YOUNG SCIENTISTS CONFERENCE MODERN ASPECTS OF BIOCHEMISTRY AND BIOTECHNOLOGY – 2019

Palladin Institute of Biochemistry, National Academy of Sciences of Ukraine
21-22 March, 2019, Kyiv, Ukraine

On March 21-22, 2019 the regular annual young scientists Conference "*Modern Aspects of Biochemistry and Biotechnology*" was successfully held in Palladin Institute of Biochemistry. The Conference was organized by the Young Scientists Council of Palladin Institute of Biochemistry with the support of ALT Ukraine Ltd –advanced laboratory technologies Company. Young scientists from Kyiv, Dnipro, Kharkiv, Chernivtsi, Ternopil, Poltava took part in the Conference as oral speakers. The scientific program of the Conference included the following sections: Translational Studies; Biochemistry; Biotechnology; Molecular Biology; Medical Biochemistry; Biochemical mechanisms of resistance to adverse environmental conditions. The workshops devoted to computer analysis of biological images; methods of biological experimental data statistical analysis; quantitative polymerase chain reaction for gene expression estimation; spectrofluorometry as a rainbow force for biochemists service were organized to broaden the research skills of young scientists.

Conference was opened by the new section 'Translational Studies'. So many reports were focused on the efforts to build on basic scientific research to create new therapies, medical procedures, or diagnostics. The members of Scientific Committee specially acknowledged those young scientists who presented data about long way from idea, it's *in vitro* approval to the *in vivo* testing and application.

The honorary awards for the best oral presentation were given to *Anna Myronova* ("CRISPRa-mediated direct cardiac reprogramming of embryonic rat fibroblasts"), Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv; *Yevgen Stohnii* ("Epitope determination of novel fibrinogen-specific antibody by limited proteolysis"), Palladin Institute of Biochemistry, NAS of Ukraine, Kyiv; *Anton Tkachenko* ("Fascin is upregulated in nasal mucosa in chronic rhinosinusitis with nasal polyps"), Kharkiv National Medical University, Kharkiv.

The honourable mention prizes for interesting scientific reports were presented to *Vira Borshchovetska* (Yuriy Fedkovych Chernivtsi National University, Chernivtsi), *Olga Revka* and *Valerija Zhovannyk* (Palladin Institute of Biochemistry, Kyiv), *Maxym Skrypnyk* (Ukrainian medical stomatological academy, Poltava).

The meeting was held in a creative and friendly atmosphere with constructive and helpful discussions. The abstracts of the oral presentations of participants will be published in the "*Ukrainian Biochemical Journal*".

The Head of the Conference Competition Commission, D. Sc., prof. Olga MATYSHEVSKA
The Head of the Young Scientists Council of Palladin Institute of Biochemistry, PhD Tetjana JATSENKO

TRANSLATIONAL STUDIES

UDC 612.015:616-003.231:616-056.52]-053.81-07

CHARACTERISTIC OF SOME OXIDATIVE STRESS ALTERATION MARKERS IN SALIVA OF YOUNG PATIENTS WITH OBESITY

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Introduction. Obesity is a global problem for public health. Obesity has become common among young people. Overweight has a strong liaison with chronic systemic inflammation and can initiate development of obesity-linked diseases like type 2 diabetes, cardiovascular diseases, dyslipidemia, and metabolic syndrome. Some links between obesity and oral health condition, especially periodontitis, were observed but the mechanism of pathological changes development is still unclear.

Methods. 154 participants of young age (18-22 years) and of both genders were divided into 4 groups – depending of their body mass index (1 – to 24.99 kg/cm^2, 2 – 25-29.99 kg/cm^2, 3 – 30-34.99 kg/cm^2, 4 – 35 and more). All patients underwent a clinical examination of the oral cavity and some anthropometrical measures were done. Samples of saliva were collected too. In saliva, we researched the content of oxidation-modified proteins and TBA-active products.

Results. In patients of the 4th group, periodontal diseases were observed in 88.3% persons, that is much higher than in other groups and the prevalence of generalized forms of diseases was the highest – 75% out of all patients with periodontal pathology. Only in the 4th group, 3 cases of chronic generalized periodontitis were observed, that is not typical for young patients that have effective mechanisms of adaptation. The prevalence of periodontal diseases in the 3th group was 82.7%, in the 2nd group – 74.4% and in the 1st group 52.8%. The level of oral hygiene strongly correlate with periodontal disease prevalence and was the worst in the 4th group.

The content of oxidation-modified proteins in the saliva of patients of the 3th and 4th group was by 1.2 times higher than in patients with normal weight where this value was 0.32 ± 0.01 mg/kg. The content of TBA-active products in saliva was the biggest in patients of the 3th group (BMI 30-34.99 kg/cm^2) 55.11 ± 3.87 mg/kg that attests the dominating of free radical alteration processes in salivary glands of patients with obesity. In other groups, content of TBA – active products was significantly lower by 1.5 times compared with patients with normal BMI, and by 1.2 lower compare with patients with BMI 25-29.99 kg/cm^2.

Discussion. Thus, in patients with obesity, we observed activation of oxidative stress alteration processes in salivary glands, that are very sensitive to internal and external changes. There was a strong correlation between BMI and oxidative stress markers – oxidation-modified proteins and TBA-active products.

Conclusions. The obtaining results showed that patients with overweight and obesity are in risk group of main diseases of the oral cavity, especially periodontal diseases, and have to receive preventive action and special medicament and non-medicament supply.

UDC 576.52

THE APPLICATION OF MESENCHYMAL STEM CELLS IN DEVELOPMENT OF A NEW GENERATION GRAFTS FOR THE BONE TISSUE DAMAGE THERAPY

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Introduction. In recent decades there has been a tendency to increase the number of diseases, as well as injuries, of the musculoskeletal system, which requires the development of effective methods for the bone tissue reconstruction. A combination of modern achievements in cellular engineering and materials science provides an opportunity to create bone implants that have all biological characteristics that would ensure complete regeneration of injured bone tissue. The new generation grafts use bioactive ceramics as a scaffold, which is an analogue of human bone tissue, and osteogenic cells to form a bone matrix. MSCs are prospective candidates for those kind cell-based therapies because of their regenerative characteristics and ability to differentiate into osteoblasts. The review of the publications about MSCs has showed that bone marrow-derived MSCs (BMSCs) and adipose tissue-derived MSCs (ASCs) have no significant difference in morphology, expression patterns of surface markers and proliferative potential, that's why we concentrated on ASCs as they are easily accessible, available, and abundant source of autologous osteogenic cells. Our main goal is to optimize the isolation of MSC from adipose tissue and select an appropriate type of bioceramic scaffold for further cultivation with MSCs.

Methods. MSCs isolation from rat's adipose tissue. ASCs cultivation in osteogenic medium with bioactive ceramics granules: Biomin GTlC-500, Biomin GTG-1, Biomin T-500 at 37 °C, 5% CO_2. Metabolic activity of MSCs was determined by the MTT test. Flow Cytometry (FACS) Analysis of expression of positive markers CD105, CD73, CD90 and negative CD34, CD 45, CD31, CD145, CD114 that are known to be expressed by ASCs and BMSCs. Quantitation of osteopontin, osteonectin, alkaline phosphatase, main osteogenic markers, mRNA levels by real-time PCR.

Results. ASCs demonstrate high level of osteogenic differentiation in special culture condition with bioactive ceramics granules that are not toxic to the cells: Biomin GTG-1 (size of granules 0.8–1 mm and consists of β-tricalcium phosphate and hydroxyapatite), Biomin T-500 (size of granules 0.4–0.6 mm and consists of β-tricalcium phosphate), analysis of the cell proliferation showed that only GTG-1 and T-500 doesn't limit cells growth. Our data confirm the presence of a common set of CD markers expressed in stromal cells from adipose tissue e.g. CD90, CD73 (>95%), CD105 (<2%). MSC's are negative for hematopoietic cell markers: CD45, CD34 (<0.2%). After 3 weeks of osteogenic differentiation with bioactive ceramics, qRT-PCR revealed an upregulation of osteogenic markers osteopontin, osteonectin, alkaline phosphatase for ASCs. ASCs showed extracellular calcium depositions visualized by Alizarin red staining, compared with control cells.

Conclusion. Adipose tissue-derived MSCs demonstrate a high potential for their use in bone grafts engineering. Our results showed that bioactive ceramics enhance ASC's osteogenic differentiation, so "T-500", "GTG-1" can be used as a new biocompatible scaffold for bone regeneration.

UDC 616-092.9

BIOCHEMICAL METHOD OF ORGANISM SUSCEPTIBILITY ASSESSMENT

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Introduction. Susceptibility Biomarkers are key tools and can provide crucial information on the complex cascade of events and molecular mechanisms underlying diseases and disorders development. One of the known biomarkers of susceptibility to chemical hazards (Williams R., 2011) and disease development (Kita T., 2007) is genetically determined activity N-acetyltransferase (NAT). However, genetic analysis is too expensive for screening and monitoring. Besides, it is shown that NAT activity could change during the lifespan due to different factor influence (Kachula S.O., 2004). Thus, the overall goal of the study was to analyze the biochemical method for the assessment of NAT activity as a susceptibility biomarker.

Methods. Experiment was done in 108 white male rats, using authors' modification of the loading test with amidopyrine. NAT phenotype (as "rapid" and "slow" acetylation type) was determined based on the rate of 4-amino-antipyrine and N-acetyl-4-aminoanthypirine excretion with urine.

Results. 60% (65) of rats belonged to "rapid", 38% (41) to "slow acetylation" type. Data of 2 rats were intermediate. The received percentages correspond to literature data concerning average rates of acetylation genotype in population.

Discussion. Evidence indicates that inherited and acquired genetic susceptibility, epigenetic modifications as well as alterations in physiological structures and functions induced by age, pathological conditions, and lifestyle factors, may lead to different phenotypic expressions from xenobiotic exposures (Ivo Iavicoli, 2016). Horvath and Hannum Epigenetic clocks show DNA methylation age as an integrated and truly universal epigenetic biomarker of aging for lifespan and healthspan (S. Horvath, 2018). In this context, the link with a set of biochemical susceptibility markers such as methyltransferases and acetyltransferases became clear. Most of the authors used to believe, that pathogenesis of that connected with the participation of NAT as a key enzyme in the chain of detoxication mechanism. However, considering data above, a participation of NAT in the process of DNA methylation considered to be an important pathogenetical chain as well. This fact would also explain linking the activity level of NAT to development of diseases (bronchial asthma (Koloskova, 2005)) and intoxications (nitrates (Korotun O., Vlasyk L., 2012) and so on), which are not pathogenically directly connected to acetylation.

Conclusions. Biochemical assessment of acetylation and methylation activity level could be used as an inexpensive, non-invasive and relevant index of organism susceptibility. Risk of a disease development, lifespan and healthspan in genetically susceptible population group can be modified by behavioral and lifestyle factors. The scientific studies of susceptibility biomarkers should include phenotype research to reach proper validation and qualification.

UDC577.161.22

NEUROTOXIC EFFECTS OF GLUCOCORTICOIDS ARE AMELIORATED BY VITAMIN D_3 TREATMENT

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Introduction. Glucocorticoid(GC)-induced neurotoxicity is a result of a long-term corticosteroids administration. Nevertheless, the molecular mechanisms underlying GC-induced brain injury and ways to prevent side effects of GC treatment remain unclear. We assumed that vitamin D_3, a para- and autocrine neurosteroid, might play an important role in protecting the brain from GC-induced damage.

Methods. Female Wistar rats were given synthetic GC prednisolone at a dose of 5 mg/kg of b.w. during 30 days with and without 100 IU of vitamin D_3. Morphopathological changes in the hippocampus were visualized using Toluidine Blue staining. Levels of caspase-3, 3-nitrotyrosine, nuclear factor κB (NF-κB), glucocorticoid receptor (GR) and vitamin D receptor (VDR) were measured by Western blotting. The 25OHD level was detected by ELISA.

Results. Prednisolone administration caused significant changes in hippocampal neurons compared to the control animal group, including an increase in perikaryon areas and a decrease in cell density of CA1-CA3 fields. In contrast, co-administration of vitamin D_3 led to a decrease in hippocampal perikaryon and nucleus mean areas compared to prednisolone group. In addition, elevated protein levels of caspase-3, 3-nitrotyrosine, NF-κB, GR and VDR were observed after prednisolone administration. Blood serum 25OHD level was shown to be reduced (by 3-fold), indicative of severe vitamin D-deficiency. Vitamin D_3 treatment partially normalized the indicators of oxidative stress and cell death.

Discussion. GCs induced compensatory response of vitamin D endocrine system, which appears in elevated VDR level. Surprisingly, the GR level was also increased, and it may be explained by the impairment of GR signaling. The levels of caspase-3, a representative cell death marker, and 3-nitrotyrosine, an oxidative stress indicator, were reduced after vitamin D_3 administration.

Conclusions. Prednisolone-elicited brain damage include changes at histological, molecular and biochemical levels. GC-induced brain injury can be partially ameliorated by vitamin D_3 treatment.

BIOCHEMISTRY

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SEROTONIN PATHWAY IN RAT BRAINS UNDER LONG-TERM PROGESTERONE ADMINISTRATION

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Introduction. The need for food and the maintenance of the balance between consumption and energy storage are found under the control of a number of nerve cells. Serotonin plays a significant role in these processes. Serotonin is synthesized in the central nervous system and in enterochromaffin cells of the intestinal epithelium. Serotonin is formed from the amino acid L-tryptophan, due to 5-hydroxylation of tryptophan, with the formation of 5-hydroxytryptophan and rapid decarboxylation of to serotonin.

It is known that the activity of serotonin can be affected by the female sex hormone progesterone. Progesterone causes an increase in the amount of adipose tissue during pregnancy. In addition, it has been shown that when passing hormone substitution therapy or long-term use of contraceptives containing progesterone, body mass significantly increases due to the accumulation of fat. Therefore, the purpose of this study was to investigate the effect of long-term administration of progesterone on the state of functioning of the pathway of biosynthesis of serotonin.

Methods. The present study used white nonlinear female rats weighing 165 ± 10 g at the beginning of the experiment. Development of experimental obesity in experimental animals was performed by subcutaneous administration of an oily solution of progesterone at a rate of 10 mg per 1 kg of body weight for 28 days. After that, the concentrations of tryptophan, 5-hydroxy-tryptophan, serotonin and activity of enzymes tryptophan-hydroxylase, tryptophan-decarboxylase in the homogenate of rat brain were measured.

Results and Discussion. It has been established that the concentration of tryptophan in the progesterone group of rats increased by 2.16 times in comparison with the control group. The first stage of the biosynthesis of serotonin is the hydroxylation of tryptophan to form an intermediate metabolite - 5-hydroxytryptophan. Tryptophan-hydroxylase activity decrease by 25% in the group of rats under long-term progesterone administration in comparison with the control group. The concentration of 5-hydroxytryptophan increased by 2.09 times in the progesterone group. According to the results, tryptophan-decarboxylase activity increased and amounted 170% of this activity in the control group of rats. The concentration of product of tryptophan-decarboxylase activity – serotonin increased by 3.25 times in progesterone group of rats.

Conclusions. Thus, as a result of our studies, we found an imbalance in the system of serotonin metabolism in the brain of rats with the development of hormonal obesity induced by prolonged administration of progesterone, which may indicate the involvement of the serotonergic neurotransmitter system in the mechanisms of the development of obesity and concomitant diseases.

UDC 577.124:616.61]:616.379 – 008.64 – 085.357:612.017.2

INFLUENCE OF MELATONIN ON ACTIVITY OF MAIN ENZYMES OF CORY CYCLE IN HEART AND KIDNEY OF RATS WITH ALLOXAN DIABETES

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Introduction. The aim was to determine the influence of melatonin on basal levels of glucose (BG) in blood, activities of glucose-6-phosphatase (G-6-Ph, EC 3.1.3.9) in kidney, pyruvate kinase (PK, EC 2.7.1.40) in the heart of alloxan diabetic rats.

Methods. Diabetes was induced in male Wistar rats by single i.p. injection of alloxan (170 mg/kg). Four days after diabetes induction, rats were divided into diabetic (untreated) and melatonin-diabetic group (10 mg/kg Sigma, USA, daily and orally for 42 days starting from 5th day). Rats with diabetes mellitus (DM1T) characterize by BG ≥ 8.0 mmol/l. Animals were sacrificed at 49th day from the beginning of the experiment in accordance with the ethical treatment of animals. The heart muscle tissue and kidney were quickly removed, rinsed in saline, blotted, weighed and homogenized. Determinations of the enzymes activities were by standard methods. Statistical analysis was performed using Statistica 10 StatSoft Inc.

Results. Melatonin injections caused a sharp decrease by 78% (on 49th day) means normalization in the elevated serum glucose level in DM1T group of rats compared with BG level before treatment. Accordance to the results obtained in the kidney of diabetic rats the activity of G-6-Ph was increased in average by 155% compared with control value.

We have established a reduction of PK activity in the heart muscle of diabetic animals on average by 50% compared with the control. According to our research, 6 weeks daily administration of melatonin to diabetic rats at 10 mg/kg of b.w. resulted in normalization of PK and G-6-Ph activities.

Discussion. It may be that the lack of melatonin can causes impairment in glucose utilization. It was detected, that melatonin stimulates glucose transport to skeletal muscle cells via insulin receptor substrate-1/phosphoinositide 3-kinase (IRS-1/PI-3-kinase) pathway, which implies, at the molecular level, its role in glucose homeostasis and possibly in diabetes.

It is logical that the activity of PK is reduced during DM1T, whether an administration of melatonin leads to increased its activity, probably due to direct activation of gene expression of glucose transporter GLUT 4, 2, 1 Reduced activity of G-6-Ph in diabetic rats under melatonin action is probably due to the fact that melatonin is in the physiological counteraction with cortisone and catecholamines.

A possible link between melatonin and insulin interaction may be in its protective effect against free radical attack of β-cells Langerhans islets in pancreas.

Conclusions. These findings suggest that melatonin reverses the catabolic consequences of total lack of insulin, potentially by decreasing of basal glucose level in the blood, activating of pyruvate kinase activity in heart and suppressing of glucose-6-phosphatase activity in the kidney of alloxan diabetic rats. This restores the events in the Cori cycle by possible activation of glycolysis in heart muscles and normalization of gluconeogenesis kidneys.

UDC 577.29

NOVEL INHIBITOR OF PLATELETS AGGREGATION FROM *Bitis arietans* VENOM

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Introduction. Inhibition of platelet aggregation may provide an effective approach for prevention of coronary artery thrombosis and rethrombosis in the genesis of acute myocardial infarction. The source of platelets aggregation inhibitors can be the snake venoms. Thus the objective of our work was the analysis of platelet inhibitory activity of chromatographically separated fractions of *Bitis arietans* venom.

Methods. Venom of *Bitis arietans* was fractionated using Q-sepharose ion-exchange chromatography followed by size-exclusion chromatography on Superdex G75 using the FPLC system (ÄKTA, GE Healthcare, USA). Analysis of the molecular weight of protein components was performed using SDS-PAGE. Determination of molecular weights of major proteins in purified preparation was performed using MALDI-TOF analysis on Voyager-DE (Applied Biosystems, USA). Aggregation of platelet-rich plasma (PRP) in the presence of platelet aggregation inhibitor was investigated using aggregometry on the AR-2110 (Solar, Belarussia).

Results. The fractionation of the whole venom of *Bitis arietans* on Q-sepharose allowed obtaining four fractions with different protein content: non-bound fraction and fractions eluted at 0.1 M, 0.2 M and 0.5 M NaCl. Screening of obtained fractions allowed us to detect two fibrinogen-specific proteases in the fraction eluted at 0.2 M NaCl. This fraction also possessed the inhibitory action on platelet aggregation; however, it did not depend on the concentration and was a consequence of proteolytic action on fibrinogen that is needed for platelet aggregation. In the same time, non-bound fraction contained specific compound that in a concentration dependent manner inhibited ADP-induced platelet aggregation. Accordin to MALDI-TOF analysis, the fraction contained two polypeptides with molecular weight 9.0 and 13.67 kDa that was in accordance to the existing reports about platelet aggregation inhibitors from snake venoms.

Discussion. Apparent IC_{50} of platelet aggregation inhibitor can be estimated as 60 μg/ml. The pre-incubation of platelet rich plasma with platelet aggregation inhibitor increased inhibitory action in a time-dependent manner and enriched maximal rate after 10 min of pre-incubation. Further incubation did not affect platelet aggregation more. Thus we can conclude that platelet aggregation inhibitor from the venom of *Bitis arietan*s can bind platelets in a reverse manner.

Conclusion. Novel inhibitor of platelets aggregation was found in the venom of *Bitis arietans*. Active fraction contained two polypeptides with molecular weight 9.0 and 13.67 kDa and effectively inhibited platelet aggregation induced by ADP. These polypeptides can be assumed as the prospective molecular platform for the development of new antithrombotic medications.

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LIPID PROFILE OF BLOOD SERUM IN MICE UNDER THE CONDITIONS OF BISPHENOL A ADMINISTRATION AND VITAMIN A OVERCONSUMPTION

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Introduction. Bisphenol A (BPA), one of the highest volume chemicals produced worldwide, is an endocrine disruptor that is extremely prevalent in our environment. Many epidemiological studies show that BPA exposure could cause various adverse health problems related to metabolic disorders in humans, including obesity, insulin resistance, type 2 diabetes, hepatic injury, dyslipidemia and cardiovascular diseases. Retinoids are involved in a wide range of biological processes, through binding and activation of nuclear receptors: RAR and RXR. RAR/RXR-mediated signaling has been implicated in the regulation of glucose and lipid metabolism. That's why the purpose of the study was to determine the lipid profile blood serum and glucose tolerance in mice under the conditions of bisphenol A administration and vitamin A overconsumption.

Methods. The experimental animals were C57BL/6J mice (wild type, WT) of 2.5-3 month age, with a body mass of 20-25 g. Bisphenol A (BPA), dissolved in corn oil (used as a vehicle), was administered *per os* daily for 3 days at a dose of 50 mg/kg body weight. Vitamin A overconsumption was modeled by gavage of retinyl acetate (Rac) in a very high dose of 3000 IU at 12 h intervals for 3 days. The glucose tolerance of the mice was examined at 24 h after BPA administration. The estimation of lipid profiles including total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol, and HDL-cholesterol, were conducted by enzymatic colorimetric methods using commercial kits.

Results. In the present study, we observed dyslipidemia in the mice received 50 mg/kg BPA represented by significant higher triglycerides (at 1.4-fold), total cholesterol (at 2.2-fold), LDL-C, VLDL-C and HDL-C (46%, 39% and 2.8-fold respectively) than those of group mice received vehicle. Administration of BPA also resulted in disruptions of glucose homeostasis, consisting in hyperglycemia (11 ± 1.11 mmol/l) and glucose intolerance of animals. The administration of pharmacological doses of retinoids leads to increases examined indicators in mice treated with BPA.

Discussion. In the present study, we demonstrate that BPA exposure in mice results in disruptions of glucose homeostasis, consisting in hyperglycemia and glucose intolerance. In addition, BPA exposure exerts disruption of lipid metabolism, mainly in cholesterol biosynthesis, associated with hypercholesterolemia and hyperlipidemia. These BPA's actions were attributed to its ability to bind to nonclassical membrane estrogen receptor as well as the G-protein coupled-receptor 30 (GPR30) and to act through nongenomic pathways. Retinoids exposure at the same time with BPA enhanced the lipogenic effect of this xenobiotic by activation the LXR regulated genes, which are known to be responsible for lipid metabolism.

Conclusions. BPA exposure results in metabolic disorders consisting in hyperglycemia, glucose intolerance, hypercholesterolemia and hyperlipidemia. Retinoids enhanced the BPA action as an obesogen.

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IP_3RS IN THE NUCLEAR ENVELOPE - AN ELECTROPHYSIOLOGICAL AND IMMUNOHISTOCHEMICAL CONFIRMATION

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Introduction. There are three IP_3Rs isoforms that differ in biophysical properties such as affinity for IP_3 and sensitivity to modulating factors. The quantitative ratio between RyRs and IP_3Rs in ventricular cardiomyocytes is 100:1 (Moschella et al., 1993) but a significant part of IP3Rs are located in the nuclear membrane or the area around the nucleus (Bare et al., 2005), while specific ryanodine binding level to the nuclear membrane is very low (Guihard et al., 1997). Such a specific arrangement of IP_3Rs potentially enables the generation of autonomous nuclear Ca^{2+}-signals that can play a key role in gene transcription regulation (Marchenko et al., 2006). The aim of this study was to investigate the expression and electrophysiological properties of IP_3Rs in the nuclear envelope.

Methods. The experiments were performed on 3-weeks old rats (Wistar and Fisher). Cardiomyocytes and Purkinje neuronal nuclei isolation were carried out as described earlier (Marchenko et al., 2005; Kotyk et al., 2018). IP_3R subtypes identification in the nuclear membrane was carried out by immunohistochemical staining using specific polyclonal antibodies. Ion current registration through the nuclear membrane was performed using patch clamp technique in the nucleus attached or excised patch configuration. The obtained results were processed mathematically and statistically using Clampfit and Origin software.

Results and Discussion. We have registered ion currents through the native nuclear membrane of cardiomyocytes, which were activated by IP_3 (0.1-20 μM) and inhibited by 2-APB (50 μM). Similar to IP_3R1 previously recorded in Purkinje neurons nuclear membrane (Marchenko et al., 2005; Fedorenko et al., 2014), their Po was potential-dependent. In both objects higher activity of IP_3Rs was observed at positive potential values, while at negative values their activity decreased. IP_3R activity depended on IP_3 concentration and reached its maximum value at 2 μM of IP_3 in the medium for IP_3Rs in Purkinje neurons nuclear membrane (Marchenko et al., 2005) and 10 μM of IP_3 for IP_3Rs in the cardiomyocytes nuclear membrane. Immunohistochemical studies confirm that IP_3R1 is the most common isoform in Purkinje neurons nuclear membrane, while IP_3R1s and IP_3R2s are expressed in the cardiomyocytes nuclear membrane. We have also registered IP_3Rs with different Ca^{2+}-ions sensitivity in cardiomyocytes nuclear membrane, where one of the receptors was inhibited by Ca^{2+} at a high concentration (classic bell-shape), while another was not inactivated even in the presence of 1 mM Ca^{2+} in the medium.

Conclusions. Our results confirm the presence of IP_3Rs with unique properties in the nuclear membrane of cardiomyocytes, which differ dramatically both from the neuronal IP_3R1 and from the genetically-expressed IP_3R2. We assume that such differences can be caused by two facts: the molecular environment influence and receptor post-translational modifications that can not be taken into account during the genetically-expressed receptors research. These differences are crucial for Ca^{2+}-signal generation through IP_3Rs.

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THIAMINE TRANSPORTER THTR-1 EXPRESSION INCREASES UNDER NUTRITIONAL THIAMINE DEFICIENCY CONDITIONS AND DOES NOT DEPEND DIRECTLY FROM THIAMINE INPUT INTO THE ORGANISM

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Introduction. It is known that the transport of thiamine cation or its electro-neutral form thiamine monophosphate to the cell is provided by several families of transporters. However, the highly affiliated thiamine-bearing carrier protein expressing itself in the nerve cells is THTR-1. It could be assumed, that the state of the thiamine transport system in nerve cells in conditions of norm and pathology can be evaluated by content and expression level of this protein. The accumulated literature data strongly suggests that almost all of the known neurodegenerative diseases are accompanied by more or less pronounced thiamine deficiency (TD). This fact does not exclude that the thiamine deficiency may be the initiating factor for the development of some of the neurodegenerative pathologies. That is why the study of changes in the thiamine metabolism system of nerve cells with TD is an important step in determining the mechanisms of neurodegenerative processes. The aim of this study was to determine changes in content and expression of THTR-1 under TD and check the reversibility of these changes by high single dose thiamine administration.

Methods. The nutritional thiamine deficiency (Av-T) model was created using the Gubler diet. The term of the diet was four weeks. Controls rats were administered with thiamine orally (200 μg/day). One day before decapitation, part of the rats was administered with thiamine according to the daily norm. The number of rats in groups was n = 3-4. The level of THTR-1 in different parts of the brain of animals (cerebral cortex, cerebellum, hippocampus) was investigated by immunoblotting. The results were visualized using the ECL set. The expression of *SLC19A2* gene was measured with semi-quantitative PCR method. Results were calculated using ΔΔCt method.

Results. The results show that in the condition of deep Av-T, the growth of THTR-1 content is observed in all investigated departments, most clearly in the cerebral cortex - 2.5 times compared with the control group. The one-time administration of vitamin per day to decapitation did not affect the protein content of any of the departments (in all three structures THTR-1 content remained elevated compared with the control group). The PCR data confirmed the pattern observed with immunoblotting and demonstrated that Av-T leads to an increase in the biosynthesis of THTR-1 at expression level in all departments, most clearly in the cerebral cortex (2.2 fold compared to control). When thiamine is administered, this indicator in the cortex and the hippocampus remains at the level of the Av-T group and only in the cerebellum is a marked tendency to normalize the level THTR-1.

Discussion. The results of this study have shown a violation of the thiamine transport in the condition of deep nutritional thiamine deficiency. It is possible that this effect is a compensatory mechanism, which is expressed in the enhancement of the synthesis of the transporter. Also, under the conditions of our experiment, we can see the heterogeneity of the brain's departments reaction: the most sensitive area of the brain in conditions of alimentary thiamine deficiency was the cerebral cortex: it showed the most pronounced increase in THTR-1,

Conclusions. The results demonstrated an increase in the content of THTR-1, already occurring at the expression level of the SLC19A2 gene, in all investigated divisions in response to alimentary avitaminosis. It also became known that these changes do not depend directly on the incidence of thiamine, since there is no response from the THTR-1 to the administration of a high single thiamine dose.

UDC 577.112.5

EPITOPE DETERMINATION OF NOVEL FIBRINOGEN-SPECIFIC ANTIBODY BY LIMITED PROTEOLYSIS

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Introduction. An epitope is the part of an antigen molecule that is recognized by antibody. Exact determination of the epitope is important for a wide range of studies with the use of monoclonal antibodies. However, in most times the site of monoclonal antibody is being determined very preliminarly. In this study, we aimed to localize the epitope of new fibrin(ogen)-specific antibody 1-5A using specific proteases targeted to the C-terminal portions of Aα-chains of fibrinogen.

Material and Methods. Protease II (PII) and Protease III (PIII) were purified from *Bacillus thuringiensis* var. israelensis IMV B-7465 culture media. Prothrombin activator that was able to cleave Aα-chain of fibrinogen was purified form the venom of *Echis multisquamatis*. Fibrinogen was purified from human blood plasma by the method of Varetska. Hydrolytic products were identified using SDS-PAGE with following Western-Blot analysis. Monoclonal antibody II-5C specific to Aα 20-78 was obtained in Protein Structure and functions department earlier. Determination of molecular weight of products was performed using densitometry soft TotalLab TL100.

Results. Previously was reported that PII cleaves Aα504-505 peptide bond producing peptide Aα505-610 (11.4 kDa) from the full-length Aα-chain and Aα505-583 (8.6 kDa) from the Aα-chain partly hydrolyzed by plasmin that normally is found in fibrinogen preparations as minor fraction. According to the Western-Blot analysis studied antibody 1-5A were able to interact with both peptides and did not interact with the remnant polypeptide. Thus we were able to conclude the epitope within Aα505-583 fragment. Protease from the venom of *Echis multisquamatis* cleaved Aα-chain of fibrinogen molecule forming three fragments with molecular weights 40, 23 and 20 kDa. Antibody 1-5A interacted with two last that were also derived from C-terminal part of Aα-chain of fibrinogen.

Discussion. PIII possessed different specificity. Using of II-5C antibody showed that after initial cleaving off 9 kDa fragments of C-terminal portions of fibrinogen Aα-chain, it cleaved the resting polypeptide on two fragments with apparent molecular weights produced three polypeptides derived from Aα-chain of fibrinogen with molecular weights 37 and 27 kDa. However, any of these peptides were detected in Western-Blot analysis with the use of the studied 1-5A antibody.

It was found that the epitope of new antibody 1-5A located in the 505-583 fragment of fibrinogen Aα-chain. However, antibody did not bind to the small C-terminal fragment generated by PIII as well as other fragments of fibrinogen Aα-chain, generated by this protease. This can be explained by the destruction of epitope after cleaving the 505-583 fragment by PIII.

UDC 577.151.6 : 612.115

INTERPLAY OF PLATELET PROCOAGULANT AND PROFIBRINOLYTIC ACTIVITY IN THROMBUS FORMATION AND LYSIS

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Introduction. Coagulation and fibrinolysis are competing, but at the same time complementary mechanisms, accurately regulated to maintain hemostatic balance. Platelets, as an interconnection point of both coagulation and fibrinolysis, play an essential role in such regulation. While platelets contribution to the enhancement of coagulation is well-known, less is known about their involvement in the process of fibrinolysis, as well as about the molecular mechanisms of connection of both processes by platelets. The aim of the present study was to investigate possible mechanisms underlying regulation of fibrinolysis by platelets and, consequently, their participation in coordination of both coagulation and fibrinolysis.

Methods. Using confocal microscopy method, under flow and static conditions, formation of platelet-fibrin microaggregates on collagen surface in real time and binding of t-PA to microaggregates obtained from the whole blood or to washed platelets, as well as fibrin clot structures obtained from PRP was analyzed. Activation of plasminogen by t-PA was estimated in a system with washed platelets using specific chromogenic substrate assay.

Results and Discussion. Using flow chamber model system to visualize the processes of fibrin formation in real time after platelets adhesion on collagen, we observed that platelets' microaggregates served as centers of the initiation of fibrin polymerization with further formation of fibrin network in a space between microaggregates. These findings were also confirmed in static conditions with clotted PRP, where fibrin network was structured around clusters of platelets which played a role of "organization centers" of clot architecture. In comparison with platelet-free plasma-derived clots fibrin fibers packing in PRP were not homogenous, being the densest on clustered platelets, thus presumably creating the structure to simplify the access of fibrinolytic factors and to make more beneficial milieu for lysis. Indeed, under flow conditions, we observed t-PA binding to platelet microaggregates. Moreover, t-PA binding (presumably through fibrin) was higher on platelets, stimulated by additional agonists, for instance, by thrombin receptor-activating peptide (TRAP) or ionomycin (ionophore used to evoke secretion). At the same time, binding of t-PA was also retained in the presence of ε-aminocaproic acid (blocker of lysin-binding sites). It is also worth noting that we observed t-PA binding to washed platelets adhered to glass, even without additional agonists - presumably through secreted inner pool of fibrin. Furthermore, when platelets were stimulated by both collagen and thrombin (generated *in situ*), there was a colocalization between bound labeled t-PA and labeled FX(a) antibodies, which can be evidence of the role of prothrombinase in binding t-PA and potential plasminogen activation. Together with the observation of t-PA binding also in the presence of tirofiban (glycoprotein IIb/IIIa inhibitor) these data suggest another, fibrin - independent mechanisms of t-PA binding. Further, to investigate the activation of plasminogen in the presence of platelets, the study was performed applying specific plasmin substrate S-2251 added to washed platelets (native or activated by thrombin) preincubated with plasminogen and t-PA. The evidence from these experiments indicated the increasing of plasminogen activation by t-PA on more activated platelets.

Conclusion. In conclusion, the evidence from this study suggests that platelets, along with the process of coagulation, are also able to enhance fibrinolysis. They regulate both fibrin formation and its subsequent lysis, moreover, can coordinate coagulation and fibrinolysis processes through the

components of the intrinsic pathway and plasminogen/plasmin system, particularly by structuring fibrin network, creating areas with different properties within thrombi, and by binding of t-PA through fibrin-dependent and -independent mechanisms. Platelets thus regulate local hemostatic balance, supporting coagulation when necessary to prevent bleeding, and, as the process of thrombi formation progresses, "switching" hemostatic balance towards fibrinolysis, augmenting plasminogen activation on the most activated platelets, therefore restricting the size and lifetime of fibrin clot.

UDC 577.29

DIGESTION OF FIBRINOGEN MOLECULE BY PROTEINASE FROM THE VENOM OF *Brachypelma smithi*

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Introduction. Venom of *Brachypelma* spiders is mainly used for the digestion of prey that most likely are insects. However several compounds of *Brachypelma* venom can also act on mammals, in particular, inhibit or activate ion channels or possess neurotoxic effects. Action of *Brachypelma* venom on human blood coagulation system was not studied. In this study, we concentrated on proteases of *Brachypelma smithi* venom to identify the fibrinogen-specific enzymes that can be used in the study of the structure of fibrinogen.

Methods. Action of crude venom and its compounds on blood plasma coagulation studied using activated partial thromboplastin test (APTT). Action of aggregation of the platelets in platelet rich plasma (PRP) studied using aggregometry on AP2110 (Solar, Belarussia). The venom of *Brachypelma smithi* was fractionated using Q-sepharose ion-exchange chromatography on the FPLC system (ÄKTA, GE Healthcare, USA). Protein composition of fractions was studied using SDS-PAGE. Fibrinogen-specific protease was detected using enzyme-electrophoresis. Peculiarities of fibrinogen digestion under the action of protease were studied by Western-Blotting using fibrinogen-specific monoclonal antibodies 2d2a and 1-5C produced in Protein structure and functions department of Palladin Institute of Biochemistry.

Results and Discussion. Ion-exchange chromatography of *Brachypelma smithi* venom on Q-sepharose allowed obtaining fraction that substantially prolonged clotting time in APTT test (110 ± 8 s against control meanings 66 ± 3 s). It also inhibited ADP-induced platelet aggregation in PRP on 15 ± 4% in comparison to control meanings. According to the data of enzyme-electrophoresis this fraction contained fibrinogen-specific protease with apparent molecular weight 35 kDa. It can cleave Aα- and Bβ-chains of fibrinogen molecule equally effective. Western-Blotting indicated that studied enzyme cleaved N-terminal portion of Bβ-chain and αC-region of fibrinogen molecule simultaneously.

Conclusion. New fibrinogen-specific protease was found in *Brachypelma smithi* venom. It possessed strong anticoagulant activity towards blood plasma and PRP. The action of the enzyme on the fibrinogen molecule is promising for obtaining new non-physiological fragments of fibrinogen molecule that can be used in study of fibrinogen structure and functions.

UDC 577.112

ADAPTOR PROTEIN RUK/CIN85 IS ESSENTIAL FOR BREAST CANCER CELLS INVASIVENESS

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Introduction. During the process of invasion and metastasis tumor cells of epithelial origin lose their inter cellular contacts, apical-basal polarity and acquire an elongated spindle-like shape, increase their motility and invasiveness. Such changes are known as epithelial-mesenchymal transition (EMT), the process associated with transcriptional reprogramming, changes in cell signaling and rearrangements of the cytoskeleton. The aim of this study was to investigate the role of the adaptor protein Ruk/CIN85 in the control of breast cancer cells invasion in vitro and the expression of main EMT markers.

Methods. As a model, we used mouse breast adenocarcinoma 4T1 cells with stable overexpression of the adaptor protein Ruk/CIN85. Matrigel invasiveness was estimated using a modified Boyden chamber. Highly-invasive M1 and M2 subpopulations of 4T1 cells were selected by Matrigel invasion assay followed by propagation of invaded cells. The expression levels of Ruk/CIN85, Vimentin and E-cadherin were evaluated using real-time PCR and Western blot analysis.

Results. Ruk/CIN85 overexpression in 4T1 cells resulted in significantly increased Matrigel invasiveness. The analysis of EMT markers expression demonstrated that Ruk/CIN85-overexpressing 4T1 cells are characterized by elevated Vimentin (Vim) expression and lack of E-cadherin (Cdh1). Analysis of gene expression revealed that highly-invasive subpopulations of 4T1 cells overexpress Ruk/CIN85 and have signs of EMT.

Discussion. In the present study, we demonstrated that adaptor protein Ruk/CIN85 is essential for breast cancer cell invasion. As well, we found that this adaptor protein modulates the expression of epithelial (E-cadherin) and mesenchymal (Vimentin) markers in 4T1 cells depending on its expression levels.

Conclusions. The data obtained indicate that adaptor protein Ruk/CIN85 regulate breast cancer cells invasiveness via control of EMT-related genes expression.

BIOTECHNOLOGY

UDC531/534/: [57+61]

FEATURES OF THE FATTY ACID PROFILE OF NON-PATHOGENIC STRAINS *AEROMONAS ICHTHIOSMIA* ONU552, *BACILLUS SUBTILIS* ONU551-DESTRUCTORS OF PHENOLIC COMPOUNDS

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Introduction. The search for new environmentally safe biological products designed to clean the environment from toxic organic pollutants remains a pressing problem of our time.

Methods. The modern method of gas chromatography using automatic MIDI Sherlock microorganism identification system (USA) based on the Agilent 7890 gas chromatograph on the composition of fatty acids confirmed the species identification of bacterial strains F-2, F-13, isolated from wastewater produced by pharmaceuticals. The non-pathogenicity of the isolated strains *in vitro* and *in vivo* was evaluated.

Results. The features of the fatty acid profile of the strains *Aeromonas ichthiosmia* ONU552 (F-2), *Bacillus subtilis* ONU551 (F-13)-destructors of phenolic compounds and antagonists of pathogenic microbiota were noted for the first time: the presence of 16:1 fatty acid strains in the cell lipids of each of the strains fatty acids 17:0 and iso and 17:0 anteiso. The minimum total content of branched fatty acids 17:0 in the form and iso and anteiso is fixed in the fatty acid profile of strain *A. ichthiosmia* ONU552 (w = 1.69%), and the maximum w = 17.35% - in strain *B. subtilis* ONU551.

Discussion. A thorough comparative analysis of the chromatographic results we obtained with the literature data showed that the strain *A. ichthiosmia* ONU552 we studied was close enough not to *A. hydrophila* with pathogenic properties, but to the non-pathogenic strain *A. sharmana* sp. nov. GPTSA-6T: chromatograms of both bacterial strains detected fatty acids of 16:0, 18:1 w7c, 12:0, 14:0, Σ14:0 3OH/16:1 iso I. A comparative analysis of the obtained fatty acid profile of the *B. subtilis* ONU551 strain did not fix the isomers of saturated fatty acids: 12:0 iso, 13:0 iso and 13:0 anteiso, which are characteristic of the pathogenic strain *B. cereus*. Detailed analysis of the fatty acid profile of the strain *B. subtilis* ONU551 showed the presence of unsaturated fatty acid isomers: 15:1 w5c (1.85%); 16:1 w11c (1.21%); 16:1 w7c alcohol (1.08%); 17:1 iso w10c (3.18%) and the sum of acids Σ17:1 iso I/anteiso B (2.57%). This distinguishes it from members of the genus *Bacillus* of group 2 (*B. sphaericus*, *B. fusiformis*, *B. insolitus*, *B. pasteurii*, *B. psychrophilus*), which are characterized by a significant content of isomers with unsaturated bonds (w = 17-28%) and made it possible to classify strain *B. subtilis* ONU551 to *Bacillus* of group 1 (*B. amyloliquefaciens*, *B. atrophaeus*, *B. licheniformis*, etc.), for which the content of unsaturated fatty acid isomers is less than 10%.

Conclusions. The identified features of the fatty acid composition of the strains *A. ichthiosmia* ONU552, *B. subtilis* ONU551 are systematized and distinguish them from other bacteria. An assessment of the pathogenicity of *A. ichthiosmia* ONU552, *B. subtilis* ONU551 strains *in vitro* (on a model of human cell culture lines - Hep2 and RD and animals - L20B) and *in vivo* (on white laboratory mice) showed that there are no bacteria-destructor strains virulent and toxigenic properties. A positive result was also obtained as a result of biotesting on hydrobionts - on *Dafnia magna* test objects for 7 days. Discovered reveals the limits of practical use of the studied strains of microorganisms in environmentally safe biotechnology for cleaning the environment from highly toxic organic pollutants.

MOLECULAR BIOLOGY

UDC 577.112:616

ANGIOGENESIS-RELATED GENE EXPRESSIONS IN SUBCUTANEOUS ADIPOSE TISSUE

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Introduction. Obesity and its metabolic complications are one of the most profound public health problems and result from interactions between genes and environment. The development of obesity is tightly connected with dysregulation of intrinsic gene expression mechanisms controlling the majority of metabolic processes, which are essential for regulation many physiological functions, including insulin sensitivity, cellular proliferation and angiogenesis.

The aim of this study was investigation of VEGF-A, PDGFC, FGF1, FGF2, FGFR2, FGFRL1, E2F8, HIF1A gene expressions at mRNA level in adipose tissue in obese men with normal glucose tolerance and glucose intolerance as compared to relative healthy (normal) people same age and sex for identification of obesity related genes as well as genes related to development of metabolic complication.

Methods. The level of studied genes expression as well as beta-actin as control gene, is determined in adipose tissue of obese adult men with obesity and normal glucose tolerance as well as with obesity and glucose intolerance using quantitative polymerase chain reaction method and "The 7900 HT Fast Real-Time PCR System" and "QuantStudio 5 Real-Time PCR System" (Applied Biosystems, США). Group of relatively healthy people is used as control. Absolute QPCR SYBP-Green Mix and specific for each human gene are used for amplification. RNA is extracted from adipose tissue samples using RNasy Lipid Tissue Mini Kit. QuantiTect Reverse Transcription Kit is used for complementary DNA synthesis.

Result and Discussion. We have shown that the expression level of VEGF-A, PDGFC, FGF2, and FGFRL1 genes is decreased in adipose tissue of obese men with normal glucose tolerance (NGT) versus a group of control subjects. At the same time, in this group of obese individuals a significant up-regulation of FGF1, FGFR2, E2F8 and HIF1A gene expressions were observed. Impaired glucose tolerance (IGT) in obese patients associates with down-regulation FGFR2 mRNA and up-regulations of FGF1, FGF2, VEGF-A and its splice variant 189 mRNA expressions in adipose tissue versus obese (NGT) individuals.

Conclusion. Therefore, our data demonstrate that the expression of almost all studied genes is affected in subcutaneous adipose tissue of obese individuals with NGT and that glucose intolerance is associated with gene-specific changes in the expression of FGF1, FGF2, VEGF-A, and FGFR2 mRNAs. These genes are possibly involved in the development of obesity and its complications.

UDC 577.2

CRISPRa-MEDIATED DIRECT CARDIAC REPROGRAMMING OF EMBRYONIC RAT FIBROBLASTS

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Introduction. CRISPR-Cas9 system represents the recently adopted tool for gene knockout experiments. It consists of a Cas9 nuclease and guide RNA (gRNA), which determines specificity of the complex. CRISPRa is an alternative approach for system application. It enables a stable gene activation through the fusion of transcription activators to the mutant Cas9 protein (dCas9). Here we applied CRISPRa approach to reach cardiac reprogramming of rat embryonic fibroblasts through the activation of Cardiac Master Regulator genes expression (*Mef2c*, *GATA4*, *Tbx5*, *MyoD*1, *Hand2*).

Methods. Using web-server CRISPR-ERA we chose several gRNAs complementary to promoter region of genes of interest and cloned them into plasmid vector phU6-gRNA. After liposomal transfection of embryonic fibroblasts, we extracted total RNA, synthesized cDNA and performed qPCR with cardiac gene-specific primers.

Results. We transfected embryonic cells with obtained phU6-gRNA vector constructions and SP-dCas9-VPR. Cells were cultivated for 48 hours after which RNA extraction, cDNA synthesis and qPCR were performed. The highest level of expression was observed when gRNA was targeted to 20 n. b. long DNA site located at position -81 (+ strand) to TSS for *Mef2c*, -50 (+) to TSS for *GATA4*, -26 (-) to TSS for *MyoD1*, -419 (-) and -588 (-) to TSS for *Hand2*, -215 (-) and -166 (-) to TSS for *Tbx5*. Subsequently, we used selected gRNAs to activate 5 genes simultaneously. We transfected cells with 2 combinations of validated gRNAs, first one containing *Hand2.1* and *Tbx5.1* and the second one containing *Hand2.2* and *Tbx5.2* gRNAs. Cells were cultivated for 3,6,9 and 27 days after which we extracted RNA, synthesized cDNA and applied qPCR to determine the levels of expression of genes of interest.

Discussion. Our research was focused on non-viral vectors approach application to reach the stable cardiac reprogramming. As it is known, cardiovascular diseases cause the death of cardiomyocytes and the replacement of dead cells by connective and adipose tissues takes place. Thus the direct reprogramming of fibroblast in the injured area into cardiomyocytes is a promising strategy to heal the affected organ. The data obtained has an important value for CRISPRa-based reprogramming strategy optimization.

Conclusions. We chose 6 specific gRNA sequences and constructed an equivalent number of phU6-gRNA cloning vectors for *Mef2c* gene, 5 for *GATA4*, 2 for *Tbx5*, 4 for *MyoD1* and 4 for *Hand2*. We examined the efficiency of CRISPRa-mediated transcriptional activation of cardiac genes and determined gRNAs that have led to the highest expression level. Upon transfection of embryonic cells with 2 combinations of selected gRNAs, we then analyzed the levels of transcriptional activation of 5 genes at once and chose the best set of validated gRNAs. We also determined the expression of *Atp2a2* and *Nppa* genes that are the markers of cardiomyocytes, which indicates the successful cardiac reprogramming of rat embryonic fibroblasts.

UDC 557.112.7:616

THE EFFECT OF IRE1 KNOCKDOWN AND SILENCING OF NAMPT ON THE EXPRESSION OF A SUBSET OF PROLIFERATION-ASSOCIATED GENES IN U87 GLIOMA CELLS

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Introduction. Endoplasmic reticulum stress signaling has emerged as a major player in cancer development, including glioma. Inhibition of IRE1 (inositol requiring enzyme-1) signaling, a key mediator of the unfolded protein response, leads to the suppression of tumor growth through metabolic reprogramming of cancer cells. NAPMT (nicotinamide phosphoribosyl transferase) is an important regulator of energy in cells and controls the NAD biosynthesis, which has a critical role in life and disease, is significantly increased in numerous malignant tumors, including glioma. The aim of this study was investigation the effect of IRE1 inhibition as well as NAMPT knockdown on the expressions of a subset of proliferation-related genes in U87 glioma cells with hope elucidating their interconnection in the progression of glioma.

Methods. We have used U87 glioma cells and their subline stably transfected with dnIRE1 construct (without kinase and endoribonuclease domains). For inhibition of enzyme NAMPT cells were transfected by specific anti-NAMPT siRNA. Glioma cells transfected by control siRNA were used as control. The expression level of NAMPT and a subset of proliferation-associated mRNAs were measured in these glioma cells by real-time quantitative polymerase chain reaction.

Results. It was shown that the expression level of NAMPT as well as NAMPT protein is strongly down-regulated in glioma cells without inhibited IRE1 signaling enzyme function and correlated with slower proliferation rate of these cells. The decreased level of NAMPT mRNA expression is associated with up-regulation of microRNA miR-182, binding sites of which are identified in 3'-region of NAMPT mRNA. It was also shown that silencing of NAMPT mRNA by specific siRNA leads to significant reduction of NAMPT mRNA and protein as well as to slower proliferation rate of U87 glioma cells. Furthermore, silencing of NAMPT mRNA down-regulates the expression level of PERK, ATF3, BRCA1, and CLU1 and up-regulates TGM2, BIRC5, and RAB5C genes. At the same time, IRE1 knockdown leads to up-regulation of BRCA1 and down-regulation of ATF6, ATF3, CLU1, BIRC5, and RAB5C genes.

Conclusion. Thus, the expression of NAMPT and some other proliferation associated genes is responsible to IRE1-mediated endoplasmic reticulum stress signaling in a gene-specific manner and that silencing of NAMPT also affects the expression of these genes, but in variable ways, indicating the differential manner of the regulation of proliferation-related gene expressions by IRE1 and NAMPT.

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ISOLATION OF EXTRACELLULAR VESICLES FROM MOUSE KIDNEY ADENOCARCINOMA RENCA CELLS AND AN ANALYSIS OF THEIR PROTEIN COMPOSITION

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Introduction. The physiology of cells in a multicellular organism is determined by the action of signals coming from the extracellular and intracellular environment, including from adjacent cells, and are perceived by membrane and cytoplasmic receptors of target cells. In recent years, it became apparent that the transmission of signaling molecules by means of so-called extracellular vesicles, exosomes (40-100 nm in size) formed by exocytosis plays a major role in intercellular communication. Discovered in all body fluids, exosomes are a source of information on a large number of processes and disorders at the cellular level. Currently, there are attempts to use them to detect and treat tumors, as well as means of active specialized immunotherapy. The purpose of this work was to evaluate the protein content of extracellular vesicles produced by mouse kidney adenocarcinoma Renca cell lines.

Methods. Exosomes were isolated from conditioned medium of Renca cells discontinuous iodixanol gradient (5-40%). Laser-correlation spectroscopy was then performed with the Malvern NanoSight device to determine the amount and size of nanoparticles in the selected samples. Obtained preparations of extracellular vesicles were analyzed by electron microscopy and western-blot analysis.

Results and Discussion. The results obtained with the NanoSight device indicate that the extracellular vesicles produced by Renca cells match exosomes in size. Visual examination of extracellular vesicles by electron microscopy indicates that the resulting particles morphologically correspond to exosomes. Western Blot analysis confirmed the presence of markers of exosomes, namely, CD63, CD81, and Alix proteins, including adaptor protein Ruk/CIN 85, in obtained preparations.

Conclusions. Extracellular vesicles were isolated from conditioned medium of Renca kidney carcinoma cells which on size morphology and the content of marker proteins (CD 63, CD 81 and Alix) corresponded to exosomes. It has been shown for the first time that adaptor protein Ruk/CIN 85 is a component of exosomes produced by tumor cells.

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MOLECULAR MECHANISMS OF HYPOXIC REGULATION OF PROLIFERATION RELATED GENE EXPRESSIONS IN GLIOMA CELLS

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Introduction. Hypoxia is one of the factors which induce the endoplasmic reticulum stress, both are an obligatory component of malignant tumor growth. The effects of hypoxia are mediated preferentially by hypoxia-inducible factor (HIF), which activates the transcription of genes that are involved in crucial aspects of cancer biology, including angiogenesis, cell survival, glucose metabolism and invasion. Moreover, blockade of IRE1, the key signal transducer of the unfolded protein response, results in significant suppression of glioma growth through changes in the expression level of genes related to the angiogenesis, apoptosis, proliferation and mitochondrial functions. The transcription factor X-box binding protein 1 (XBP1) is a key component of the endoplasmic reticulum stress response and controls close to one thousand genes responsible for tumor growth including hypoxia-inducible genes.

Methods. We used the bioinformatic methods for identification of HIF- and XBP1-binding sites in the promoter region of cancer growth-related genes. We used U87 glioma cells and their subline with blockade of IRE1 function by dnIRE1-construct. The expression level of studied genes was determined in glioma cells by real-time quantitative polymerase chain reaction.

Results and Discussion. We studied the effect of hypoxia on the expression level of glioma growth-related genes such as ME2, NNT, IDH2, MDH2, GOT2, FAM16A and AIFM1 in relation to inhibition of the endoplasmic reticulum stress mediated by IRE1 signaling. It was shown that the expression level is significantly down-regulated in glioma cells without IRE1 enzyme function, except IDH2, NNT and FAM162A genes, which expression level is strongly up-regulated. At the same time, inhibition of IRE1 leads to suppression of expression level of AIFM1 gene, which is involved in apoptosis and autophagy signaling pathways. We showed that hypoxia affects the expression level of most studied genes in a gene-specific manner and that effect of hypoxia on the expression of some genes is modulated by IRE1. Binding sites for HIF and XBP1 were found in most studied genes and some of these binding sites can recognize both transcription factor.

Conclusions. The expression of all studied genes is responsible for IRE1 signaling enzyme function in a gene-specific manner, because inhibition of IRE1 significantly affects their expression. Results of our investigation demonstrate that hypoxia may affect the expression of genes through binding sites for HIF and XBP1. Therefore, IRE1 can modify the effect of hypoxia on gene expressions through induction of XBP1.

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MEDICAL BIOCHEMISTRY

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ANGIOGENESIS REGULATORS AND MMP ACTIVITY IN TROPHIC AND HYPERTONIC ULCERS OF MILD TISSUES

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Introduction. Chronic ulcers are the common complication of diabetes (trophic ulcers) and unusual complication of hypertension (Martorell syndrome). Diabetic chronic wounds are the result of angiopathies, while Martorell ulcers appear due to diastolic arterial hypertension. The exact molecular mechanisms of delayed healing of these ulcers are still unknown. The aim of this study was to assess and compare angiostatin and VEGF levels and MMPs activities in fluids from acute vs chronic diabetic wounds of mild tissues and bioptates of Martorell hypertensive ischemic leg ulcer.

Methods. The exudates of chronic diabetic wounds (n = 8) and fluids of acute surgical wounds (n = 9) were collected by topical negative pressure (-80-125 mm Hg). The Martorell ulcer bioptates (n = 5) were taken from different regions of ulcer of one unique patient, and histologically normal surrounding (granulating) tissue specimens served as a control. Informed written consent was obtained from all patients. Plasminogen and its proteolytic fragments (angiostatins) and VEGF levels were evaluated by western blot. Gelatin zymography was applied for the determination of MMPs activity.

Results. In exudates of diabetic wounds, the elevated levels of angiostatins were determined as compared with acute wound fluids. The [angiostatin/plasminogen] ratio in chronic diabetic wounds was 6 times higher compared to that of acute wounds. Martorell ulcer tissue was also characterized by an increased amount of angiogenic inhibitor. Dramatic elevation of VEGF was observed in both ulcer types. Significant activation of MMP-2 and -9 was shown in exudates of chronic diabetic wounds, unlike that of surgical wounds. Similarly, high collagenolytic MMP activities occurred in Martorell ulcer tissue.

Discussion. It is known that MMPs are responsible for plasminogen cleavage to angiostatins, which specifically inhibit endotheliocyte proliferation, migration and vessel formation. There is similar molecular canvas of MMP-2, -9 activity and angiostatin/plasminogen ratio for Martorell disease compared to control as for diabetic wounds. Therefore, the high permanent activities of MMPs may cause the overproduction of angiostatins and degradation of extracellular matrix proteins. Likely, VEGF production, which is inherent for both chronic diabetic wounds and Martorell ulcers, is unable to provide sufficient wound neovascularization due to counteracting action of angiostatins, thus contributing to healing delay.

Conclusions. Despite the different pathogenesis of diabetic and hypertensive (Martorell) ulcers, they have similar biochemical changes, such as increased MMPs activities and imbalance of angiogenesis regulators. Obtained results demonstrate that observed changes may be considered to play a essential role in restriction of blood vessel recovery and wound healing delay. Understanding of molecular processes in delayed wound healing can lead to more optimized management of chronic ulcers of different etiology.

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FASCIN IS UPREGULATED IN NASAL MUCOSA IN CHRONIC RHINOSINUSITIS WITH NASAL POLYPS

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Introduction. Chronic rhinosinusitis is a long-lasting (over 12 weeks) inflammation of the sinonasal tissue. The disease can either be associated with the formation of non-cancerous outgrowths called nasal polyps or develop without them. The former is called chronic rhinosinusitis with nasal polyps (CRSwNP). Numerous efforts have aimed at elucidating the mechanisms of its development. However, its pathogenesis is not fully understood. In particular, the role of an actin-bundling protein fascin involved in cell motility is under debate in CRSwNP. Thus, our aim was to evaluate the expression of fascin in the nasal tissue of patients with CRSwNP.

Methods. We evaluated fascin expression in nasal tissues of 11 patients with CRSwNP and 7 healthy individuals. Diagnosis of CRSwNP was verified using clinical and instrumental methods in accordance with ‘‘EPOS 2012: European Position Paper on Rhinosinusitis and NPs 2012” recommendations. Fascin expression was assessed immunohistochemically using antibodies manufactured by Thermo Fischer Scientific (UK).

Results. Fascin was weakly expressed in the nasal tissue of control individuals, whereas fascin upregulation was observed in nasal epithelial cells of polyp tissue in CRSwNP. In addition, fascin expression was stronger in the lamina propria of patients with nasal polyposis compared with the conditionally healthy controls.

Discussion. Our findings of fascin overexpression may suggest that fascin-expressing nasal epithelial cells have the increased ability to migrate, participating in tissue repair in CRSwNP, since it has been reported that CRSwNP is associated with the damage to the nasal epithelial layer as a result of inflammation with the formation of atrophic areas. We believe that the increased migratory capacity of fascin-positive nasal epithelial cells can be used as a compensatory mechanism to close the gaps found in the epithelial layer of the inflamed nasal mucosa.

Conclusions. Fascin is overexpressed in the nasal tissue of patients with CRSwNP.

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ACTIVITY OF NAD$^+$-DEPENDENT ENZYMES OF THE KREBS CYCLE UNDER THE CONDITIONS OF DIFFERENT NUTRIENTS SUPPLY

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Introduction. Up to date the possible mechanisms for the formation of various metabolic disturbances under the conditions of nutrients imbalance remain quite relevant. A modern diet consists mostly of simple carbohydrates and saturated fats with a simultaneous deficiency of a complete protein, which may be an important factor contributing to the induction and progression of the metabolic disorders. One of the key mechanisms for the dysmetabolic changes is the disturbances in the energy supply of the cell. Since the NAD$^+$-dependent reactions of the Krebs cycle are the key reactions that ensure the maintenance of the NADH level required for the cell, the aim of our research was to determine the activity of isocitrate dehydrogenase, α-ketoglutarate dehydrogenase and malate dehydrogenase in conditions of high-sucrose and high-sucrose/low- protein diet.

Materials and Methods. The experiments were conducted on white rats aged 2-2.5 months, divided into groups: I – rats maintained on a complete semi-synthetic diet; II – rats maintained on a high-sucrose diet (hS); III – rats maintained on a high-sucrose/low-protein diet (hS/lP).

Results. It is shown that in animals maintained on an hS diet, there was a tendency to decrease in isocitrate dehydrogenase and malate dehydrogenase activity in the mitochondrial fraction of liver by 28% and 23%, respectively. In addition, α-ketoglutarate dehydrogenase activity was reduced by 60%. At the same time, in rats maintained on a hS/lP diet the activity of malate dehydrogenase decreased by 6 times, isocitrate and α-ketoglutarate dehydrogenase – by 5 times compared to control.

Discussion. There is literature data describing a relationship between the content of sucrose in the diet and the degree of destructive changes in liver. In particular, it was shown that hepatocyte damage is manifested by the appearance of swollen cells with excessive lipid droplets in a cytoplasm, swelling of nuclei and mitochondria, infiltration of the liver tissue by leukocytes and formation of an inflammatory focus. Moreover, it is known that under the conditions of a hS diet there is an imbalance between the metabolite contents in the cell combined with a failure of the regulatory mechanisms in various tissues, primarily liver and muscle. The decrease in the enzymatic activity in liver mitochondria of rats maintained on a hS diet indicates an inhibition of the Krebs cycle reactions. It may result in a decrease in NADH, which is essential for the functioning of the respiratory chain. The established changes may be related to both destructive changes in liver switching of the ways to use the acetyl-CoA. It is interesting to note the fact of the significantly decreased enzymatic activity in the liver mitochondria of animals, which were kept on a hS/lP diet. Probably, in the deficiency of protein in the diet and formation of an amino acid imbalance in animals from this group, not only the structural-functional changes of the studied enzymes, but also an increased use of Krebs cycle metabolites in the reactions of the substitutable amino acids synthesis are observed.

Conclusions. Thus, under the conditions of a high-sucrose diet, there is a decrease in the activity of NAD$^+$-dependent enzymes of the Krebs cycle in the liver, which is most pronounced in animals maintained on a low-protein/high-sucrose diet. The obtained results may be used to develop a strategy for correction of the metabolic disturbances in conditions of different nutrients supply.

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BIOCHEMICAL MARKERS OF EXHALED BREATH CONDENSATE IN CHILDREN DIAGNOSED WITH PNEUMONIA

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Introduction. Community-acquired pneumonia (CAP) in children is one of the frequent respiratory system diseases, especially as the result or the complication of acute respiratory infections. At the same time, there is little to no research data on non-invasive methods of pulmonary parenchyma inflammation diagnostics that could be used for fast diagnosis verification or severity assessment.

Methods. At the Region Children Clinical Hospital at Chernivtsi, a complex examination has been conducted on 63 children diagnosed with CAP. The average age of the children was 8.7 ± 0.6 a.u., 54.0% were male and 61.9% were citizens of rural area. Samples of EBC have been collected. The measurement of following biochemical markers was conducted: total protein content (Lowry et al.), 2,4-dynitrophenylhydrazine aldehyde and ketone derivatives of neutral and alkaline nature (Dubinina O.E. et al.), catalase activity (Koroliuk M.A. et al.), proteolysis activity assay by azoalbumin, azocasein and azocol lysis (Kucharchuk O.L.), nitrogen monoxide metabolites levels (Jemchenko N.L., modified method by Gojenko A.I.).

Results and Discussion. Research has shown that in EBS samples from children with CAP total protein content rose insignificantly in comparison to the age norm (3.2 g/l) and amounted 3.40 ± 0.34 g/l, simultaneously with the activation of both alkaline (71.5 ± 9.0 E 430 mmol/g of protein) and neutral (7.90 ± 0.86 E 370 mmol/g of protein) protein oxidative modification. In patients with CAP, a reliable decrease of catalase activity (48.90 ± 7.98 μmol/min×mg of protein compared to average age norm 88.90 ± 8.17 μmol/min×mg of protein, $P < 0.05$) was found; the specificity of pneumonia verification test in the presence of catalase activity higher than 80,0 μmol/min×mg of protein reached 83.3%, odds ratio 14.0, post-test probability 81.6%. The proteolysis activity lysis rate amounted to 1.50 ± 0.09 ml/h measured by azoalbumin lysis, 1.30 ± 0.11 ml/h measured by azocasein lysis and 0.21 ± 0.03 ml/h measured by azocol lysis. The average nitrogen monoxide metabolites content in EBS amounted to 45.40 ± 5.48 μmol/l, the specificity of the test based on this marker's value higher than 40,0 μmol/l reached 83.3%, odds ratio 6.0, post-test probability 76.6%.

Conclusions. Consequently, there has been discovered an activation of proteolysis and protein oxidative modification simultaneously with the inhibition of antioxidant system in children diagnosed with CAP.

BIOCHEMICAL MECHANISMS OF RESISTANCE TO ADVERSE ENVIRONMENTAL CONDITIONS

UDC 577.155

GLUCOSE AND PROTEIN IN THE BLOOD SERUM OF THE DICE SNAKES FROM DIFFERENT ECOSYSTEMS

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Introduction. Dice snake (*Natrix tessellata* (Laurenti, 1768)) inhabits a large area including Europe, North Africa and Asia. In Ukraine, *N. tessellata* is found in the steppe zone and occasionally in the forest steppe area. That species is obligatory aquatic. Therefore, the dice snake may be a reliable "tool" to study the anthropogenic effects in aquatic ecosystems. Blood biochemical parameters are known to be a good indicator of an organism condition. The dice snake blood biochemistry is still poorly studied.

Methods. The study was conducted on 16 adult *N. tessellata* specimens, which were caught in July 2014–2016 in ecosystems of the Dnipro River. Group I of snakes was collected in natural ecosystems of the National Nature Park "Velykyi Lug". Group II – in the Maiorova gully (Maiorka village, Dnipropetrovsk region) and group III – in anthropogenically transformed ecosystems of the Sanitary Protection Zone of Prydniprovska Thermal Power Plant. Biochemical indices of the blood serum were studied by standard clinical methods. There were determined concentrations of glucose, total protein, albumin and globulin. We also calculated the albumin/globulin ratio in the serum. We used nonparametric Kruskal-Wallis and Mann-Whitney U tests for comparative analysis of the studied groups (Statistica 12.0, StatSoft Inc., 2013).

Results. Significant differences between the groups of snakes in the studied parameters: concentrations of glucose, albumin, globulin, and albumin/globulin ratio were substantiated by the Kruskal-Wallis test ($P < 0.05$). The levels of albumin, globulin, glucose and albumin/globulin ratio groups I and II are similar and don't have statistic differences according to the Mann-Whitney U test. Glucose level in the serum increased 2.5 times in the snakes group III in comparison with the control populations (groups I and II, $P < 0.05$). Globulin concentration increased by 18% contrary to the control groups ($P < 0.01$). The albumin concentration and albumin/globulin ratio were reduced by 22% ($P < 0.01$) and 32% ($P < 0.01$) respectively as compared with the relevant parameters in the groups I and II. The differences in total protein concentrations between snakes of the studied groups are not statistically significant according to the Kruskal-Wallis test ($P > 0.05$).

Discussion. Unchanging level of total protein in the blood serum of snake from different ecosystems excludes an inflammation, starvation and dehydration in the snakes from anthropogenically transformed ecosystems. But decline of the albumin/globulin ratio speaks in favour of a metabolic disorder. The albumin concentration decrease as well as the increase of the glucose and globulin levels point to the development of liver disorder that may probably be explained by the anthropogenic load and environmental pollution.

Conclusions. The data obtained demonstrate statistically significant differences in the *N. tessellata* blood serum biochemical parameters between anthropogenically disturbed ecosystems and the near-natural ones. These differences may be caused by the liver disorders and need further research.

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BIOCHEMICAL RESPONSES OF BIVALVE MOLLUSKS TO THE COMBINE EFFECT OF PHARMACEUTICALS, HERBICIDE AND HEATING

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Introduction. The toxicity of pharmaceuticals for the untargeted organisms attracts the growing concern. Although these substances are introducing in the environment at low concentrations (ng-µg per l), their combined impact jointly with the climate changes can induce unpredicted changes in the metabolic activity and its regulation in the wildlife. The aim of this study was to evaluate the specificity of the biochemical responses in the aquatic sentinel organism under the single and combine exposures to typical stressors.

Methods. Freshwater mussels Unio tumidus were treated with drugs diclofenac (Dc, 600 ng·l^{-1}), nifedipine (Nf, 700 ng·l^{-1}), or herbicide glyphosate (Gl, 33.8 µg·l^{-1}) separately at the 18o C and jointly at the 18 °C (DcNfGl) and 25 °C (DcNfGlT) during 14 days. The utilised concentrations were corresponding to the environmentally realistic levels. The indices of stress and apoptotic activities were evaluated in the digestive gland, and lysosomal membrane stability and nuclear abnormalities - in hemocytes.

Results. The most common response was the down-regulation of glutathione S-transferase activity. It decreased in all exposures (up to 3 times). In the combine exposures, the total level of glutathione (GSH) increased. However, the concentration of oxidized glutathione (GSSG) and GSH/GSSG ratio did not differ from the control value in any exposure. The level of metal-keeping and stress-related protein metallothionein enhanced in all exposures, except Nf. The cholinesterase activity decreased in the exposures to Gl and DcNfGl, detecting the typical effect of the phosphonate. However, heating diminished this response. The activity of the main executor apoptotic enzyme, caspase-3, was increased in the co-exposures, particularly, to DcNfGl+T (up to 2 times). The activity of cathepsin D in the lysosomes increased by the exposure to Dc, but decreased by the Nf and Gl (up to 4 times). The efflux of Cathepsin D from the lysosomes in digestive gland was magnified in the co-exposure to DcNfGl+T by 2 times. The determining of the lysosomal membrane permeability in hemocytes detected its elevation by Nf and co-exposures. However, the level of hemocytes with the micronuclei and other nuclear abnormalities was not changed in all exposures.

Discussion. These changes attest the promoting of apoptosis induced both by cytosolic and lysosomal signals by the heating. Importantly, the response to Nf in the applied in this study low concentration and under the effect of micromolar concentration, studied in mollusk earlier, differed substantially.

Conclusions. To summarize, the heating had the most disturbing effect on the responses of stress and biotransformation of the xenobiotics and distorted the specific responses for the single compounds in the studied model organism.

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THE EFFECT OF FEED ADDITIVES HUMIC NATURE ON MOLECULAR MECHANISMS OF SECOND HEMOSTASIS IN LABORATORY ANIMALS AFTER THE INFLUENCE OF COMBINED STRESS

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Introduction. The effect of any type of stress (psychological, immobilization, chemical, physical, etc.) on a living organism is accompanied by a short-term or long-term activation of processes that trigger cascaded adaptation reactions to adapt and normalize the processes of its functioning. It is known that the degree of adaptation and the nature of changes in the body for the effects of stress depends on the type of stress factors, their duration and strength.

Coagulation hemostasis is a step-by-step process for the activation of serine proteases, which ends with the formation of a fibrin thrombus and depends on a large number of plasma and cellular factors of blood coagulation. Long-term effects, even of weak stress factors, can be summed up, resulting in emotional tension and pathological conditions in the living organism. With long-term stress, there is an exhaustion of the functional reserves of the organism, which causes the violations of many functional systems, including hemostatic system. It is known that the most sensitive link of the hemostasis system is the phase of activation of plasma hemostasis.

Humic substances are products of a multistage transformation of organic matter in nature and the preservation of carbon and energy from dissipation. It is known that humic substances can exhibit antioxidant properties, which are primarily due to the presence of a large number of functional groups. It is important, however, that humic substances do not accumulate in the body, but metabolize to end products.

The purpose of this experiment is the effect of natural antioxidants, fodder additives humic nature on the indicators of coagulation system after the influence of combined stress.

Materials and Methods. The experiment was carried out on white, sexually-mature, young male rats, which were divided into 6 groups of 5 animals in each. Group 1 (control) - intact animals. Rats 2, 3, 4, 5, and 6 groups simulated combined stress. Animals of the 2 groups were deduced from the experiment the day after the simulation of combined stress. For animals of 3, 4, 5 and 6 experimental groups, water, feed additives and vitamin E were administered orally with a special dosage unit, individually for 18 days, after which the animals were withdrawn from the experiment. In rats, citrate plasma thrombin time, prothrombin time, Quick prothrombin test, number of fibrinogen by Rutberg were determined. Prothrombin ratio and prothrombin index were obtained calculation method.

Results and Discussion. Introduction to the diet in the post-stress period of natural antioxidant, feed additive Humilid normalized the processes of fibrinous thrombus formation, namely: the processes of the external and internal mechanism of activation of prothrombinase and the prothrombin level increased on the contrary. At the same time, Vitamin E after stress exerted its influence only in the first stage of the formation of a prothrombinase complex, which includes external and internal mechanisms. In the case of the animal feed additives of humic nature, Eco Impulse Animal, it has a different ambiguous effect on each of the links of the hemostasis system after the action of a complex of stress factors and requires further research.

Conclusions. Established, the feed additive of humic nature Humilid, as an antioxidant, triggers mechanisms for the normalization of the system of hemostasis of rats, namely, it approximates the reference values of the indicators that are characteristic of all levels of coagulation of blood, such as active partial thromboplastin time, prothrombin time and thrombin time, reduces the content of fibrinogen.