PAGE in the presence of 0.2% mercaptoethanol. The intensity of non cross-linked protein bands was estimated using densitometric software TotalLab TL100. Alternatively polymeric fibrin clot was removed from incubation media and dissolved in 0.125% acetic acid. Optical density of dissolved fibrin was monitored by spectrophotometer POP (Optizen, Korea).

Results and Discussion. SDS-PAGE demonstrated that 0,5 IU/ml of TG can cross link Aα-chain of fibrin. It's fibrin-specific activity was estimated as 5.0 ± 0.6 ug/min at the initial stages. Longer incubation or using of samples of TG with higher activity leaded to cross-linking of all three chains of fibrin. Polymeric fibrin is being cross-linked by TG effectively; however the sites of cross-linking are differed from those known for factor XIIIa. The cross-linking of fibrin by TG is time- and concentration-dependant.

Modified spectrophotometric method allowed us to obtain the calibration curve for the estimation of TG activity in International Unit based on the fibrin-specific activity. Estimation of activity was accurate in the range of concentrations from 0.17 to 0.8 IU/ml. This calibration curve allows estimating the enzymatic activity of commercially available TG.

Conclusions. Polymeric fibrin is useful substrate for estimation of TG activity. The simple and effective method for TG activity was developed and approved.

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REDOX BALANCE IN WHITE RATS' SPLEEN IN THE DYNAMICS OF EXPERIMENTALLY DEVELOPED CARCINOGENESIS

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Introduction. The problem of malignant growth is one of the most relevant in medicine and biology. Despite accomplishments in the learning of the causes and features of oncological disease, the frequency and mortality from them continue to increase. The tumors artificially induced by certain carcinogens in laboratory animals provide an opportunity to explore various aspects of carcinogenesis that cannot be effectively studied directly on the human body. One of them is the dimethylhydrazine model, which is an effective tool for the study of the characteristics of chemically induced carcinogenesis. The aim of the work was to study the changes of oxidation-reduction balance in the spleen tissue under chemically induced carcinogenesis

Methods. The research was conducted on 100 mature white rats with body weight of 185-190 g., kept in standard vivarium conditions. Carcinogenesis was modeled according to the method of V. P. Deryagina (2009). The concentration of TBC-active products, diene and triene conjugates (DC,TC), Schiff base was studied in the homogenate of the spleen; activity of catalase (CAT), Superoxide dismutase (SOD), Glutathione peroxidase (GP), Glutathione reductase (GR) were studied by the methods of Vlaslo V. V. (2012).

Results. Under conditions of induced oncogenesis, a significant 1.2 times increase was detected in DC concentration during the 1st month of administration, 1.4 times – in the 2nd month, 1.6 times – in the 3rd and 4th months, 1.7 times – in the 5th and 6th months; introduction of this substance raises the DC concentration during the 7th month 2.3 times ($P < 0.001$) in comparison with the same indicator of the control group of animals. A similar growth trend was observed during determination of TC concentration under conditions of induced oncogenesis. Throughout 1, 2, 3, 4, 5, 6, 7 months, the concentration of SB significantly increases 1.1; 1.3; 1.5; 1.7; 1.8; 2.02 and 2.2 times, respectively, in comparison with the same indicator in the control group of animals. Under the conditions of experimental carcinogenesis, an increase in the content of TBC-active products in the spleen tissue homogenate was observed at all periods of the study ($P < 0.001$). Under DMH-induced carcinogenesis, the activity of CAT was significantly decreasing in the 1st (3.3 times), the 2nd (3.2 times), the 3rd (3.3 times), the 4th (3.7 times), 5th (4.7 times), 6th (6.4 times) and 7th month of oncoprocess modeling 7.4 times compared to the same indicator in the control animals group. It has been experimentally established that during development of the oncological process, the activity of SOD in the spleen tissue is significantly increasing in the first months of DMH injection, whereas, starting from 5 months, it decreases. The lowest activity of SOD was observed in the 7th month of administration – 2.9 times ($P < 0.001$).

The activity of the GP significantly increases in the 1st (by 14.6%) and the 5th (by 18.1%) month, while it decreases by 66.2% in the 7th month of carcinogen introduction. The activity of GR in the 1st month of observation increased significantly by 16.3%, while in all subsequent periods there was a tendency towards a systematic reduction of GR, with the lowest activity in the 7th month (by 69.1%) compared with the activity of the enzyme in the control group of animals.

Conclusions. In conditions of chemically induced carcinogenesis, the disturbance of the oxidation-reduction equilibrium was established due to the accumulation of products of lipoperoxidation and TBC-reactive substances and reduction of antioxidant enzymes activity.

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FATTY ACIDS COMPOSITION IN BLOOD SERUM LIPIDS OF STERLETS OF DIFFERENT AGE

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Introduction. As the only sturgeon living in freshwater, sterlet is important for breeding in industrial fish farms, where lately cases of early mortality of this species have been detected. Hence, in order to preserve the species, it is important to study the parameters of fatty acid (FA) composition of lipid fractions of blood serum of sterlet.

Materials and Methods. Here we present changes of fatty acid composition in different lipid fractions of blood serum of sterlets of different age (namely two-, three- and nine-year-old) with the masses 0.3-0.4, 0.5-0.6 and 5-6 kg for the age-groups of fish, respectively. Fatty acid (FA) composition was determined using gas-chromatography on HRGC 5300 (Italy) in Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine (NASU).

Results. Fatty acid composition of sterlet blood serum is presented by saturated and unsaturated high-molecular weight carboxylic acids, mostly palmic, stearic, oleic and linoleic. In the phospholipid fraction, there was a moderate increase in saturated and monounsaturated fatty acids and a slight decrease in polyunsaturated fatty acids depending on the age of fish. As for free fatty acids, there was a drop in the saturated ones depending on the sterlet age. Among the free fatty acids of sterlet blood serum, we identified 28 acids, of them 39, 35 and 30% were saturated in 2-, 3-year-old and mature fish, respectively. Monounsaturated FA content was 14, 23 and 23% in 2-, 3-year-old and adult sterlet fish, and polyunsaturated FA content – 46, 41 and 36%, respectively. The data can be used for the theoretical verification of correcting supplementary feed and premixes.

Conclusion. Thus, for the first time, we studied the fatty acid composition of blood serum of sterlet of different age. Thus, according to the experimental results of studies of phospholipids in the blood serum of sterlets, there was a significant increase in the content of saturated and monounsaturated fatty acids and a decrease of the level of polyunsaturated fatty acids depending on fish age. Regarding free fatty acids, it was shown a decrease in saturated fatty acids with age.

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GLUTATHIONE STATUS IN RAT'S LIVER EXPERIMENTALLY INDUCED UNDER INFLUENCE CHROMIUM CITRATE

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Introduction. The trace elements can affect the glucose metabolism and have an effect on the oxidative stress in diabetes mellitus (DM) type 2. Chromium, as a trace element, improves glucose tolerance, plays an important role in metabolism of carbohydrates, proteins, fatty and nucleic acids. Results of the research indicate that adequate chromium intake may be important for the prevention of DM (Martin J. et al., 2006).

The aim of the research was to find out the effect of various amounts of the organic compound of chromium citrate on glutathione status in the liver tissue of rats with alloxan-induced diabetes.

Methods. Rats weighing 100–120 g were divided into 4 groups: I - control, II, III, IV – research. Rats from groups I and II were given pure water without any additives; animals from groups III and IV were given water with the solution of chromium citrate in the amounts of 0.1 and 2.0 mg/ml of water during one month. Experimental diabetes mellitus (EDM) was induced in the animals from groups II, III, IV after a 24-hour fasting period by intraperitoneal administration of 5% solution of alloxan monohydrate in the amount of 150 mg/kg of body weight.

Results. The content of reduced glutathione (GSH) and the activity of glutathione peroxidase (GPx) decreased significantly by 68.04 and 28.43% respectively during EDM. While the activity of glutathione reductase (GR) significantly increased compared to the control group.

GSH and activity of GPx increased significantly in group III by 112.2 and 63.64% and in group IV – by 26.69 and 20.40% compared to the group with EDM under the influence of vanadium citrate.

When chromium citrate was administered into the drinking water of rats, activity of GR decreased significantly in groups III and IV by 29 and 45.56% compared to group II.

Discussion. Induction of DM in group II determines the excessive formation of reactive oxygen species (ROS), which interacting with others compounds, lead to the development of oxidative stress and inhibition of the activity of the glutathione link of antioxidant defense. In particular the decreased content of GSH and activity of GP.

Chromium citrate as an additive stabilizes the activity of antioxidant enzymes. Obviously, chromium, as a mediator of insulin, has the ability to increase glucose uptake by cells and also exacerbates the expression of the synthesis of antioxidant enzymes (Anderson R., 2007).

Conclusions. Administration of chromium citrate helps to restore the balance between the formation of ROS and the activity of antioxidant enzymes. Chromium citrate can be used to prevent the onset of secondary diabetic complications.

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UDC 577.1

MAPPING OF RESIDUES OF FIBRINOGEN αC-REGION CLEAVED BY PROTEASE FROM THE VENOM OF *AGKISTRODON HALYS HALYS*

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Introduction. Fibrin(ogen) αC-region (Aα220-610) is involved in fibrin polymerization, binds platelet GPIIbIIIa receptor, VLDLR of endothelial cells, plasminogen, tPA, α2-antiplasmin, apolipoprotein, etc. Thus preparation of αC-derived polypeptides could be important issue for studying the role of different parts of the region in physiological processes. Being strongly specific to distinct peptide bonds of proteins, proteases are the promising tool for preparation of such fragments that possess the features or preserve the structure of the whole molecule. That is why the aim of our work was to study the action on fibrinogen of the protease purified from the venom of *Agkistrodon halys halys.*

Methods. Hydrolysis products of fibrinogen by protease from the venom of *A. halys halys* were analysed by SDS-PAGE under reducing conditions with further immunoprobing using the mouse monoclonal 1-6B (anti-Aα509-610) and II-5C (anti-Aα20-78) antibody. Polypeptide, generated at initial synthesis was purified using HPLC chromatography (Agilent 1100, USA) on phenyl-functionalized silica gel (250x4.7 mm, 4.3 ml, Dupont Instruments, Corp., USA), filled with ZORBAX SB-Phenyl. Protein was eluted by 2 M (NH4)2SO4 using linear gradient. MALDI-TOF analysis of purified fibrinogen hydrolysis products was performed using a Voyager-DE Pro (Applied Biosystems, USA). Its accurate molecular weight was calculated using Data Explorer 4.0.0.0. For the identification of peptide the trypsinolysis with following MALDI-TOF analysis was performed.

Results and Discussion. SDS-PAGE showed that protease from the venom of A. halys halys cleaved preferentially the Aα-chain of fibrinogen. Western-blot analysis carried out using monoclonal antibodies allowed us to detect the product with apparent molecular weight of 20 kDa that corresponded to the C-terminal part of Aα-chain of fibrinogen molecule. MALDI-TOF analysis of product of initial hydrolysis of fibrinogen by protease allowed detecting that the main peak occurs at 21,12 kDa. According to “Peptide Mass Calculator” this peptide corresponded to fragment Aα414-610 of fibrinogen molecule. This suggestion was confirmed by analyzing the products of tripsinolysis, protein sequence coverage was 94%.

Conclusions. It was shown that protease from the venom of *A. halys halys* cleaves the peptide bond AαK413-L414. Its application allowed us to obtain unique non-physiological product Aα414-610 that represents mainly the C-terminal subdomain of fibrin(ogen) αC-region.

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CHANGES OF THE PERFORMANCE PARAMETERS OF THE HEART OF RATS FOR ARTIFICIAL HYPOBYOSIS

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Introduction. One of the important issues of today is the search for alternative methods of anesthesia. One of these methods is the state of artificial hypobiosis. Mandatory conditions for the creation of an artificial carbon dioxide hypobiosis along with hypoxia and hypothermia are hypercapnia.

One of the most important indicators of the functional state of the heart is the condition of the macro- and micronutrient composition. The purpose of the study was to investigate changes in the elemental composition of the rat heart in the state of artificial hypobiosis compared with the control.

Methods. Experiments were conducted in accordance with the requirements of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, France, 1985), on the general ethical principles of experiments on animals adopted by the First National Congress of Bioethics in Ukraine (2001). In experiments, white non-breeded male rats weighing 180-200 g were used, which were maintained under standard vivarium conditions. The animals were divided into groups: control (intact animals) and experimental group (state of artificial hypobiosis). The number of animals in each group is $n = 8$. Measurement of the content of macro- and micronutrients was carried out using a mass spectrometric ionization method in inductively coupled plasma on an IRIS Interband II XSP device manufactured by Thermo Scientific, USA.

Results. The trial showed a decrease in the calcium content in the heart of rats in the state of artificial hypobiosis. Its decrease in the hypopoietic state is explained by inhibition of the activity of enzymes and slowing down the frequency of muscle contractions. At the same time, in the state of artificial hypobiosis, the content of such elements increases: Potassium, Sodium, Ferum.

Conclusions. The study of the role of the micro-macronutrient composition is necessary in order to understand the ways of adapting mammals to low temperatures, as well as finding ways to support long-term and safe hypobiosis. Other macro- and trace elements do not change in conditions of hypobiosis, which is quite positive, since this condition does not cause a significant change in the homeostasis of a living organism.

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ADAPTOR PROTEIN RuK/CIN85 INDUCES EPITHELIAL-TO-MESENCHYMAL TRANSITION IN HUMAN A549 LUNG ADENOCARCINOMA CELLS

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Introduction. Due to its aggressiveness, non-small cell lung cancer (NSCLC) remains the major cause of cancer-related mortality through the world. The reversible process of epithelial-to-mesenchymal transition (EMT) is responsible for increased migration and invasiveness of cancer cells and is also important in metastasis. Interplay between extracellular signals and consequent modulation of receptor-mediated signaling networks provides a fine control of morphofunctional changes associated with EMT. Cells undergoing EMT lose expression of epithelial markers (e.g., E-cadherin) and gain expression of mesenchymal markers (e.g., vimentin) through differential expression and activation of transcription factors including Twist1, ZEB1, ZEB2 and Snail. The pro-oncogenic adaptor protein is a multi-modular scaffold protein that regulates spatiotemporal organization of supramolecular signaling complexes involved in the control of cell proliferation, migration, invasion and metastasis. In the present work, human A549 lung adenocarcinoma cell line was used as a model of NSCLC to study the role of Ruk/CIN85 in EMT *in vitro.*

Methods. Sublines of A549 cells overexpressing Ruk/CIN85 were obtained by transfection with pRc/CMVRuk1 plasmid. Ruk/CIN85 expression in A549 cells was suppressed by infection with lentivirus encoding Ruk/CIN85-specific shRNA. Stable transfectants/infectants were obtained by selection in the presence of specific drugs, G418 and puromycin, respectively. Expression levels of Ruk/CIN85 in modified cells were determined by both Western-blot analysis and qRT-PCR. Proliferation was evaluated by direct cell counting with trypan blue and MTT test. Profiling of EMT-related transcription factors mRNAs expression was studied using qRT-PCR.

Results. At first, A549 cells sublines with different Ruk/CIN85 expression levels were generated. It was demonstrated that cells with Ruk/CIN85 overexpression proliferate faster than control cells; on the contrary Ruk/CIN85 suppression led to a decrease in cell proliferation. There was a clear correlation between the level of Ruk/CIN85 expression and morphological changes in obtained sublines. Cells with Ruk/CIN85 up-regulation acquired the features inherent of the mesenchymal phenotype whereas adaptor protein suppression resulted in epithelial phenotype. The results of qRT-PCR analysis showed that Ruk/CIN85 overexpression in A549 cells led to the 10-fold increase in the expression levels of Twist1 and Zeb2 mRNAs. At the same time, Ruk/CIN85 knockdown resulted in a decrease of Zeb2 and vimentin mRNAs and simultaneous increase of Snail1 mRNA level.

Conclusions. The obtained results pointed to the potential regulatory role of Ruk/CIN85 in mechanisms underlying EMT in NSCLC.

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UDC 577. 34

SIGNAL FUNCTION OF ENDOGENOUS HYDROGEN PEROXIDE IN RESPONSE OF PLANTS TO ULTRAVIOLET RADIATION

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Introduction. Endogenous hydrogen peroxide (HP) is the most common and long-lived form of active oxygen and a signal molecule in plant cells. This allows it to be used to evaluate the stress response of mesophyll cells to ultraviolet radiation, that are the main targets of its action. The purpose of our research was to study the dynamics of endogenous HP content in pea (*Pisum sativum* L.) and corn (*Zea mays* L.) leaves due to the effects of chronic irradiation by ultraviolet B (UV-B) radiation.

Methods. Young corn and pea plants with two mature leaves were irradiated with UV-B at dose of 2 and 6 kJ/m^2 a day with power of 1 W/m2 during 12 days. Control plants were protected by glass filter from the UV-B radiation influence. Endogenous HP content was measured in leaves by sulfate-titanium method.

Results. It is established that the effect of chronic UV-B irradiation of pea plants at a dose 2 kJ/m2 a day decreased endogenous HP content slightly at the first day of experiment and increased at the second day and then stabilized. Analyses of HP level in corn plants did not detect significant changes in the HP content at doses 2 kJ/m^2 during the entire period of UV-B treatment. Chronic irradiation of pea and corn plants with UV-B dose 6 kJ/m^2 a day caused a double increase in the endogenous HP content on the 3rd and 6-8th days of action. During the 4-5th days of chronic UV-B irradiation exposure at a dose 6 kJ/m^2 a day HP level decreased.

Discussion. The irradiation dose of 2 kJ/m^2 per day corresponds to the natural UV-B level for temperate climate territories. Enhanced sensitivity of pea plants to the UV-B radiation is increased by the horizontal orientation of the leaf blades and opened growth point, that caused fluctuations of endogenous HP content in the mesophyll cells and followed by its stabilization. Corn leaves cells were not susceptible to the low dose of ultraviolet radiation, that could be caused by their vertical orientation and closed growth point. Chronic irradiation of pea and corn plants with UV-B dose of 6 kJ/m^2 per day was stressful as revealed by the sharp changes in the endogenous HP content. The changes in the endogenous HP concentration indicate the changes of cells metabolism and can be a stress marker.

Conclusions. It is shown that the effect of chronic UV-B radiation on pea and corn plants caused changes in the endogenous HP level that is a sensitive indicator for diagnosing the plant stress response to ultraviolet radiation as obligate component of solar radiation.

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THE COMBINATION OF NO DONOR AND FERULIC ACID EFFECT ON THE ELICITATION OF WHEAT TOLERANCE AGAINST BIOTIC STRESS

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Introduction. One of the alternative ways to protect agricultural plants from crop losses caused by fungal infections is the usage of biotic elicitors. Nitric oxide NO as a signal molecule plays an important role in plant responses to biotic stress. The aim is to research the ability of enhancing ferulic acid (FA) effect as a biotic elicitor to induce tolerance of wheat plants against fungal diseases by addition donor of NO signal molecule.

Methods. The content of endogenous hydrogen peroxide (HP) was measured in leaves by sulfate-titanium method. Winter wheat plants (cv. Oberig myronivskij and Svytanok myronivskij) were inoculated by *Septoria tritici* Rob et Desm. leaf blotch infection at booting phase three days after 0.1 mM solution of FA and 0.5 mM solution of sodium nitroprusside as donor NO treatment. The powdery mildew caused by *Erysiphe graminis* f.sp. *tritici* DS Em. Marchal was detected in field trials. The extent of disease development according to Saari-Prescott scale, morphometric parameters and yield structure were analyzed.

Results. It is shown that combination of treatment by FA with NO donor reduced the disease symptoms by 1-3 points, decreased the HP content under biotic stress in wheat plants by 70%. The processes of morphogenesis were stimulated and yield increased by 15-25%.

Discussion. Hydrogen peroxide (HP) is a link in the plant protection system and is used as a stress level indicator. During the fungal and plant development, their metabolisms counteract and FA could stimulate the plant cell wall building acting as a source. That process needs HP for peroxidase enzyme. Nitric oxide also influences HP content. So tolerance to fungal diseases in wheat could be enhanced by combination with donor NO via control of HP content. The reaction of wheat plants depended on genetic characteristics of different cultivars and nutrition type of phytopathogens.

Conclusions. The data obtained suggest that combination of FA with donor NO could be used as more effective combination than biotic elicitor. They decreased the degree of lesions in leaf area caused by leaf blotch and powdery mildew infection and stimulated the increasing of yield.

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ADAPTOR PROTEIN Ruk/CIN85 IS A NOVEL MOLECULAR COMPONENT OF EXTRACELLULAR VESICLES PRODUCED BY TUMOR CELLS

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Introduction. Nanosized membrane vesicles, termed "exosomes", are secreted by most cell types under both physiological and pathological conditions, especially by tumor cells, and were proposed to be actively involved in intercellular signaling (Yang & Robbins, 2011). There are data that proteins Alix and Tsg101 (Schmidt et al., 2005; Segura-Morales et al., 2005) involved in the formation of MVB (multivesicular bodies) and currently recognized as marker proteins of exosomes, as well as cortactin, which stimulates the secretion of exosomes (Lynch et al., 2003; Sinha et al., 2016), are the binding partners of adaptor protein Ruk/CIN85. In addition, we have shown previously that up-regulation of the adaptor protein Ruk/CIN85 in breast cancer cells is involved in the stabilization of the transcription factor HIF1α (Samoylenko et al., 2010). Taking into account these data, the main aim of our study was to elucidate the role of Ruk/CIN85 in biogenesis of extracellular membrane vesicles (EVs) produced by tumor cells and assess the influence of hypoxia conditions on this process.

Methods. Renca cells (mouse renal cell carcinoma) were cultured under standard conditions. The hypoxic environment was created by incubating the cells in a standard 5% CO_2 incubator infused with N2 to create a constant 1% O_2 environment. Normoxia was defined as 21% O_2 environment supplemented with 10% CO_2. To obtain Ruk/CIN85-overexpressing cells, Renca cells were transfected with pRc/CMV2-Rukl plasmid encoding the full-length form of Ruk/CIN85 or empty vector using Lipofectamine 2000 reagent followed by selection of stable transfectants in the presence of Geneticin. EVs were isolated by concentration of conditioned medium with Centricon Plus-70 followed by ultracentrifugation at 100 000 g. The number and size of EVs were assessed using NanoSight device and morphology – by electron microscopy. The protein content of EVs was studied by Western-blot analysis.

Results. To achieve the main aim of our work, we created subline of Renca cells stably overexpressing the adaptor protein Ruk/CIN85. Using Western-blot analysis of whole cell lysates, it was demonstrated that up-regulation of Ruk/CIN85 in Renca cells results in a significant increase in the expression level of Alix protein. Importantly, Ruk/CIN85 and Alix expression levels were decreased under hypoxic conditions in both control and Ruk/CIN85-overexpressing cells. Higher content of Ruk/CIN85, concomitantly with marker proteins Alix and CD81, was detected in EVs preparations isolated from conditioned medium of Ruk/CIN85-overexpressing cells in comparison with control ones. Under hypoxic conditions, increased levels of Ruk/CIN85 and CD81 were observed in EVs produced by control cells while decreased levels - in EVs produced by Ruk/CIN85-overexpressing cells. At the same time, hypoxia caused decrease of Alix protein content in EVs from both cell types.

Conclusions. It was demonstrated that the adaptor protein Ruk/CIN85 is a newly identified component of exosomes produced by tumor cells. The potential role of Ruk/CIN85 in the control of protein composition of exosomes under conditions of normoxia and hypoxia was established.

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