NATIONAL ACADEMY OF SCIENCES OF UKRAINE
PALLADIN INSTITUTE OF BIOCHEMISTRY

THE UKRAINIAN BIOCHEMICAL JOURNAL

Volume 90, Special Issue, 2018 **Kyiv**

FEBS3+ Meeting – XIth Parnas Conference – Young Scientists Forum "Biochemistry and Molecular Biology for Innovative Medicine"

Contents

Dear participants of the FEBS3+ Meeting – XIth Parnas Conference – Young Scientists Forum 'Biochemistry and Molecular Biology for Innovative Medicine'

On behalf of the Organizing Committee of the XIth Parnas Conference it's my honour and pleasure to greet you and to invite you to join the PARNAS Conference-2018 in Kyiv, the beautiful capital of our country. Welcome to Ukraine!

Many things have been changed in the world and in Biology since the first Parnas Conference in 1996 in Lviv, and the Conference itself has been changed. During these 22 years, the Parnas Conference became an International scientific forum organized now by three societies – Ukrainian Biochemical, Polish Biochemical and Israel Society for Biochemistry and Molecular Biology. Though these three societies serve alternatively as hosts, the Parnas Conference was never considered being a close trilateral event but a wide and open International Conference with a special emphasis on young scientists. Since the last Conference in Wroclaw in 2016 the Parnas Conference became a Young Scientists Forum with financial support of FEBS and as the FEBS3+ Meeting.

General opinion is that all ten previous Parnas conferences were successful, and I wish to thank all my colleagues who had contributed so much in the organization of these conferences. In particular, I wish to express our deep gratitude to Professor Rostyslav Stoika who had initiated Parnas Conferences, to Professor Andrzej Dzugaj and Professor Jolanta Baranska – former presidents of the Polish Biochemical Society, to Professor Michael Eisenbach, former President of the Israel Society for Biochemistry and Molecular Biology as well as to Professor Israel Pecht - former FEBS General Secretary and Professor Adam Szewczyk - former FEBS Congress Counsellor for their support and contribution into the Parnas Conferences organization.

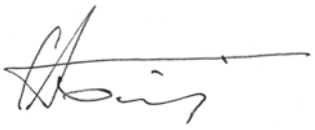
Almost everybody at our Conference is well aware why it bears the name of Jakub Parnas (1884-1949). But still I wish to remind that this outstanding scholar was born in Austro-Hungarian Empire in a village in Drogobytch region, which is in recent Ukraine but after the First WW became in Poland, was studying sciences in Germany, France and Switzerland, and was working in France and in Poland (in Warsaw and mainly in Lviv) until 1939. In all fairness, he is considered to be the founder of Polish Biochemistry. Due to the known events in 1939 Parnas became a Soviet citizen still living and working in Lviv (in the then Ukrainian Soviet Socialist Republic) until 1941 when Nazis occupied Lviv, and he was evacuated to Ufa and later to Moscow. For some time in Ufa he was collaborating with our Institute of Biochemistry, which was evacuated there from Kyiv during the WW II. Due to his outstanding contribution to Biochemistry, Parnas was highly estimated in the Soviet Union being elected to the USSR Academy of Sciences and Academy of Medical Sciences and decorated with highest awards. But it was USSR under Stalin and on January 28, 1949 he was arrested by KGB and next day died in KGB prison because of diabetes crisis or heart attack.

Indeed it was a personal tragedy but also a huge loss for the world's science. That's why establishing the Parnas Conferences on modern Biochemistry is our Biochemical Societies' homage to this great scholar and personality.

Please allow me again, on behalf of the Organizing Committee, to wish you all fruitful and enjoyable stay in Kyiv.

Yours sincerely

Professor Serhiy Komisarenko,
President of the Ukrainian Biochemical Society,
Chairperson of the Organizing Committee



PRE-CONFERENCE WORKSHOP

INNOVATIVE MEDICINE DOES NOT EXIST WITHOUT KNOWLEDGE TRANSFER

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Introduction: Most scientists are self-directed, and they desire to make discoveries independently. In the 21st Century, this situation is changing rapidly and nowadays researchers and research institutions look for collaborations to enable capacity building and offer multiple opportunities that surpass the limitations of a single institution and scarce resources.

Methods: Develop – It is the nature of research Scientists to develop new methods, markers, tests, and instruments. *Aware* – Scientists realize the scientific value of their research and apply for protection of their research output. *Protect* – Technology transfer professionals initiate Intellectual Property Protection. *Accept* – Scientists publish their results to motivate other scientists, advance their research, and gain acceptance of novelty by the scientific community. *Investigate* – Bringing into medical practice the newly developed diagnostic or treatment modalities starts with market research to investigate the market value and benefit for medical practice. *Demonstrate* – Initiate clinical research to demonstrate that the invented methods, markers, tests, or instruments can perform the predicted non-harmful, well-defined medical applications. *Agree* – The medical community accepts, and the interested companies realize the market opportunities. *Benefit* – Investigate the cost versus benefit of the new application in medical practice.

Results: Researchers: are *aware* of the intellectual property (IP) potential of their research; *protect* their results and make intelligent decisions to avoid premature publication; understand the basics of IP management to maximize the economic benefit from public research; *develop* new products and services; publish results to investigate whether the scientific community; accept the novelty of the research work; *investigate* the relationship between basic research and clinical research; *demonstrate* the advantages of the novel drugs, markers, diagnostic and treatment methods in clinical research; *agree* to transfer results of the translational research into the practice; and have a clear view of the cost *benefit* of healthcare technologies to make diagnostic procedures and therapies more effective.

Discussion – Conclusion: The ongoing process of knowledge integration is removing territorial borders and fostering research collaborations to expedite laboratory research transformation into practice via the steps of Knowledge Transfer: Develop: (1) Aware, (2) Protect, (3) Accept, (4) Investigate, (5) Demonstrate, (6) Agree, and (7) Benefit. The Knowledge Transfer Chain™: *Develop – Aware – Protect – Accept – Investigate – Demonstrate – Agree – Benefit (DAPAIDEB)* helps the advancements of biochemistry and molecular biology gained through various laboratory techniques to develop new ways to treat patients and are key for Innovative Medicine.

Acknowledgment: We thank Cedars - Sinai Medical Center's International Research and Innovation in Medicine Program, the Association for Regional Cooperation in the Fields of Health, Science and Technology (RECOOP HST Association) for their support.

PLENARY LECTURES

GLOBAL BIOSECURITY IN THE 21ST CENTURY

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Widening access to affordable enabling technologies alongside ever-increasing technical capability is surely a good thing for molecular biologists. Yet policymakers are increasingly taking note of the dual-use potential of the life sciences, concerned that biotech (and biology in general) poses a great risk to human and environmental health and security. In the same way that cybersecurity emerged to secure digital technologies, biosecurity is emerging as an integral component of the 21st-century biotechnology toolkit.

All biologists have a stake in a being part of a new drive to make biology more secure. This talk provides an overview of ongoing developments in the biosecurity field, which has rapidly expanded in scope and manpower in the last decade to deal with matters relating to DNA synthesis regulation, synthetic biology and terrorism, invasive species and pandemic pathogens, as well as DIY CRISPR kits and unregulated gene therapy. Special attention will be given to developments in the governance of CRISPR genome editing, and the loopholes being exploited to bring advanced genome editing to settings far beyond the lab and the clinic.

SINGLE CELL SEQUENCING IN SPACE AND TIME

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Abstract is not presented

KEY-NOTE LECTURES

PROTEIN COALATION AND ANTIOXIDANT FUNCTION OF COENZYME A

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Coenzyme A (CoA) is a key metabolic integrator in all living cells. CoA and its thioester derivatives (acetyl CoA, malonyl CoA, HMG CoA etc.) participate in diverse anabolic and catabolic pathways, allosteric regulatory interactions and the regulation of gene expression. Dysregulation of CoA/CoA derivatives biosynthesis and homeostasis has been associated with various human pathologies, including metabolic disorders, cancer and neurodegeneration.

We have recently discovered a novel post-translational modification involving covalent attachment of CoA to cellular proteins. Cell-based and animal models were employed to demonstrate that protein CoAlation is a reversible post-translational modification induced by oxidizing agents and metabolic stress in prokaryotic and eukaryotic cells. Furthermore, we developed a strategy for the identification of CoA-modified proteins in cells and tissues which allowed us to identify by MS-MS a diverse range of CoAlated proteins. Protein CoAlation alters the molecular mass, charge, and activity of modified proteins, and prevents them from irreversible sulfhydryl overoxidation. A selected panel of identified proteins were shown to be CoAlated *in vitro* and *in vivo* in ways that modified their activities. Based on these findings, we propose that under physiological conditions CoA functions to produce metabolically-active derivatives, but has a potential to act as an antioxidant in response to oxidative or metabolic stress. Current advances and future perspectives in this emerging field of research will be discussed.

CHAPERONIN NANO-MACHINES: ALLOSTERY AND FUNCTION

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Chaperonins are nano-machines that are built of two back-to-back stacked heptameric rings. They assist protein folding by undergoing large conformational changes that are controlled by ATP binding and hydrolysis. In the *E. coli* cell, only about 60 different proteins require GroEL for efficient folding. In the first part of the talk, I will describe work that was aimed at determining the properties that distinguish GroEL clients from non-clients. In the second part of the talk, I will describe new approaches for establishing allosteric mechanisms. Using these approaches, it was possible to show that the chaperonin GroEL from *E. coli* undergoes concerted intra-ring conformational changes whereas its eukaryotic homolog CCT/TRiC undergoes sequential intra-ring conformational changes. The impact of these different allosteric mechanisms on the folding functions of GroEL and CCT/TRiC will be discussed.

IMMUNE EVASION MECHANISMS IN CLASSICAL HODGKIN LYMPHOMA: TOWARDS NEW IMMUNOTHERAPIES

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Classical Hodgkin lymphoma (cHL) is a B cell malignancy diagnosed in ~20,000 new patients in North America and Europe each year. Classical HLs include small numbers of malignant Reed–Sternberg (RS) cells within an extensive inflammatory infiltrate. Despite the abundant inflammatory infiltrate in cHL, there is little evidence for an effective immune response against tumor cells. Recent studies, undertaken to explain this paradox, demonstrated that R-S cells are genetically hardwired to suppress effective immune response. High NF-κB activity, triggered by mutations in this pathway, promotes the secretion of cytokines such as CCL5, CCL17 (TARC) and CCL22, which attract Th2 and Treg cells. R-S cells exhibit frequent abnormalities in the antigen presentation pathway (β2-microglobulin loss, C2TA fusion genes) that decrease MHC I and MHC II surface expression. Our group found that RS cells selectively overexpressed the immunoregulatory glycan-binding protein, galectin-1 *(LGALSI*), through an AP1-dependent enhancer, thus suppressing anti-tumor Th1 responses and favoring expansion of Treg cells (CD4+CD25 high FOXP3+). In addition, RS cells overexpress PD-L1 and PD-L2 ligands as key targets at the 9p24.1 amplification peak in cHL lines and primary Hodgkin Reed-Sternberg cells.

Since the NFkB and JAK/STATs pathways control the expression of multiple immunoregulatory proteins, therapeutic targeting of NFkB and STATs might decrease their expression, favoring immunogenicity of R-S cells. NFkB and STATs are directly regulated by the oncogenic PIM1/2/3 serine/threonine kinases. We found that PIM1/2/3 were ubiquitously expressed in primary and cultured RS cells. Genetic or chemical PIM inhibition decreased cap-dependent protein translation, blocked JAK-STAT signaling, and markedly attenuated NFkB-dependent gene expression. PIM inhibitor decreased the expression of multiple molecules engaged in developing the immunosuppressive microenvironment, including galectin-1 and PD-L1/2. In coculture experiments, T cells incubated with a PIM inhibitor – treated RS cells exhibited higher expression of activation markers than T cells coincubated with control RS cells. These findings indicate that PIM kinases in cHL exhibit pleiotropic effects, orchestrating tumor immune escape and supporting RS cell survival. Inhibition of PIM kinases decreases RS cell viability and disrupts signaling circuits that link RS cells. Thus, PIM kinases are promising therapeutic targets in cHL.

Taken together, RS cells are genetically and functionally programmed to blunt host anti – tumor responses. Importantly, we have shown that a detailed understanding of these mechanisms can be effectively translated to game-changing therapies for cHL patients.

YOUNG SCIENTISTS' TALKS

SYMPOSIUM I. INTRACELLULAR SIGNALING

NEW VIEW ON DEVELOPMENT OF DIABETIC POLYNEUROPATHY

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Peripheral neuropathy is one of the most common complications of diabetes. Previously, we showed that administration of Kokarnit for 9 days led to the restoration of basic dynamic parameters of skeletal muscle contraction in rats with diabetic polyneuropathy. However, pathogenesis of diabetic polyneuropathy and relationship with vitamins are still unclear. So, the aim of this study was to investigate the mechanism of diabetic polyneuropathy development and involvement of vitamins in this pathology.

Methods. The experiment was conducted on 240 white non-linear male rats, which were divided into 4 groups. Group 1 – intact animals, in animals of groups 2-4 – diabetic neuropathy was induced by i.p. injection of streptozotocin in dose 65 mg/kg. Diabetes in rats was confirmed by the presence of hyperglycemia. On the 30th day of the experiment glucose loading test for confirmation of diabetes presence were conducted. After confirmation of diabetes, for 9 days rats in group 2 was administered saline, rats in group 3 – 0.5% lidocaine hydrochloride and rats in group 4 – Kokarnit (1 mg/kg, i/p) dissolved in 0.5% lidocaine hydrochloride (World Medicine) at a dose of 1 mg/kg. The drug contains 20 mg of nicotinamide, 50 mg of cocarboxylase, 500 μg of cyanocobalamin, 10 mg of disodium trihydrate adenosine triphosphate (ATP).

After autopsy in efferent n. tibialis we determined the content of sorbitol, cholesterol, phospholipids, free fatty acids, triglycerides, the levels of VEGF, bFGF, NGF, Caspase-3, NF-Kb, t-PA and content of TBA-active products of lipid peroxidation and activity of antioxidant enzymes (superoxide dismutase and catalase).

Results. The development of diabetes type I in rats led to an increase in n. tibialis of sorbitol content, the level of Caspase-3, NGF, VEGF, t-PA and to increased content of TBA-active products of lipid peroxidation and changes in the activity of antioxidant enzymes. This indicates the involvement of the studied parameters in the pathogenesis of diabetic polyneuropathy. Introduction of Kokarnit to rats with diabetic neuropathy restored the imbalance in the pro-/-antioxidant system, decreased the bFGF and NGF levels and didn't influence the levels of sorbitol, Caspase-3, VEGF, t-PA and NF-Kb compare to the rats with diabetic neuropathy.

Conclusion. Development of diabetic polyneuropathy was associated with changes in the antioxidant system and proteins level. Kokarnit doesn't influence all links pathogenesis of diabetic polyneuropathy. The positive effect of Kokarnit also can be related to changes in levels of bFGF and NGF in n.tibialis and with its antioxidant properties.

STRUCTURAL AND BIOPHYSICAL CHARACTERIZATION OF CHLORIDE INTRACELLULAR CHANNELS INHERENT FLEXIBILITY

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Chloride intracellular channels (CLICs) are a family of unique metamorphic proteins. While exhibiting both soluble and transmembrane forms, their cellular roles remain largely unknown. Here, we present the high-resolution crystal structures of the human CLIC5 and mouse CLIC6. The structures reveal that both family members share a monomeric conformational arrangement with a high degree of structural conservation with other CLICs. Moreover, Small Angle X-ray Scattering (SAXS) analysis under reducing conditions demonstrates a monomeric distribution in solution, reminiscent of the crystallized form. Importantly, the ensemble optimization method revealed that CLICs can sample multiple conformations in solution, supporting the metamorphic classification of the family. Finally, we provide evidence for the ability of both CLIC5 and CLIC6 to undergo deferential oxidation-dependent oligomerization in solution, which is highly enhanced by the presence of membrane mimetics. These results provide insights into the inherent flexibility of CLICs and provide mechanistic clues into their possible regulation by changes in cellular redox potential.

MITOCHONDRIAL POTASSIUM CHANNELS AND CARBON MONOXIDE SIGNALING

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Carbon monoxide (CO) is an endogenously produced gaseous transmitter, which is involved in the maintenance of cellular homeostasis. A wide range of CO signaling events is incited by a mitochondrial cascade of metabolic and ROS signaling, which eventually contribute to the cellular life-or-death decision. The mechanisms of its action are largely unknown, but one of the pathways is attributed to possible regulation of the activity of large-conductance potassium channel in the inner membrane of mitochondria (mitoBK). To address modulation of mitoBK by CO, we evaluated the effect of CO-saturated solution in the mitoBK single-channel patch-clamp recordings of mitochondria isolated from human astrocytoma cells. Subsequently, we assessed the pharmacological action of several structurally different CO-releasing molecules (CORMs), which are typically used in the studies of CO regulation of cell membrane BK channels. While the CO-saturated solution has not exerted significant modulation of the channel activity without application of exogenous heme, the effects of CORMs were vastly disparate and varied by the class of compound applied. Therefore, our observations raise an issue of specificity of CORMs and their appropriateness in the study of mitochondrial ion channel pharmacology.

This work was supported by the Polish National Science Centre (grant no. 2015/17/B/NZ1/02496) and MSCA-COFUND #665735 (Bio4Med).

SYMPOSIUM II. THE BIOCHEMISTRY OF THE CELL

TRANSGENERATIONAL EFFECTS OF LATE REPRODUCTION – AN INSIGHT FROM MULTIOMICS

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An average parental age of children birth is increasing in modern society. Here we used a power of drosophila model to study the transgeneration effects of late reproduction. Initial flies were kept till late reproductive age where a reduction of laid eggs was more than 90%. These eggs were used to generate flies of the next generation. This protocol was repeated for 5 generations. Initial and experimental flies were bred for about 100 generations on regular food and then were tested for lifespan, stress resistance and reproduction. In addition, an expression of multiple genes was measured by RNA-seq and varied proteins and metabolites by LC-MS/MS.

Experimental flies of both sexes have significantly reduced lifespan on all diets tested. Also, these flies are more sensitive to starvation with reduced fecundity in females. Transcriptome analysis showed that expression level of more than 120 genes is changed by more than two-fold. Gene clusters within GO categories related to regulation of development are significantly increased in flies of both sexes. Significantly decreased genes belong to categories related to transcription, translation and energetics. KEGG analysis of transcriptome shows activation of Wnt, Hippo, FOXO and Notch pathway with downregulation of those related to aminoacid and fat metabolism. The quantities of more than 1500 proteins are affected in selected flies. Protein amounts of GO categories and pathways related to aminoacid and carbohydrate metabolism are increased. However, those for metabolism of fatty acids and response to stresses were decreased in both gene ontology and pathways. All these changes are connected to rearrangement of metabolism. A significant increase of metabolites of the glycolytic pathway with reduced those of TCA shows that those flies became more anaerobic and generate more energy by glycolysis.

Thus, reproduction at a later age has the huge impact by affecting physiology, transcriptome, proteome and metabolism in offspring after 100 generations.

UNLOCKING THE COMBINATORIAL EPIGENETIC CODE AT A SINGLE-MOLECULE LEVEL

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Different combinations of histone modifications have been proposed to signal distinct gene regulatory functions, but are poorly addressed by existing technologies. We recently published a novel high-throughput single-molecule imaging technology to decode combinatorial modifications on millions of individual nucleosomes (Shema et al., Science, 2016). We apply the technology to study combinatorial modification states of pluripotent stem cells and lineage-committed cell, as well as explore the epigenome of cancer cells. We combine this proteomic platform with single-molecule DNA sequencing technology to simultaneously determine the modification states and genomic positions of individual nucleosomes. This single-molecule technology can shed light on fundamental questions in chromatin biology and epigenetic regulation. It also has the potential to uncover combinatorial transcription factor interactions on regulatory DNA at a single-molecule level, as well as adaptations for single cell analysis.

MECHANISMS OF SHORT- AND LONG-TERM MECHANOSENSING OF MATRIX RIGIDITY

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Cells sense and respond to mechanical characteristics of their environment in a variety of ways. In particular, the rigidity of the extracellular matrix (ECM) to which cells adhere is a critical determinant of the most fundamental cellular processes, including cell migration, differentiation, death, and growth. In order to test the rigidity of the ECM, cells apply cytoskeletal-based forces to it; however, there are fundamental aspects of this 'mechanosensing' process that are poorly understood. In my talk, I will discuss our recent studies in which we aim at understanding the kinetics of mechanosensing. In particular, we aim to comprehend how short-term mechanosensing events lead to long-term cellular decisions on proliferation.

SYMPOSIUM III. CELL SIGNALING IN CANCER

SLAMF1/CD150-MEDIATED SIGNALING IN CHRONIC LYMPHOCYTIC LEUKEMIA B CELLS

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SLAMF1/CD150 is differentially expressed in chronic lymphocytic leukemia (CLL) B cells. It was shown that higher cell surface CD150 expression level is associated with a more favorable outcome for CLL patients. However, the mechanisms that underlie different biological properties of CLL B cells depend on CD150 expression are not fully understood. In the present study we focused on the characterization of CD150-mediated signaling pathways in CLL B cells. We found that expression levels of CD180, CD95, CD20, CD22, CD48 and HLA-DR were significantly upregulated in CD150 positive (csCD150+) compared to CD150 negative (csCD150-) CLL B cells. Moreover, CD150 demonstrated the highest level of colocalization with CD180 receptor on the cell surface membrane of CLL B cells. The basal levels of tyrosine phosphorylation and phosphorylation of serine/threonine-specific motifs that are substrates for AMPK, Akt, PKA, PKC, CDK kinases were higher in csCD150+ than in csCD150- CLL B cells. Ligation of CD150 on CLL B cells led to activation of pro-survival Akt, mTOR, ERK1/2, JNK1/2 and p38MAPK signaling pathways. However, simultaneous crosslinking of CD150 and CD180 displayed inhibitory effect on activation of Akt/mTOR signaling pathway, as well as MAPK pathways. The CD150- and CD180-mediated decreasing in Akt kinase activity had the strongest effect on mTORC1 pathways rather than on FOXO3a or GSK3β. Furthermore, we showed that both CD150 and CD180 were involved in regulation of transcription factors expression in CLL B cells. Ligation of CD150 and CD180 alone or in combination upregulated IRF8 and PU.1 while downregulated the IRF4 mRNA expression. Signaling via CD150 or CD180 alone elevated the level of BCL6 mRNA. Strong downregulation of IRF4 mRNA was observed after CD150, CD180 or CD150+CD80 ligation on CLL B cells. We found that in CLL B cells CD150 is a negative regulator of SPIB while CD180 is involved in the upregulation of EBF1 expression level. Moreover, CD180 ligation on CLL B cells caused an increase in CD150 mRNA level that is one of the EBF1 target genes. Taken together, CD150- and CD180-mediated signaling may be a restraining factor for neoplastic CLL B cells propagation in more than 50% of CLL cases where these receptors are coexpressed by blocking spreading of pro-survival Akt/MAPK signaling pathways and regulating transcriptional factors expression in CLL B cells.

COUPLING-ENHANCED ROBUSTNESS IN A DISORDERED PROTEIN NETWORK

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Cell homeostasis effectively buffers variations in salt concentrations. With ± 100 mM, the remaining fluctuations are typically small and not detrimental for protein function. We found that this paradigm is violated for intrinsically disordered proteins. The affinities between the mutually interacting disordered zipper domains of Myc, Max, and Mad3 exhibit ultra-sensitive salt dependencies. Single-molecule experiments with the labeled domains spot a salt-driven redistribution of electrostatic and hydrophobic interactions in the bound and disordered states even though the sequences are inconspicuous in terms of net charge, charge patterning, and hydrophobicity. However, coupling the binding reactions dampens this sensitivity and restores robustness. This dashpot effect roots in the high sequence homology between the domains and two other members of the superfamily (MondoA and Mlx) show a similar behavior. Protein networks based on disordered homologous sequences can, therefore, be surprisingly robust towards solution conditions, a crucial prerequisite for synchronized gene expression patterns across heterogeneous cell populations.

ADAPTOR PROTEIN RUK/CIN85 IS A KEY REGULATOR OF EPITHELIAL-MESENCHYMAL PLASTICITY OF BREAST CANCER CELLS

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Epithelial-to-mesenchymal transition (EMT) is a reversible process underlying tumor invasiveness and metastasis. EMT is triggered through up-regulation of transcription factors Snail1, Slug, Twist, ZEB1/2, in response to hypoxia, TGFβ, Notch, Wnt signaling etc. Adaptor proteins serve as molecular platforms for multimolecular complexes assembly and thereby regulate cell signaling. Adaptor protein Ruk/CIN85 was demonstrated to be overexpressed in several types of tumors (including breast cancer) in comparison to surrounding conditionally normal tissues. This adaptor has more than 200 interaction partners and plays critical role in important cellular processes such as ligand-induced endocytosis of RTKs, intracellular vesicular trafficking, adhesion, motility, and survival. In this study, we investigated the effect of Ruk/CIN85 on the epithelial-mesenchymal plasticity of murine breast adenocarcinoma 4T1 cells.

As a model, we used sublines of mouse breast adenocarcinoma 4T1 with stable overexpression and downregulation of full-length form of adaptor protein Ruk/CIN85. Motility and invasiveness *in vitro* were estimated using a scratch test and Boyden chamber assay, respectively. The metastatic potential *in vivo* was analyzed using experimental and spontaneous metastasis models. Expression of EMT-related genes was evaluated by Qiagen RT2 Profiler PCR Array and additionally proved by Western-blot analysis, confocal microscopy or qPCR.

Ruk/CIN85-overexpressing 4T1 cells were characterized by increased motility, invasiveness and metastatic potential, while downregulation of Ruk/CIN85 led to opposite effects. Up-regulation of Ruk/CIN85 in 4T1 cells resulted in dramatic changes in EMT-related genes expression: induction of main EMT markers, such as key transcriptional factors SNAIL, Twist, Zeb1/2 as well as vimentin, Lcn2, reprogramming and inflammation-related genes, but decreased expression of ECM degradation enzymes, cellular adhesion molecules. Ruk/CIN85 knockdown in 4T1 cells led to increased expression of epithelial marker E-cadherin, as well as extracellular proteases and adhesion molecules.

The obtained data suggest the role of adaptor protein Ruk/CIN85 as a potent regulator of EMP in breast cancer cells.

This study was supported by the State Fund For Fundamental Research (project F83).

SYMPOSIUM IV. LIGAND-RECEPTOR BINDING IN CELL FUNCTIONS

MEDICAGO ABCG TRANSPORTER INVOLVED IN SIGNALING MOLECULES SECRETION DURING ARBUSCULAR MYCORRHIZA FORMATION

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Arbuscular mycorrhiza (AM) represents one of the most ancient and widespread symbiotic interactions. The establishment of this beneficial association is tightly regulated by the exchange of chemical signals between symbionts. We are raising the question about the role of plant membrane proteins belonging to the ABC (ATP-binding cassette transporters) family in the secretion to the rhizosphere the plant-derived signaling molecules promoting AM. Analyses of transcriptomic and phylogenetic data allowed us to identify/propose ABC proteins in model legume plant *Medicago truncatula* that potentially participate in the transport of strigolactones (SL) – well recognized AM branching factors. In Medicago genome, we have identified MtABCG23 being a homolog of the PhPDR1 an ABCG protein that was described as a strigolactones exporter in *Petunia hybrida*. We showed that *MtABCG23* exhibited root specific expression and similarly to *PhPDR1* was up-regulated by phosphate starvation and synthetic SL (GR24). A spatial expression pattern analyses with GUS and GFP reporter genes revealed that *MtABCG23* promoter is active in root tip where SL are biosynthesized and in the cortical cells, consistent with MtABCG23 postulated function as strigolactone exporter from root to soil. Further phenotypic analysis involving the grid method revealed that *mtabcg23* mutants displayed reduced interaction with AM fungus compared with the wild-type plants. The latter was additionally confirmed at a molecular level using fungus- and plant-derived marker genes which expression reflect colonization rate. Finally, root exudate from *mtpdr23* stimulates to a smaller extent than WT, SL-inducible germination of *Phelipanche ramose* seeds.

National Science Centre 2015/17/D/NZ3/03625.

NOVEL ANTICANCER DRUGS FOR CIRCUMVENTING MULTIDRUG RESISTANCE AND ENHANCEMENT OF THEIR ACTION THROUGH TARGETED DELIVERY BY NANOSCALE CARRIERS

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Multidrug resistance (MDR) is considered the major factor which is responsible for over 90% of failures in the treatment of cancer patients. That's why search for novel anticancer drugs possessing innate ability to circumvent cancer drug resistance remains an actual task for modern pharmacology and medicine. Landomycin antibiotics are extremely promising candidates for this role. Uniquely shaped benz(a)anthracene tetracyclic framework with an angularly condensed ring is considered the most characteristic feature of landomycins, which distinguishes them from other aromatic polyketides. Also, it may be responsible for innate circumvention of MDR by these antibiotics, leading to early cytosolic hydrogen peroxide burst accompanied by activation of caspase-7 and PARP-1 cleavage upstream of mitochondria (Panchuk et al, 2017). Landomycin A (LA), possessing hexasaccharide side chain, is the most active representative of landomycins possessing strong anticancer activity towards murine NK/Ly lymphoma in vivo and relatively low side effects compared to doxorubicin (Kozak et al, 2018). LA is soluble in pure ethanol (10 g/l) and thus has to be injected intraperitoneally or intravenously in aqueous alcoholic solutions (2% v/w), but they are unstable due to rapid drug degradation. In order to solve this problem, a novel stable water-soluble pharmaceutical form of LA was developed on the base of poly(2)-oxazoline nanocarrier, which should possess enhanced bioavailability and decreased side effects. *In vitro* studies revealed that immobilization of LA on polymeric carrier enhanced its cytotoxic activity 1,5-fold towards drug-resistant tumor cells of HL-60/adr line (MRP-1+) tumor cells and 2-fold towards HL-60/vinc line (P-gp+), while on parental HL-60 cell line no difference between LA and LA-polymer complex was observed. These phenomena also positively correlated with the 2-fold decrease in ROS production in drug-resistant cells under the action of LA complex with poly(2)-oxazoline nanocarrier, thus indicating on antioxidant properties of this nanocarrier, which may be of high importance *in vivo* by diminishing potential side effects of LA. Further studies of therapeutic properties of LA-polymer complexes on murine tumor models are in progress.

MECHANISM OF SIGNALING AND BIOLOGICAL ROLE OF NICOTINIC ACETYLCHOLINE RECEPTORS IN MITOCHONDRIA

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Nicotinic acetylcholine receptors (nAChRs) located in the outer membrane of mitochondria regulate cytochrome *c* (Cyt *c*) release (known as the first stage of mitochondria-driven apoptosis) under the effect of apoptogenic agents like Ca^{2+} or H_2O_2. The nAChR signaling in mitochondria is ion channel-independent and involves intra-mitochondrial kinases. The aim of the present study was to delineate the mechanism of mitochondrial nAChRs signaling and to evaluate their role under different physiological conditions.

Methods. Mitochondrial and non-mitochondrial fractions were isolated from either the liver or brain of C57Bl/6, a7-/-, a3+/-, a7b2-/-, b4-/- mice or Wistar rats. The level of nAChR subunits was studied by Sandwich-ELISA using subunit-specific antibodies. The apoptotic resistance of mitochondria was evaluated based on the level of Cyt *c* released under the effect of Ca^{2+} or H_2O_2.

Results. By using nAChR subunit-specific ligands, like α7-specific allosteric modulator PNU120596, β2-specific allosteric modulator dFBr or α3β4-specific noncompetitive antagonist 18-MC, it was found that α7 subunits affect mainly CaKMII-dependent signaling pathway, β2 subunits are connected to Src-dependent pathway and α3β4 signaling can be stimulated by the binding of the α3/β4 intersubunit site in mouse mitochondria. The absence of α7-/-, β2-/- or decrease of α3 subunits in mutant mice did not critically affect mitochondria sustainability to Ca^{2+} due to compensatory expression of α9 and/or b4 subunits. Neuroinflammation caused by either LPS injection or immunization with α7(1-208) resulted in decreased level of α7 nAChRs in the mouse brain accompanied by decreased mitochondria sustainability to Ca^{2+}. In contrast, the increase of a7-, a3-, α4- and, especially, a9-containing nAChRs was found in the rat liver mitochondria 3-6 h 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. The study shows that signaling of mitochondrial nAChRs can be stimulated by the ligand binding to either extracellular orthosteric or transmembrane allosteric site, the different nAChR subunits being connected to different signaling pathways in mitochondria. Therefore, the presence of several heteromeric nAChR subtypes in mitochondria provides a reliable protection from different apoptogenic factors. The level of α7 nAChRs is critically important to protect the brain 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.

SYMPOSIUM V. FROM CELL TO ORGANISM

AP1/AXL AXIS DETERMINES PI3K EFFICACY IN ESOPHAGEAL AND HEAD AND NECK CANCERS

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AXL expression and activation confer resistance to multiple anti-cancer agents including of inhibition of the Phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), thus uncovering the molecular mechanism that regulates AXL expression in cancer cells is urgently needed. In this work, we demonstrated that AXL expression levels determine the sensitivity of the human papilloma-virus positive and negative (HPV^{pos} and HPV^{neg}) head and neck and esophageal squamous cell carcinoma cell lines (HNCC and ESCC) to the isoform-specific inhibitors of the PI3K, BYL719, *in vitro* and *in vivo.* AXL expression is regulated by the AP-1 transcription factor, as knockdown of AP-1 members (c-JUN and FOS) resulted in a reduction of AXL expression, which was concomitant with enhance sensitivity to BYL719 *in vitro.* The association in the expression of AXL and c-JUN was validated in cohorts of head and neck cancer patients and in 17 HPV^{pos} and HPV^{neg} HNC and ESCC cell lines. Pharmacological inhibition of the c-Jun N-terminal kinase, JNK, using SP600125 had additive or synergistic anti-tumor activity against 13 PIK3CA- mutated and HPV^{pos} tumor cells lines *in vitro* when combined with BYL719. *In vivo*, the efficacy of drug combination showed a potent anti-tumor activity in two HNC cell lines and two patient-derived xenografts. A significant reduction of tumor cell proliferation and further inhibition of mTOR was observed when mice treated with the combination of SP600125 and BYL719 compared to a single agent. Collectively, our data suggest that AXL is transcriptionally regulated by AP-1, and reducing its expression using JNK inhibitors is a new therapeutic strategy that needed to be tested in patients with HPV^{pos} or PIK3CA-mutated HNSCC and ESCC patients.

A NOVEL MECHANISM REGULATING CELL PROLIFERATION AND ORGAN SIZE

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Apoptosis culminates in the activation of caspase-3, which plays an important role in implementing the cell death program. Here, we reveal a non-apoptotic role of caspase-3 as a key regulator of cell proliferation and organ size. Caspase-3 is specifically activated in the proliferating cells of the sebaceous gland, but does not instruct cell elimination. Deletion or chemical inhibition of caspase-3 diminishes cell proliferation, decreases cell number and reduces sebaceous gland size *in vivo*. Exploring the underlying mechanism, we demonstrated that α-catenin is cleaved by caspase-3, thus facilitating the activation and nuclear translocation of yes-associated protein (YAP), a vital regulator of organ size. Accordingly, activation of caspase-3 leads to YAP-dependent organ size augmentation. Finally, we showed that X-linked inhibitor of apoptosis protein (XIAP) serves as an endogenous feedback antagonist for the caspase-3/YAP signaling module. Taken together, we report here a molecular mechanism wherein the apoptotic machinery is refocused to regulate cell proliferation and orchestrate organ size.

NOVEL ASPECTS IN THE REGULATION OF CELL MOTILITY VIA S6K1

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Background: Ribosomal protein S6 kinase 1 (S6K1) is involved in the regulation of protein synthesis, cell cycle, metabolism, and cell growth. In mammalian cells, S6K1 has several isoforms: p85S6K1, p70S6K1, p60S6K1, p31S6K1, which were shown to have different structural and functional features. Recent studies have linked S6K1 to the regulation of cellular migration, but the precise mechanism is not known.

The aim of the project was to study the subcellular localization of S6K1 during cancer cells migration and the impact of different S6K1 isoforms on the cell motility.

Methods: Human breast cancer cell line MCF-7, histological sections of human breast cancer and normal tissue were used. Subcellular localization was studied by immunofluorescence analysis and confocal microscopy. Scratch assay and 3D culture models were used to analyze cell migration. MCF-7 cell lines with knockout of different S6K1 isoforms were generated by CRISPR/cas9 system. Protein interactions were studied by co-immunoprecipitation and computational analysis.

Results: Analysis of histological sections revealed nuclear localization of S6K1 in breast malignant cells and mainly cytoplasmic one in conditionally normal cells. Moreover, S6K1 re-localized from cytoplasm to the nucleus during migration of MCF-7 cells from multicellular spheroids onto growth surface. Immunofluorescence analysis of S6K1 and its possible targets in migrating cells demonstrated the co-localization between transcription factor TBR2 and S6K1 in MCF-7 cells. For the first time, interaction between S6K1 and TBR2 was revealed by co-immunoprecipitation.

To study the involvement of different S6K1 isoforms into the regulation of cell motility MCF-7 cell lines with downregulation of all S6K1 isoforms (*p85-/p70-/p60-MCF-7*) or only two of them (*p85-/p70-/p60+MCF-7*) were generated and characterized. It was revealed that *p85-/p70-/p60+MCF-7* cells had fibroblast-like elongated shape, while wild-type MCF-7 and *p85-/p70-/p60-MCF-7* had typical epithelial-like cell shape. *In vitro* scratch assay showed that *p85-/p70-/p60+MCF-7* had accelerated migration activity, while *p85-/p70-/p60-MCF-7* migrated slower than wild-type MCF-7 cells.

Conclusions: It was shown that subcellular localization of S6K1 depends on the density and locomotor activity of the breast cancer cells. Obtained data allowed assuming that p60S6K1 could be involved in the regulation of adhesion and migration in MCF-7 cell line with the different impact of S6K1 isoforms.

SYMPOSIUM VI. MOLECULAR BIOLOGY OF BACTERIA AND VIRUSES

MYSTERIOUS *orf63, orf61* AND *orf60a* AS A POTENTIAL REGULATOR OF LAMBDOID PHAGES DEVELOPMENT

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In recent years, there is a lot of reports about infections by Shiga toxin-producing *E. coli* (STEC) strains. Among them, the most dangerous are enterohemorrhagic *E. coli* (EHEC). These strains are carriers of genes coding for Shiga toxins, which are located in genomes of Shiga toxins-converting bacteriophages (Stx phages). The genes coding these toxins are silent in lysogenic bacteria, and prophage induction is necessary for toxin production. This process is provoked by mitomycin C, UV-irradiation and H_2O_2.

The available literature and research of our team indicate that products encoded in the *exo-xis* region of the lambdoid bacteriophages may be involved in the regulation of phage development. This region contains highly conserved open reading frames (ORFs) and genes. These genetic elements could affect the regulation of the bacterial cell and phage lytic development, but molecular mechanisms of activities of this are still unknown.

In view of this, we decided to determine the influence of the uncharacterized open reading frame from the *exo-xis* region on lambdoid phages development using recombinant prophages, λ and Stx phage-$\Phi24_B$. The phage mutants (lacking particular orfs) were constructed by homologous recombination.

Based on the preliminary results of the experiments, it can be conclude that *orf63, orf61* and *orf60a* from the analyzed region have a special role in the control of phage development, especially at the stage of the lysis vs. lysogenization decision. This elements may change the time of prophage induction with the use of hydrogen peroxide. The most interesting effect was observed with use phage $\Phi24_B$. The removal of these elements also influenced phages adsorption on host cell surface and survival of the *E. coli* bacteria after infection with analyzed phages. The interesting effects on lysogenization of *E. coli* bacteria and intracellular lambdoid phage lytic development were also observed.

These elements and product of their expression might be considered as potential targets for anti-EHEC drugs.

COMPARISON OF EFFECTIVENESS OF BACTERIOPHAGES AND ANTIBIOTICS AGAINST *SALMONELLA ENTERICA* SEROVARS COMMON IN POULTRY

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Salmonella enterica is a pathogen responsible for one of the most common foodborne disease – salmonellosis. Every year more than 100 million people suffer from infections caused by *Salmonella* rods all around the world, and about 90 thousand cases end with a patient's death. The primary sources of infection are contaminated poultry meat and eggs, as farming birds develop a carrier state and the bacteria can be then transfered to eggs and other chicken-derived food products. *Salmonella enterica* is also able to form a biofilm on various surfaces, therefore it can cause even more problems in the food industry. The main mean for prevention against *S. enterica* colonization of birds intestines are antibiotics added to chicken feed or water. However, this causes a rapid development of antibiotic resistant strains that can be observed in recent years. Therefore, alternative ways of preventing *S. enterica* outbreaks are being researched. One of such methods may be the use of bacteriophages, viruses that infect bacteria. However, due to the fact that *S. enterica* is subdivided into more than 2500 serotypes, it is hard to find a phage that will be equally effective against all of them.

Our research group isolated bacteriophages infecting *S. enterica* serotypes from feces of farming birds: ducks, chickens, pigeons, turkeys and gees. The aim of our work was to compare the effectiveness of a phage and commonly antibiotics (tetracycline and streptomycin) in the eradication of *S. enterica* serotypes commonly found in farming birds. We observed that the effectiveness of a single phage and phage cocktail depended on used *S. enterica* serotype, while antibiotics tended to be equally effective against all tested serotypes. Interestingly, we have also observed that some of the phages were more effective in the eradication of biofilm formed by *S. enterica* compared to their use in liquid cultures.

IDENTIFICATION OF ENDOGENOUS RETROVIRUS *ENV* GENES IN GENOME OF *GALLUS GALLUS* AND THEIR EXPRESSION ANALYSIS DURING EARLY EMBRYOGENESIS

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Introduction. Retrovirus *env* genes being "captured" in genome of viviparous ancestors plays a critical role in placental development involving in the cell–cell fusion and maternal-fetal immune tolerance. The aim of the study was to identify the *env* genes in genome of *Gallus gallus* and study their expression during early embryogenesis.

Methods. The search for retroviral env genes was carried out in *Gallus gallu*s genome assembly version 5.0. downloaded from the NCBI database. All open reading frames (ORF) longer than 450 bp were selected from downloaded genomic sequences using Unipro UGENE 1.30 and checked against the HMM model of TLV-coat motif. Then all TLV-contained ORFs were checked to contain canonical Env domains and motives using HMM search. The expression of putative *Gallus gallus env* genes (*Gal-Env*) in chicken embryo was studied using RT-PCR. Sanger sequencing was used to confirm RT-PCR products. WISH was performed with corresponding DIG-labelled RNA probes.

Results. As a result of the *in silico* search, 70 ORFs encoding TLV-coat were detected. The HMM algorithm was used to select 10 genes (*Gal-Env1-10*) whose proteins had canonical Env domains and motives: signal peptide (SP), CXXC motif, furin cleavage site, fusogenic peptide (FP), immunosuppressive domain and transmembrane (TM) peptide. FP in Gal-Env1-10 are flanked by cysteine amino acids and all of these genes have C-X_6-CC motive that are the key features of avian gamma-type envelopes. Incorporation of Gal-Env1-10 into a phylogenetic tree demonstrates that they are distinct from previously identified Env proteins and that all Gal-Env cluster with avian alpharetroviruses ALV and RSV.

Gal-Env1 and Gal-Env4 were selected for further *in vitro* and *in ovo* study based on the most significant e-values of the presence of canonical Env domains and motives. The expression of *Gal-Env1* and *Gal-Env4* was confirmed by WISH in various embryonic and extraembryonic tissues during early embryogenesis until day 6 of development. It should be noted that *Gal-Env1* and *Gal-Env4* were expressed in extraembryonic mesodermal cells in contrast to endodermal and ectodermal ones. Interestingly, the expression of *Gal-Env1* was revealed in embryonic somites that could suggest its role in myoblast fusion during myogenesis.

Conclusions. Functional endogenous retrovirus env genes acquired by *Gallus gallus* and expressed in different embryonic tissues including extraembryonic membranes. This data suggest that the role of *env* is not restricted only to placentation and that functional integration might have occurred in more archaic oviparous species.

SYMPOSIUM VII. METABOLIC ENGINEERING IN THE CONSTRUCTION OF THE ADVANCED BIOTECHNOLOGICAL PRODUCERS

ROLE OF THE PEROXISOMES AND PENTOSE PHOSPHATE PATHWAY IN XYLOSE ALCOHOLIC FERMENTATION IN THE THERMOTOLERANT YEAST *OGATAEA POLYMORPHA*

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Yeast peroxisomes are responsible for the catabolism of methanol, ethanol, fatty acids, purines, and in some organisms, also for sugar metabolism. However, the role of these organelles in xylose catabolism and fermentation remained unknown. In this work, we studied effects of the lack of peroxisomes on xylose growth and alcoholic fermentation in the thermotolerant methylotrophic yeast *Ogataea polymorpha* versus non-methylotrophic yeast *Scheffersomyces stipitis* and xylose-utilizing strain of *Saccharomyces cerevisiae*. It was found that strains of *O. polymorpha* deficient in peroxisome biogenesis *pex3Δ* and *pex6Δ* are characterized by normal growth on xylose, whereas alcoholic fermentation of this pentose was almost totally suppressed. Growth and fermentation of glucose in the mentioned mutants were unimpaired. Strains of *O. polymorpha* with overexpressed key peroxisomal enzyme alcohol oxidase were constructed. It was found that they show increased ethanol production from xylose relative to the wild-type strain. It was found that overexpression of alcohol oxidase leads to an increase in the expression of *DAS1* and *TAL2* coding for peroxisomal transketolase (dihydroxyacetone synthase) and transaldolase, respectively, which were found to be important for xylose alcoholic fermentation in *O. polymorpha*.

We found that peroxisomal transketolase and transaldolase in *O. polymorpha* are required for xylose alcoholic fermentation but not for growth on this pentose. Mutants with knock out of *DAS1* and *TAL2* normally grew on xylose though were defective in its conversion to ethanol. The mutant of *O. polymorpha* with knock out of *TAL1* normally grew on glucose and did not grow on xylose; this defect was restored by overexpression of *TAL2*. Conditional mutant *pYNR1-TKL1* with expressed cytosolic transketolase gene under control of ammonium repressible nitrate reductase promoter *YNR1* did not grow on xylose and poorly grew on glucose media supplemented with ammonium. Overexpression of *DAS1* only partially restored defects of *pYNR1-TKL1* mutant. Separate overexpression or co-overexpression of *DAS1* and *TAL2* in the wild-type strain increased ethanol synthesis from xylose 2-4 times with no effect on glucose alcoholic fermentation. Overexpression of *TKL1* and *TAL1* also elevated ethanol production from xylose. Finally, co-overexpression of *DAS1* and *TAL2* in the best isolated *O. polymorpha* ethanol producer from xylose led to an elevated level of accumulated ethanol up to 16.5 g/l at 45 °C or 30-40 times more than is produced by the wild-type strain.

FROM BY-PRODUCT TO MAIN PRODUCT – BIOSYNTHESIS OF CITRIC ACID BY METABOLIC ENGINEERED YEAST *YARROWIA LIPOLYTICA*

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Yarrowia lipolytica is an unconventional yeast, which can efficiently produce many value added compounds such as lipids, erythritol or organic acids. Because of that, the yeast was subjected to numerous genetic modification in order to improve its properties. In recent years, the topic that undergoes particularly fast development was erythritol production from glycerol. The proteins involved in the metabolic pathway of glycerol assimilation, become a target of metabolic engineering, that significantly improved erythritol production. Interestingly, these modifications also positively influence the synthesis of citric acid. In this study we present the latest research on erythritol metabolism that also improves citric acid production.

This work was realized as the Ph.D. research program 'Innowacyjny Doktorat' (no. D220/0007/18) financially supported by Wrocław University of Environmental and Life Sciences.

GLYCEROL SYNTHESIS AND BIOCONVERSION IN YEASTS

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Glycerol is widely used in cosmetic, food, pharmaceutical industries. Profound amounts of glycerol are generated as a by-product of biodiesel production, but it contains hazardous contamination (e.g. methanol, heavy metals). More refined glycerol can be produced during fermentation by yeast *Saccharomyces cerevisiae*. Crude glycerol, in turn, could be converted to value-added compounds, e.g. to fuel ethanol, by methylotrophic yeast *Ogataea polymorpha*.

In *S. cerevisiae* glycerol synthesis occurs from dihydroxyacetone phosphate (DHAP) by subsequent action of glycerol-3-phosphate dehydrogenase (Gpd1) and glycerol-3-phosphate phosphatase (Gpp2). Synthesized glycerol is exported via a channel formed by aquaglyceroporin Fps1. Competing reaction is DHAP isomerization to glyceraldehyde-3-phosphate by triose phosphate isomerase (Tpi1). Enzyme acetolactate synthase (Ilv2) can convert pyruvate to acetolactate and CO_2 thus decreasing amount of pyruvate available for alcohol dehydrogenase reaction which competes for NADH with Gpd-reaction. The *S. cerevisiae* strain BY4742 was subjected to the following genetic modifications: attenuation of *TPI1* gene promoter; expression of fused *GPD1-GPP2* ORF, modified gene *FPS1* and truncated gene *ILV2* under the control of a strong *ADH1* promoter. The best constructed strain produced more than 20 g of glycerol/l under micro-aerobic conditions and 16 g of glycerol/l under anaerobic conditions.

In *O. polymorpha,* initial steps of glycerol catabolism are catalyzed by glycerol dehydrogenase (Gcy1) and dihydroxyacetone kinase (Dak1) or glycerol kinase (Gut1) and glycerol-3-phosphate dehydrogenase (Gpd1). Obtained DHAP is converted to phosphoenolpyruvate, which is then either used in the TCA cycle or converted to ethanol through the action of pyruvate decarboxylase (Pdc1) and alcohol dehydrogenase (Adh1). The mechanism of glycerol transport into *O. polymorpha* cells is unknown, but in the yeast *Pichia pastoris*, this compound is efficiently imported through the channel formed by Fps1 protein.

The *O. polymorpha* strain NCYC495 was subjected to the following genetic modifications: genes *ADH1, PDC1,* gene *FPS1 P. pastoris* and pairs *GCY1-DAK1* or *GPD1-GUT1* were overexpressed under the control of the strong *GAP1* promoter. Obtained recombinant strains produced up to 10.2 g of ethanol/l from pure glycerol or 3.1 g of ethanol/l from crude glycerol.

SYMPOSIUM VIII. STUDYING BIOLOGY IN SINGLE-MOLECULE RESOLUTION

THE SECRET LIFE OF NEW POLYVALENT STAPHYLOCOCCAL PODOVIRUS phiAGO1.3

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Phage phiAGO1.3 is a polyvalent *Staphylococcus* lytic podovirus (Gozdek et al., 2018). It adsorbs quickly to its host cells, has a short propagation time, cannot transfer genetic material between bacteria by transduction and has a broad host range. Thus, phiAGO1.3 could be interesting for potential application in phage therapy. Surprisingly, we observed that under certain conditions the exposure of *Staphylococcus aureus* cells to phiAGO1.3 can lead to the establishment of an alternative relationship of the phage with its host, namely the emergence of a mixed population in which bacteria and bacteriophages are in a more or less stable equilibrium, and remain in stable ratios even following several serial passages – an equivalent of multiple generations. This interaction is reminiscent of the so-called phage carrier state cultures. So far several virulent phages have been suggested to have the ability to reside in their hosts and postpone lysis in order to allow their carrier cell to segregate a phage-infected and phage-free siblings. However, it has not been shown for staphylococcal phages. In the phage carrier state cultures, the majority of bacteria are resistant; however, some sensitive variants appear which, when infected by the extrinsic phage, enable a continuous production of bacteriophages in a population (Lwoff, 1953). Thus, the equilibrium of sensitive and insensitive bacteria could be maintained in the presence of a large phage population. The continuous presence of phage favors the emergence of phage resistant variants of host cells. Importantly, cells isolated by us as resistant to phiAGO1.3 are sensitive to obligately lytic staphylococcal myoviruses.

Acknowledgments: This work was supported by funds from the National Science Centre, Poland, research grant “Preludium” (UMO2012/07/N/NZ2/01596).

LESSONS FROM SELENIUM CHEMISTRY TO STUDY PROTEIN SCIENCE

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Selenium occurs rarely in natural proteins, but is becoming a commonly used element in unnatural contexts to aid in the study of protein structure and function. In its natural context, selenium's role remains uncovered in half of the 25 human selenoproteins. With the aid of chemical protein synthesis, a full characterization of many of these proteins looms close on the horizon. In unnatural contexts, selenium serves as a traceless handle in native chemical ligations and as a folding chaperone. New amino acids containing selenium allow previously unfavorable protein synthesis to occur with good yield. These lessons also allow us to study protein chemistry without the use of selenium. We will discuss selenium's contributions to protein chemistry thus far, as well as its potential in future applications.

SUBSTRATE REMODELING BY Hsp70 CHAPERONES – WHAT CAN WE LEARN USING NMR

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The Hsp70 chaperone system is ubiquitous, highly conserved, and involved in a variety of different processes that are integral to cellular homeostasis. Its function relies on nucleotide-dependent interactions with client proteins, yet the structural features of substrates in their Hsp70-bound state remain poorly understood.

We used nuclear magnetic resonance (NMR) spectroscopy to structurally characterize a small, folding-competent protein domain (TRF1) in complex with *E. coli* Hsp70 (DnaK). Backbone chemical shifts of Hsp70-bound TRF1 showed that the client protein is globally unfolded, but still contains a significant portion of the secondary structure (up to 40% helicity in regions distal from the binding site) even when bound to Hsp70.

Utilizing Chemical Exchange Saturation Transfer (CEST) NMR experiments, we demonstrated that the patterns in substrate helicity mirror those found in the unfolded state in the absence of denaturants, except near the site of chaperone binding. Therefore, Hsp70 binding does not appear to change the structural propensities encoded by the substrate primary sequence. Next, using a novel 1H-based CEST PRE experiment that we developed, Hsp70 binding was found to modify the energy landscape of the substrate by preventing non-native long-range interactions that are otherwise present in the unbound, unfolded conformation and which can lead to protein misfolding and aggregation.

Moreover, looking directly at the substrate residues located in the Hsp70 binding pocket, we showed that there are multiple conformations of the TRF1, substrate protein, bound to Hsp70 and that there is a significant amount of heterogeneity in the bound ensemble. Overall, Hsp70 binds to four different sites on this short, 53-residue protein, with three of the binding sites separated by more than ten residues.

Overall our results suggest that Hsp70 binding can significantly bias the folding pathway of client substrates such that secondary structure forms first, followed by the development of longer-range contacts between more distal parts of the protein. In addition, its promiscuous binding to client protein may serve to generate different initial starting points for the client protein to start folding during the chaperone cycle, with some of the starting structures more amenable to proper folding than others. Protein molecules that do not fold to the native state upon release, still have a chance to re-enter the chaperone cycle by binding via another site, thereby increasing their chances of ultimately folding correctly.

SYMPOSIUM IX. BIOCHEMICAL MECHANISMS IN DISEASE

TARGETING KEY SIGNALING FACTORS AS A WAY TO CONTROL MICROGLIAL ACTIVATION AND INDUCTION OF NEUROINFLAMMATION

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Neuroinflammation is a co-occurring phenomenon during pathological processes in the nervous system. A key player in this process is microglia. As moderate activation of microglia is beneficial, excessive one, however, leads to more severe degeneration of tissue and inhibition of its endogenous regeneration. One way to prevent this situation is to modulate or inhibit microglia activation.

In aim of this study was to use gene silencing technique to influence microglial activation. By targeting key proteins – NF-κB, MyD-88 and TRIF, we intended to decrease inflammatory signaling network.

Gene silencing was optimized on the stable murine microglia BV-2 cell line. Before stimulation with lipopolysaccharide (LPS), cells were transfected with designed siRNA sequences. Efficacy of transfection was assessed by evaluating the expression of NF-κB, MyD-88, TRIF as well as IL-1β, IL-6, TNF-α, TREM1, TREM2 at mRNA and protein level. Optimized sequences of siRNA were then used on primary microglia.

Our results showed that siRNA can successfully inhibit activation of microglia *in vitro* after stimulation with LPS. A significant decrease was observed in the expression of signaling proteins. However, depending on the targeted factor, different decrease patterns were observed for IL-1β, IL-6 and TNF-α. Thus, the mixture of siRNA was combined to achieve the most successful effect.

Our results provide a new method to successfully limit microglia activation with siRNA technique. This approach will be further used in vivo, in our models of Parkinson's disease and hypoxia-ischemia encephalopathy, in which severe inflammation is observed.

Acknowledgments: The project was supported by the research grant from the Jagiellonian University Medical College: 2015/17/B/NZ5/00294, K/DSC/003575.

SECRETORY AUTOPHAGY IS A NOVEL DEFENSE MECHANISM IN THE INTESTINE

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Intestinal Paneth cells limit bacterial invasion by secreting antimicrobial proteins, including lysozyme. However, invasive pathogens can disrupt the Golgi apparatus, interfering with secretion and compromising intestinal antimicrobial defense. Here we show that during bacterial infection, lysozyme is rerouted via secretory autophagy, an autophagy-based alternative secretion pathway. Secretory autophagy was triggered in Paneth cells by bacteria-induced endoplasmic reticulum (ER) stress, required extrinsic signals from innate lymphoid cells, and limited bacterial dissemination. Secretory autophagy was disrupted in Paneth cells of mice harboring a mutation in autophagy gene Atg16L1 that confers increased risk for Crohn's disease in humans. Our findings identify a role for secretory autophagy in intestinal defense and suggest why Crohn's disease is associated with genetic mutations that affect both the ER stress response and autophagy.

THE ROLE OF PERIPHERAL DOPAMINERGIC SYSTEM IN THE PATHOGENESIS OF INFLAMMATORY BOWEL DISEASES

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Our research is dedicated to the investigation of peripheral dopaminergic system and role of D2- and D3-dopamine receptors (DR) in the pathogenesis of inflammatory bowel diseases. Ulcerative colitis (UC) is the chronic inflammatory disease of the unknown etiology. Little is known on the role of the peripheral dopaminergic system in the pathogenesis of UC, despite the evidence that dopaminergic hypoactivity may result in excessive inflammation. The development of inflammation under the conditions of experimental UC is associated with a violation of dopaminergic signaling in the colon. The peripheral dopaminergic system causes a predominantly negative effect on the experimental UC flow, which is mediated by changes in phagocytic activity and profile of granulocytes and peripheral blood monocytes; and increased endothelial permeability. It was determined that in rats with experimental colitis dopamine-mediated signaling was impaired in colon mucosal layer, which was shown by the decrease of tyrosine hydroxylase (TH), monoamine oxidase-B (MAO-B), dopamine transporter (DAT) and diverse changes of D2- and D3-dopamine receptors (DR) protein levels. In biopsy materials of patients with non-specific ulcerative colitis (UC) changes in TH, D2DR and D3DR protein levels were also observed. It was shown for the first time that destruction of peripheral dopaminergic neurons has led to the improvement of UC features. One of the mechanisms, which underlies the protective role of the dopaminergic system in IBD pathogenesis, is the integrity of the colon barrier. The protection of endothelial barrier is partly maintained by the activation of central D2DR. It was proven that the use of drugs with dopaminergic activity, in particular, D2DR and D3DR agonists, is effective in ulcer healing during experimental UC and could be implemented into clinical practice.

POSTER SESSION I. BIOLOGICALLY ACTIVE COMPOUNDS

RECIPROCAL EFFECT OF CURCUMIN ON CYTOSKELETON OF RPE CELLS STRESSED WITH HYDROGEN PEROXIDE

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Age-related macular degeneration (AMD) recognized as one of the main cause of irreversible vision loss in the elderly population. Oxidative stress generation is associated with aging events and may be the leading mechanism of blindness. Retinal dysfunction is linked to oxidative damages caused both aging changes and disregulation of cellular signaling pathways. There are no available methods to cure AMD. To avoid a progression of AMD remains widely used strategy. Retinal pigment epithelial (RPE) cells are critically vulnerable to oxidative damages in view of its high specialization. Antioxidants could protect surviving and functional potency of retinal cells. Moreover, the protection of RPE cells against oxidative damage may be an effective strategy for the amelioration of early AMD as well as aging decline of the vision. Curcumin is a natural polyphenolic antioxidant isolated from turmeric (*Curcuma longa*) with neuroprotective activities. Curcumin attenuates cellular reactivity through antiproliferative effect and could potentiate selective cell death depend on reactivity or/and damage range. However, the effect of curcumin against oxidative damages of RPE cells cytoskeleton remains unknown. The aim of the study was to clarify the cytoprotective effect of low dose of curcumin in hydrogen peroxide-stressed RPE cells in vitro.

The human RPE cells were stressed with 300 μM H_2O_2 (HP) for 24 h. Both treated and untreated cells were exposed for 24 h with 2 μM curcumin. The expression of cell cycle regulator RPA70, epithelial-mesenchymal transition marker vimentin, and β-actin were analyzed with western blot.

An exposure of RPE cells to HP induced meaningful ($P \leq 0.05$) increment of RPA70, vimentin intermediate filaments and opposite decrease β-actin content. The treatment with 2 μM curcumin of PRE cells exposed to HP restored the disregulation all of them. An exposure to curcumin alone led to neither RPA70 nor cytoskeleton changes. Observed results showed the reciprocal regulation of cytoskeleton in normal and stressed RPE cells. Moreover, low dose of curcumin attenuates both filamentous proteins and RPA70 expression as potential regulator of dynamic rearrangement of RPE cytoskeleton and epithelial-mesenchymal transition in stressed RPE cells.

HEAD-TO-HEAD COMPARISON OF CALIX[4]ARENE BIS-PHOSPHONIC ACID C-145 AND IT'S SULFUR-CONTAINING ANALOG AS HEMOSTASIS EFFECTORS

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C-145 (octasodium salt of calix[4]arene-tetra-methylene-bis-phosphonic acid) was previously considered as a specific anti-polymerization agent that affects fibrin polymerization and does not notably influence other parameters of the coagulation system. C-145S (octasodium salt of thiacalix[4]arene-tetra-methylene-bis-phosphonic acid) possessing wider hydrophobic hole was expected to be more effective antithrombotic agent than C-145. The aim of the present work was to compare the action of both organic compounds on fibrin polymerization, fibrinolysis, platelets and endothelial cells.

The change of turbidity during fibrin clot formation induced by APTT-reagent and digestion induced by tPA was estimated. Turbidity study was used for the estimation of polymeric fibrin hydrolysis by plasmin in the presence of thiacalix[4]arene C-145S and calix[4]arene C145. Effects of thiacalix[4]arene C-145S and calix[4]arene C145 on the activation of Glu-plasminogen by streptokinase were studied using chromogenic substrate S2251. Platelet aggregation study was performed using aggregometry. Stimulated Ca^{2+} efflux from endoplasmic reticulum and cytoplasm were determined using specific Ca^{2+}-sensitive probes targeted to the endoplasmic reticulum (Mag-Fluo-4) and cytoplasm (FURA-2) by spectrofluorimetry.

Both C-145 and C-145S decreased the final turbidity of clot and prolonged clot lysis time in blood plasma in comparison to control value. C-145 was shown to be the more effective fibrinolysis inhibitor when studied in the model system of polymerized fibrin desAB. C-145S but not C-145 induced concentration changes of Ca^{2+} in the cytoplasm of resting platelets and significantly inhibited (up to 30%) Ca^{2+} efflux from the endoplasmic reticulum of platelets activated by ADP. Both C-145 and C-145S stimulated the proliferation of endothelial cells of PAE cell line. The effect of C-145S was more prominent.

In conclusion, calix[4]arene C-145S proved to be the more potent inhibitor of fibrin polymerization in comparison to C-145, which suggested earlier as the anticoagulant agent. C-145S proved to have much more outlined inhibitory action on Ca^{2+}-signaling in platelets and stimulatory effect on endothelial cells proliferation. Thus C-145 remained the most prospective molecular platform for the development of antithrombotic agent.

The research was a part of Project N64 "Study of calix[4]arenes as hemostatic, antifibrinolytic and antithrombotic agents." according to targeted complex program of NAS of Ukraine for fundamental research "Fundamental issues of new nanomaterials and nanotechnologies creation".

IN VITRO STUDIES ON ANTICANCER AND ANTIVIRAL PROPERTIES OF A HIGH MOLECULAR WEIGHT FRACTION ISOLATED FROM COELOMIC FLUID OF EARTHWORM *DENDROBAENA VENETA*

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Recently, it has been shown that some compounds of the coelomic fluid of earthworm *Dendrobaena veneta* may exhibit antimicrobial and anticancer activity. Therefore, we examined the effect of the high molecular weight fraction (HMWF) obtained from the *D. veneta* coelomic fluid on cell viability (NR and LDH assays) and proliferation (MTT assay) of two colon cancer (CC) cell lines (HT-29, LS180) and normal colon epithelial cells (CCD841 CoTr). Moreover, we assessed the morphological changes in HMWF-treated cells by means of MGG staining. Additionally, we examined the antiviral activity of HMWF against HHV-1 and EMCV at different stages of cell infection (pre-penetration, post-penetration, and pre-post-penetration) by a virus replication inhibition assay using virus-sensitive cell lines SiHa and L929. The changes in the virus titer ($CCID_{50}$/ml) were calculated with the statistical method (according to the arithmetical method of Reed and Muench). Moreover, cell viability after infection with the HMWF-treated or HMWF-untreated viruses was determined (MTT assay).
We found that HMWF exerted anti-proliferative effects in both CC cell lines; however, the HT-29 cells were more sensitive to the HMWF treatment (IC_{50} = 38.43 μg/ml). Additionally, HMWF did not exhibit a cytotoxic effect against normal colon epithelial cells. On the other hand, the exposure of HHV-1 and EMCV to HMWF at all tested stages of infection did not affect significantly the viral titers. However, cell viability decreased significantly after infection with the HMWF-treated viruses in comparison to the viability of cells infected with HMWF-untreated viruses.
In conclusion, our studies indicated the anticancer potential of the HMWF obtained from the *D. veneta*. Although HMWF did not show direct antiviral activity, it accelerated the death of infected cells. Thus, it may limit the spread of virus infection *in vivo.*

MOLECULAR MECHANISM OF FACTOR Xa-INHIBITORS INTERACTION

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Cardiovascular - related diseases being the major cause of mortality in developed countries demand the development of new anticoagulant drugs. Factor Xa (FXa) interconnecting internal and external blood coagulation pathways plays the important regulatory role in blood coagulation functionality. Therefore, FXa is considered one of the main targets for therapeutic blood investigations.
However, it is important not only to conduct biological tests, but also to discover and understand the key interactions between protein and ligand.
In the present study, we provide a detail description of the mechanism of molecular interaction between factor Xa of blood coagulation cascade and its direct inhibitors.
For our research several software packages and databases were used. Structural compounds of Xa inhibitors taken from ChEMBL and filtered according to ADME parameters that resulted in decreasing Enamine database from 2.5m to 1.75m compounds. Also, stereoisomers were generated for them and the structures were translated into a format suitable for docking.
A Schrodinger program was used to prepare the binding site of the FXa from PDB crystal structures (entry 1KSN and 1F0S). After analysis subpockets' structure and interactions with native ligands, 4 main constraints within S1 and S4 were posed and docking was performed.
Molecular docking was based on 2 pharmacophore models: either presence of an amine component as a imitation of arginine or a polarized aromatic fragment or a halide occupying the volume of S1.
This resulted in a virtually generated target library of 1397 inhibitors, based on all aforementioned criteria. Those compounds belong to different chemical classes.
Selected series for each model were tested using a modified RVV test (RVVT) and inhibitor activity. Biological screening on purified FXa within compounds showed activity ranging 40-60%. The most effective among them inhibitors were used in the RVVT on human blood plasma. The result of the test emerged efficiency of 17 substances within 70-100% and 2 inhibitors reached the highest capability to reduce FXa activity.
Further, related substances with the highest and the lowest efficiency were identified and dynamic analysis was performed based on them. Such test led to discovering the mechanism of interaction within subpockets and influence of different functional groups on a compound's ability to decrease enzyme's activity.

NOVEL 4-THIAZOLIDINONE DERIVATIVES CHANGE THE ACIDIFICATION OF SYNAPTIC VESICLES IN RAT BRAIN NERVE TERMINALS

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The search for new anticonvulsants with high activity and low toxicity is one of the important tasks of medical/pharmaceutical chemistry. The design and synthesis of novel 4-thiazolidinone derivatives as potentially new neuroactive compounds and anticonvulsants was carried out. Leading compounds Les-2659, Les-2769, Les-3836, Les-3105, Les-2615, Les-2658, Les-2670 were selected after *in vivo* screening for anticonvulsant activity and acute toxicity.

Here, we examined the effects of these leading compounds on acidification of the synaptic vesicles in isolated rat brain nerve terminals (synaptosomes). Acridine orange (AO), a pH-sensitive fluorescent dye was used in the experiments for monitoring synaptic vesicle acidification, which is an important component of the electrochemical proton gradient. The application of AO to synaptosomes resulted in partial quenching of the fluorescence signal due to dye accumulation in synaptic vesicles that was considered as 100%. We demonstrated the absence of significant changes in the emission spectrum of AO after adding each of the investigated compounds at a concentration of 100 μM. Les-2769, Les-3836 and Les-3105 caused an increase in the fluorescence dye intensity by 15.2 ± 1.72% ($P < 0.01$), 30.56 ± 4.18% ($P < 0.01$) and 23.57 ± 2.64% ($P < 0.01$) that indicating a decrease in acidification of synaptic vesicles. Les-2659, Les-2615 and Les-2670 evoked a weak reduction in acidification of synaptic vesicles, increasing the fluorescence dye intensity by 8.24 ± 1.94%, 5.83 ± 1.8% and 1.55 ± 0.42%, respectively. Les-2658, in contrast to other compounds, caused an increase in acidification of synaptic vesicles, decreasing the fluorescence dye intensity by 16.38 ± 1.81% ($P < 0.01$).

Therefore, the effects of novel 4-thiazolidinone derivatives on the acidification of the synaptic vesicles of the nerve terminals were revealed. The synaptic vesicle acidification as well as the plasma membrane potential, is crucial parameters, the changes of which can modulate transporter-mediated uptake/release and exocytotic release of neurotransmitters. Further comprehensive *in vitro* and *in vivo* studies of new synthesized 4-thiazolidinone derivatives are promising for the creation on their basis of more advanced medicines and the discovery of new ways to overcome neurological disorders and the development of a strategy for neuroprotection.

This work was provided by the grant support of the State Fund For Fundamental Research (# F76/13-2018).

ANTICANCER ACTIVITY OF NEWLY SYNTHESIZED RESVERATROL DERIVATIVES

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Stilbenes, 1,2-diphenylethen derivatives, including resveratrol and combretastatins, are compounds of anticancer feature especially active against tumor angiogenesis. Fosbretabulin, disodium phosphate salt of combretastatin 4 (CA-4), in combination with carboplatin, is on the last stages of clinical tests in patients with thyroid cancer. The mode of action of these compounds includes suppression of angiogenesis through interfering with tubulin (de)polymerization, thus making them vascular targeting agents (VTAs).

We have previously synthesized new five *E*-2-hydroxystilbenes and seven dibenzo[*b,f*]oxepins in Z configuration, with methyl or nitro groups at varied positions. The aim of the present work was to evaluate the anticancer activity and molecular mechanism of action of these compounds. Four cell lines: fibroblasts EUFA30 and human embryonic kidney HEK293, representing healthy tissues, and cervical cancer HeLa and glioblastoma U87, representing cancer cells, were subjected to four stilbenes and seven oxepins. Two of this compounds, JJR5 and JJR6, showed relatively high activity against cancerous cells tested and were selected for further investigations. They induced apoptosis with sub-G1 or S cell cycle arrest and PARP cleavage, with no visible activation of caspases 3 and 7. Proteomic differential analysis of stilbene-treated cells led to the identification of several proteins involved almost exclusively in cell cycle management, apoptosis, DNA repair, and stress response, e.g. oxidative stress, like γ-glutamylcyclotransferase, UV excision repair protein RAD23 homolog B, or heme oxidase HO-1. We concluded that JJR5 and JJR6 compounds are worth further investigation and can be a base for structure modification(s) to obtain even more active compounds.

This work was supported by the National Science Centre, Poland, UMO-2012/07/B/ST5/03194 and UMO-2011/03/B/NZ4/02425 for ALKBH studies.

APOPTOSIS INDUCTION IN HUMAN LEUKEMIC CELLS WITH C_{60} FULLERENE LED-MEDIATED PHOTODYNAMIC THERAPY

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Recent progress in nanobiotechnology has attracted interest to a biomedical application of the carbon nanostructure C_{60} a fullerene since it possesses a unique structure and versatile biological activity. C_{60} fullerene potential application in the frame of cancer photodynamic therapy (PDT) relies on better understanding of the interaction of nanostructure with cancer cells. The aim of this study was to evaluate C_{60} fullerene (Ritter U. et al., 2015) biochemical effects on human leukemic cells (CCRF-CEM) in combination with high power single chip light-emitting diodes (LEDs) light irradiation of different wavelengths.

With the use of immunocytochemical staining and liquid chromatography-mass spectrometry we showed that pristine C_{60} fullerene was taken up by human leukemic CCRF-CEM cells from the media in a time-dependent manner, reaching a maximum intracellular level of almost 250 ng/10^6 cells at 24 h of incubation. The assessment of accumulation and distribution of nanoparticle within the cell revealed that C_{60} fullerene was predominantly localized within mitochondria (72%). The comparative analysis of a potential phototoxicity of C_{60} fullerene in human leukemic cells using LEDs of different wavelengths showed that the highest anticancer effect was achieved at 10 J/cm^2 405 nm. Neither C_{60} fullerene nor 405 nm LED light alone impaired cell viability, while their combined action was followed by a 10-fold increase of reactive oxygen species generation and 46% cell viability decrease. Absolute loss of ATP content and a 4-fold increase of apoptosis-executive caspases 3/7 activity indicated on the activation of the mitochondrial apoptotic pathway. On top of that, the externalization of plasma membrane phospholipid phosphatidylserine was detected in more than 50% of cells suggesting the execution of the late apoptotic stage.

Our work proved C_{60} fullerene ability to induce apoptosis of leukemic cells after photoexcitation with high power single chip 405 nm LED as a light source. This underlined the potential for application of C_{60} nanostructure as a photosensitizer for anticancer therapy.

THE CYTOTOXICITY OF CADMIUM ION SMALL CONCENTRATIONS IN BONE MARROW CELLS DURING PROLONGED CULTIVATION

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It is known that cadmium ions have the property of accumulating 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 a medium containing small doses of cadmium ions - 0.1-10 μM/l 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.

Studies were carried out on a monolayer of bone marrow cell culture from the femur of 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 h 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 the CASPlab software. The single-strand DNA concentration was determined by Molecular Probes Qubit ssDNA Assay Kit.

It was 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 led 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 led 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.

We are heartily thankful to our supervisors, Prof., Dr.Sc. Ye. Persky and PhD, Associate Prof. Yu. Kot for their guidance during the development of this experimental work.

GLIA-SPECIFIC REACTION IN THE DIFFERENT BRAIN AREAS OF RATS AFFECTED WITH PITUITRIN AND IZADRIN

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The study of the heart-brain interactions under conditions of myocardial damage is important in predicting future cardiovascular events and development of neurological complication. Glia-specific proteins are used as markers of glia, especially in brain damage. Myelin basic protein (MBP) is the main protein component of the oligodendroglia. With the destruction of myelin, MBP is released and can reflect the level of demyelination. Glial fibrillary acidic protein (GFAP), an intermediate filament marker from fibrous astrocytes found mostly in the white matter, is one of the key proteins that characterize the behavior of astrocytes. The aim of our study was to investigate the level of MBP and GFAP in different brain areas of rats under the effect of pituitrin-izadrin-induced cardiomyopathy (PIICM).

We used 12 6-month-old Wistar rats that were divided into two groups ($n = 6$). Group 1 – control rats maintained under standard condition, group 2 – rats with the pituitrin-izadrin-induced myocardial attack. The administration of pituitrin and izadrin was performed as follows: intraperitoneal administration of 0.5 U/kg of pituitrin, followed by the subcutaneous introduction of 100 mg/kg izadrin after 20 min, followed by another izadrin injection 6 h later, and 24 h later the administration of pituitrin and izadrin in the same doses as mentioned above. The animals were decapitated under anesthesia (thiopental, 60 μg/kg), cerebellum, cerebral cortex, thalamus and hippocampus were isolated, which were then used to produce protein fractions. The levels of GFAP and MBP in obtained fractions were measured with competitive ELISA. Statistical analysis was performed with ANOVA. Reliable data were considered at $P < 0.05$.

The reduction of level of myelin basic protein in the cerebellum (from 4.5 ± 0.3 to 3.03 ± 0.3 μg per 100 mg of tissue), thalamus (from 3.08 ± 0.4 to 1.50 ± 0.07 μg per 100 mg of tissue) and hippocampus (from 3.1 ± 0.3 to 2.2 ± 0.2 μg per 100 mg of tissue) of rats under the PIICM suggested the nerve fibers demyelization and reduced functional activity of oligodendroglia. It was coincident with astrocytes reactivation. The level of both soluble and filamentous forms of GFAP was increased up to 1.2-1.3 times in studied brain areas of rats with PIICM.

Obtained data allow suggesting that PIICM can provoke the complication of brain function by induced demyelization and reactive astrogliosis.

IMPACT OF ANTIOXIDANTS SELENOMETHIONINE AND D-PANTETHINE ON FUNCTIONAL STATUS OF GLUTATHIONE SYSTEM IN MULTIDRUG RESISTANT TUMOR CELLS UNDER DOXORUBICIN TREATMENT

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A rapid development of multiple drug resistance (MDR) in cancer cells to chemotherapy is considered one of the main problems of current oncology. Thus, development of novel approaches for effective circumvention of MDR is of key importance to modern pharmacology and cell biology. Previously (Panchuk et al, 2014) we showed that antioxidant compounds selenomethionine (SeMet) and D-Pantethine (D-Pt) possess weak cytoprotective effect *in vitro* under doxorubicin treatment of tumor cells, sensitive to chemotherapy, but enhanced cytotoxic activity of this drug towards MDR cell lines. Taking into consideration that both antioxidants may be involved in regulation of glutathione biosynthesis, the main aim of current study was to investigate the impact of SeMet and D-Pt on cellular glutathione system under the action of doxorubicin (Dx) in tumor cells possessing different mechanisms of drug resistance. Human promyelocytic leukemia cells of HL-60/wt line were addressed together with their HL-60/Adr (MRP-1 overexpression) and HL-60/vinc (P-gp overexpression) sublines. It was shown that cells of HL-60/vinc subline were characterized by a 10-fold reduced basal level of oxidized (GSSG) and total glutathione (tGSH) compared with the cells of HL-60/wt line. On the contrary, a 2-fold increase of GSSG level and a 1.5-fold increase in tGSH concentration were observed in untreated HL-60/adr cells. Dx treatment led to a 1.5-fold enhancement of GSSG level in wild-type cells and a 2-fold increase of this index in HL-60/vinc cells. However, in HL-60/adr cells, Dx caused a 2.5-fold decrease in GSSG concentration and had no influence on the level of tGSH. SeMet and D-Pt in combination with Dx led to a 1.5-fold reduction of GSSG and tGSH levels in HL-60/wt and HL-60/vinc cells, but possessed a little effect on these indices in HL-60/adr cells. We did not observed any significant changes in the activity of glutathione peroxidase in all three cell lines, while the basal level of glutathione S-transferase was increased 2-fold both in HL-60/adr and HL-60/vinc lines. Dx treatment further increased this index 2.5-fold in HL-60/vinc cells, but co-treatment of cells with SeMet or D-Pt effectively lowered activity of GST back to basal level. Thus, GST-inhibiting properties of SeMet or D-Pt may explain the enhancement of cytotoxic activity of Dx by 30% towards P-glycoprotein overexpressing tumor cells under co-incubation with these antioxidants. Further studies of synergistic effects of SeMet, D-Pt and Dx on drug-resistant animal tumor models are in progress.

CURCUMIN PREVENTS CYTOSKELETON DISREGULATION AND ASTROCYTE REACTIVITY INDUCED BY LIPOPOLYSACCHARIDE

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Neurodegenerative diseases are characterized by inflammation, a lack of neural cell viability, oxidative stress generation and signaling pathways disregulation. All of them in neural tissue closely related to glial cells dysfunction. Astrocytes are crucial to provide CNS homeostasis and neuronal survival. Reactivity of astrocytes is a molecular and metabolic marker of number CNS pathogenesis that accompanied with both glial fibrillary acidic protein (GFAP) and cytokines overproduction. Curcumin potently modulates cellular response through anti-inflammatory signaling. The protective effects of curcumin discovered in various cells. However, the effect of curcumin on the cytoskeleton of astrocytes remains unknown. The aim of the study was to elucidate the effects of curcumin on astrocyte reactivity in LPS-inflammatory *in vitro* model.

Reactivity of primary astrocyte cell cultures was stimulated with 0.01 μM LPS. Stimulated and nonstimulated cells were exposed to 2.0 μM curcumin 24 h. Astrocyte reactivity was determined as an expression GFAP and NF-kB.

The treatment of primary astrocytes with LPS induced in primary astrocytes minor (17%) lack of cell viability. There was no antiproliferative effect in astrocytes exposed to curcumin. However, the treatment LPS-stimulated astrocytes with curcumin restored cell viability to the level of control cells. LPS induced a meaningful increase of GFAP, NF-kB and PARP expression (1.9, 1.7 and 1.6 times respectively). The treatment of nonstimulated astrocytes with curcumin provoked low nonstatistic changes all of them. Whereas, there was determined pronounced effect of curcumin in LPS-stimulated astrocytes. Curcumin prevented upregulation of GFAP, NF-kB and PARP (1.7, 1.4 and 1.3 times accordingly) compare to LPS-stimulated cells.

Observed protective effects of curcumin may be related to antioxidant and antiproliferative capability of this polyphenol. LPS provoke plural mechanisms that linked with transcriptional activity of NF-kB and as result the cytokines secretion. The effects of curcumin may realize through NF-kB and PARP suppressing as the regulator astrocyte reactivity.

Presented data demonstrate the neuroprotective effectiveness of low dose of curcumin against abnormal reactivity in primary rat astrocytes, including amelioration of cell viability, cytoskeleton state and transcriptional regulation cellular response.

THE ACTIVITY OF THE ENZYMES OF THE STONE MOROKO UNDER THE INFLUENCE OF THE HEAVY METAL IONS

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The biological effects of heavy metal contamination of the aquatic environment are, first of all, in the direct toxic effects on hydrobionts, which lead to damage to their physiological systems. The purpose of our study was to determine the influence of nickel and lead ions on the activity of enzymes in the hydrocarbon metabolism of the Stone moroko.

Experimental part was carried out with stone moroko, which were divided into 3 groups (n = 15): 1 – control group; 2 – fish with the addition of Ni^{2+} – 0.04 mg/l (4 MAC); 3 – fish with the addition of Pb^{2+} – 0.15 mg/l (1.5 MAC). The experiment lasted for 21 days. The activity of succinate dehydrogenase (SDG) was determined by the Vecsey method. The activity of lactate dehydrogenase (LDH) was determined using the LDH sets (Filisit-Diagnostika, Ukraine). The data were statistically analyzed using the Excel program for the t-test, with $P < 0.05$.

It is established that the activity of LDH increases by 37.4% due to the impact of nickel ions. LDH activity in the control group was at the level of 65.64 ± 5.10 nM NADN/mg of protein·min, with the nickel LDH has increased up to 97.06 ± 9.80 nM NADN/mg protein·min. There was a tendency to increase LDH activity by 13.6% at the action of the indicated concentration of lead, but no statistical significance with the control group of fish was detected. The increase of the enzyme is primarily due to increased glycolytic processes in the muscle tissue of fish. Since nickel actively influences cell activity, it has been triggered by anaerobic processes and a violation of mitochondrial oxidation. In turn, decreased activity of EDA and LDH growth affects the accumulation of pyruvate and lactate in fish tissues, which may contribute to acidosis of tissues. The decrease in the activity of EDA due to the impact of nickel and lead ions by 33.4 and 41.5%, respectively was determined. The activity of EDC in the control group was 1.67 ± 0.16 nM of succinate per mg protein·min. The influence of lead indicates a decrease in the activity of EDC to 0.99 ± 0.09 nM of succinate per mg protein·min, and the influence of nickel – up to 1.13 ± 0.05 nM of succinate per mg protein·min.

It is known that heavy metals are absorbed in the body, which further contributes to tissue damage and causes dysfunction of specific organs. This is reflected in the changes in the enzyme activity. First of all, it concerns enzymes involved in the regulation of energy metabolism. These changes could cause disturbances in oxidative-reducing processes in the muscle tissues of the stone moroko.

DETERMINATION OF THE AMOUNT OF MALONDIALDEHYDE IN THE LIVER OF THE PUMPKINSEED AS A MARKER OF LIPID PEROXIDATION

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Pumpkinseed is one of the new alien species of waters in Ukraine, which came to our water bodies only 40 years ago, but has already increased the number and biomass. The character of this species is the ability to live under the conditions of chronic toxicological effects. Such adaptation of the pumpkinseed is manifested through physiological and biochemical plasticity of the species. One of the indicators that allow estimating the level of the species adaptation to the toxicological effect is the determination of the intensity of lipid peroxidation, which can be determined in tissues by the accumulation of malondialdehyde (MDA) – one of the end products of peroxidation. This indicator helps evaluate both the activity of the metabolism in the organism of fish and the degree of its imbalance in adverse environmental conditions.

Samples of fish were taken in the summer of 2017 in the waters of the Samara Bay of Zaporizke Reservoir, which is under significant anthropogenic load due to the dumping of mine waters. The object of the study was five-year-old, sexually mature individuals of the pumpkinseed. The concentration of TBC-active products in the homogenate from the liver tissue of the pumpkinseed was measured by the color reaction of malonic dialdehyde (MDA) with thiobarbituric acid (TBC) under high temperature and acidic media, resulting in the formation of a trimethine complex containing one MDA molecule and two TBC molecules.

According to the results of the study, the content of malondialdehyde in the liver of males of the pumpkinseed was 18.9% lower than that in females, and the level of MDA in the liver reached 11.06 ± 0.98 mM/g ($n = 10$).

Reduced amount of MDA in liver tissue of pumpkinseed males can be explained by more intensive clearance of free radicals formed in the body of the fish as a result of aerobic metabolism, which reduces the formation of TBA-active products. LPO is an important indicator of lipid metabolism, active metabolic and regulatory factor, reflecting the accumulation of intermediate and final metabolites, so the results may indicate an appropriate defensive mechanism of the organism in the physiological and biochemical level to the effect of various stress factors. Similar results were shown in studies on the analysis of LPOs on carp, trout, silver carp, and monkey goby.

INFLUENCE OF PLANT-DERIVED EXTRACTS ONTO CANCER CELLS AND PLATELETS

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Wild plants *Viscum album* (Mistletoe) and *Chelidonium majus* (Celandine) are known source of biologicaly active compounds that are used in traditional medicine for preventing of bleeding or curing cancer diseases. That is why the aim of present study was analyzing of effects of water extracts of *Viscum album* and *Chelidonium majus* leaves on isolated human platelets and adenocarcinoma cells of mammal gland, MCF-7.

Water extracts of leaves of two medicinal plants were obtained and concentrated using lyophilization on TelStar LyoQuest (Spain). The compositions of both extracts characterized using HPLC Agilent 1100. ADP-induced platelet aggregation in the presence of studied mixtures was studied by aggregometry using Solar2110 (Belorussia). Effects of both extracts on the proliferation of cancer cells were estimated using MTT-test.

It was demonstrated that *Chelidonium majus* extract taken in concentration 3 μg/ml induced platelet activation that leads to moderate aggregation (rate of aggregation 10%). Rate of ADP-induced aggregation of platelets was increased in the presence of *Chelidonium majus* extract (in 1.5 times) and inhibited (on 1.2 times) in the presence of *Viscum album* extract in comparison to control meanings (30%).

Also *Chelidonium majus* extract taken in concentration of 3 and 1.5 μg/ml demonstrated cytopathogenic effect on MCF-7 cells. Addition of extract decreased the surviving of MCF-7 coulter cells determined using MTT-test on 40%.

Plant deriving metabolites analyzed in this study were shown to be efficient effectors of platelet reactivity and possessed moderate platelet-activating (*Chelidonium majus*) or inhibiting (*Viscum album*) effects. Also, *Chelidonium majus* possessed anti-proliferative activity towards MCF-7 cancer cells. Further studies of biological effects of these plants extracts can be promising for drug development.

This work was carried out in the frame of the basic theme of the Palladin Institute of Biochemistry of NAS of Ukraine “Study of regulation mechanisms of blood coagulation and fibrinolysis interplay with vascular and platelet hemostasis”.

MODULATING THE INNATE IMMUNE RESPONSE TO INFLUENZA A VIRUS H1N1 (A/FM/1/47) BY COMPLEXES OF OLIGORIBONUCLEOTIDES WITH D-MANNITOL

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Rapid replication of influenza A virus and lung tissue damage caused by exaggerated pro-inflammatory host immune responses lead to high numerous deaths. Oligoribonucleotides-D-mannitol (ORNs-D-M) complexes possess both antiviral and anti-inflammatory activities. Inhibiting the neuraminidase activity and hemagglutinin–glycan interaction of the influenza virus is a mechanism of anti-influenza activity of the ORNs-D-M. The aim of the research was to study the ORNs-D-M influences on up-expression of the innate immune genes induced by the influenza virus infection in mice lungs as a mechanism of anti-inflammatory activity of the complexes at the influenza infection. Genes expression was determined by RT-qPCR and Western Blot assays. The influenza infectious titers were investigated using $TCID_{50}$ assay. In the present studies, we found out that the ORNs-D-M reduce the influenza-induced up-expression of Toll-like receptors (TLRs) (*tlr3, tlr7, tlr8*), nuclear factor NF-kB (*nfkbia*, *nfnb1*), cytokines (*ifnε, ifnk, ifna2, ifnb1, ifnγ, il6, il1b, il12a*, *tnf*), chemokines (*ccl3, ccl4, ccl5, cxcl9, cxcl10, cxcl11*), interferon-stimulated genes (*oas1a*, *oas2*, *oas3, mx1*) and pro-oxidation (*nos2*, *xdh*) genes. The ORNs-D-M inhibited the mRNA overexpression of tlr3, tlr7, tlr8 induced by influenza virus, suggesting that they impair the upregulation of NF-kB, cytokines, chemokines, ISGs, pro-oxidation genes induced by influenza virus by inhibiting activation of the TLR-3, TLR-7, TLR-8 signaling pathways. By impairing activation of the TLR-3, TLR-7, TLR-8 signaling pathways, the ORNs-D-M can modulate the innate immune response to influenza virus infection.

IDENTIFICATION AND CHARACTERIZATION OF POTENTIAL MOLECULAR DRUG TARGETS OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* USING *IN SILICO* APPROACHES

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Staphylococcus aureus is one of the most common bacterial pathogens whose antimicrobial resistance has increased worldwide and has become the major cause of treatment failure in many countries. The development of novel antibiotics against this infectious agent is a very significant challenge for modern medicinal chemistry. That's why *in silico* methods of predicting novel drug targets have emerged.

The objective of this study was the identification of novel putative drug targets of methicillin-resistant *S. aureus* (MRSA) through subtractive proteome analysis.

Proteomes of several annotated methicillin-resistant (MRSA) strains, i.e., MRSA ATCC BAA-1680, H-EMRSA-15, LA MRSA ST398, MRSA 252, MRSA ST772, UTSW MRSA 55, were taken to form the initial set.

The proteome analysis of the MRSA strains in several consequent steps (indicated in Table) resulted in identification of two molecular targets – diadenylate cyclase and D-alanyl-lipoteichoic acid biosynthesis (DltB) protein. Using homology modeling approach we built three-dimensional structures of these proteins and identified their ligand-binding sites.

T a b l e. Subtractive proteome analysis of MRSA

Analytical step of the analysis	*Total number of proteins as the outcome*	*Short description of the step*
Non-homologous proteins	172	Protein BLAST against human proteome
Essential proteins	45	Protein BLAST against the Database of Essential Genes
Proteins after KEGG analysis	28	KAAS protein annotation to metabolic pathways
Potentially novel drug targets	22	Search in the DrugBank database
Membrane-bound proteins	2	Utilizing PSORT v. 3.0.2, CELLO v. 2.5, iLoc-Gposand Pred-Lipo tools

Therefore, we identified two molecular targets of methicillin-resistant *Staphylococcus aureus* – diadenylate cyclase and DltB protein, which meet the requirements of being essential, membrane-bound, non-homologous to human proteome, involved in unique metabolic pathways and new in terms of not having approved drugs. 3D models of these proteins suggest the existence of at least one binding site in diadenylate cyclase and two binding sites in DltB protein. Thus, these candidate molecular targets can be considered for further rational drug design of novel therapeutic agents for treatment of MRSA infections.

SALT OF HEAVY METALS AS EFFECTORS OF PEPTIDEHYDROLASE FROM *DROSOPHILA MELANOGASTER*

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The purpose of the presented work was to investigate the biochemical properties and functional activity of the alkaline peptidehydrolase in the intestine of Drosophila in the ontogenesis of the flies, with keeping them under standard conditions and in the presence of heavy metal salts (Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}) in the environment.

The anilidase activity of alkaline peptidehydrolase was determined by the hydrolysis of 1.0 mM chromogenic substrate N, α-benzoyl-L-arginine-p-nitroanilide (BAPNA), in 0.1 M glycine-NaOH buffer pH 9.0 at 382.5 nm. The esterase activity of the alkaline peptidehydrolase was determined by the hydrolysis of 1.0 mM N, α-benzoyl-L-arginine-ethyl ether (BAEE) in 0.1 M glycine-NaOH buffer pH 9.0 at 253 nm. Specific activity of alkaline peptidehydrolase was determined in mU related to 1 mg of total protein of the investigated tissue extract or enzyme preparation. The influence of metal cations on the activity of alkaline peptidehydrolase was investigated using solutions of chlorides of Co^{2+}, Cu^{2+}, Zn^{2+} и Cd^{2+} at the final concentration: 0.2, 0.4, 1.0 and 2.0 mM.

Pre-incubation with 0.2-0.4 mM $CoCl_2$ and $CuCl_2$ increased the activity of the alkaline peptidehydrolase of the original extract of tissues of the Drosophila larvae of the Normal line, respectively, by 21.6 and 11.2% and led to a decrease in purified enzyme activity by 10.8 and 27.5%. In the presence of 1.0 and 2.0 mM $CoCl_2$, the activity of both purified and non-purified enzyme was suppressed by more than 50%. Reduced purity of enzyme activity by 88-95% and complete inhibition of the activity of the crude enzyme were established by pre-incubation with 2.0 mM $CuCl_2$ and $ZnCl_2$ solutions. The activity of the alkaline peptidehydrolase of the extract of the Muller-5 line increased by 15.0-25.7% in the presence of 0.2 mM $CoCl_2$ and $CuCl_2$. In the presence of 1.0-2.0 mM $CoCl_2$, the purified and untreated enzyme activity was suppressed by 20 and 67%, respectively. Complete inhibition of the activity of crude and purified enzyme was determined by pre-incubation with 2.0 mM of $ZnCl_2$ solution and 2.0 mM of $CuCl_2$ solution. The obtained data indicate inhibition of activity of alkaline peptidehydrolase of Drosophila larvae of both lines in vitro with high concentrations of chlorides Co, Cu, Zn and Cd.

The influence of metal cations on the activity of alkaline peptide glycosylates of larvae of both lines of Drosophila can be explained by non-specific irreversible inhibition associated with the adsorption of metal ions on the surface of the protein molecule and their interaction with functional active groups, which leads to conformational changes in the enzyme molecule or their effect on the active center.

INHIBITION EFFECT OF CALIX[4]ARENES ON PLASMIN ACTIVITY

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Background. Plasminogen over activation, plasmin hyperactivity and low level of plasmin inhibitors in the circulation lead to the range of hemorrhagic disorders and bleedings. Development of effective low molecular weight plasmin inhibitors opens up new prospects in hyper fibrinolysis medication. Calixarenes are perspective class of low-toxic compounds which can be functionalized and used as biologically active substances due to their ability to form supramolecular complexes with biological molecules. Calix[4]arenes methylene bisphosphonic acids have strong negative total charge and presumably can interact with fibrinolytic system enzymes, modulating their activity.

Materials and methods. We assessed the ability of calix[4]arenes functionalized by various number of phosphonic acid remnants to inhibit fibrinolysis (human plasmin amidolytic and fibrinolytic activity, plasminogen activators activity).

Results. Calix[4]arenes C 296, C-425, C-427 and C-145 with respectively 2, 4, 3 and 8 phosphonic groups, specifically inhibit fibrin clot lysis by plasmin in a dose-dependent manner and plasmin activity inhibition is proportional to the number of phosphonic acid remnants. C-145 (sodium salt of calix[4]-arene-methylene-bis-phosphonic acid) is a most effective plasmin inhibitor (competitive mechanism, K_i = 0.26 μM). At the same time, ¼ part of the calixarene – 4-hydroxyphenyl-methylene-bis-phosphonic acid – does not effect plasmin fibrinolytic activity, that indicates the requirement of whole calix[4]-arene molecule for the enzyme inhibition. However, C-145 does not affect amidolytic plasmin activity toward low molecular weight chromogenic substrate. Moreover, C-145 does not inhibit plasminogen activation by tissue-type activator and urokinase on platelet surface, but attenuate newly formed plasmin activity.

Probably such calix[4]arene selectivity to fibrillary substrate like fibrin is provided by complex formation between 8 negatively charged phosphonic groups in upper ring and positively charged substrate recognition exosites of plasmin active site.

Conclusions. Calix[4]arene C-145 effectively inhibit plasmin fibrinolytic activity is perspective agent for hyper fibrinolytic disorders treatment.

Acknowledgment. The research was a part of Project N64 ”Study of calix[4]arenes as hemostatic, antifibrinolytic and antithrombotic agents.” according to targeted complex program of NAS of Ukraine for fundamental research “Fundamental issues of new nanomaterials and nanotechnologies creation”.

INFLUENCE OF SUPPRESSION SYNTHESIS OF NITRIC OXIDE ON BIOCHEMICAL PARAMETERS OF BLOOD

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Nitric oxide (NO) is an important factor that characterizes the state of the environment, but also is a powerful bioregulator in organisms of humans and animals.

There are a lot of studies about the effects of nitric oxide on the gastrointestinal tract, but the state of organs in case of insufficiency NO does require in-depth study.

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). Suppression synthesis NO caused by a 6- and 12-day intraperitoneal injection of Nω-nitro-L-arginine (L-NNA) at a dose of 40 mg/kg (groups II and III). We anesthetized the rats with a lethal dose of ketamine hydrochloride solution (220 mg/kg) and collected blood. The statistical processing of the data was carried out the Mann-Whitney U test.

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

MDA concentration increases by 37 and 62% at 6 and 12 daily injected L-NNA respectively. Concentration of ceruloplasmin is growing by 17 and 79%. Activity of the α-amylase reliably changes: it is reduced by 2 times at 6-day course and increase by 2 times at 12-day compared with control. The activity of trypsin doubles at the six-day L-NNA introduction, but it is not significantly different from control at twelve-day. The concentration of protein-bound hydroxyproline is changing unreliably, but there is a tendency to increase quantity.

Inhibiting the synthesis of nitric oxide stimulates the formation of oxidative stress. Also, hepatocytes are destroyed at 12-day course of injections L-NNA. Deficiency of NO violates the secretion of pancreatic enzymes.

THE NEW SMOOTH MUSCLE CELL CONTRACTION AGENT – CALIX[4]ARENE C-956

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Plasma membrane Ca^{2+},Mg^{2+}-ATPase (PMCA) functions as a fine tuner of cellular calcium concentration and plays a pivotal role in the termination of Ca^{2+} signal in uterine myocytes. Taking onto consideration of its critical contribution to the maintenance of Ca^{2+} homeostasis in cells, this enzyme can be ased as an important pharmacological target.

Previously we have found that calix[4]arene C-90 selectively inhibited PMCA of myometrium. The aim of our investigating was searching of new selective and more efficient inhibitor of PMCA among calix[4]arenes. That is why we have studied the influence of structure analogs of calix[4]arene C-90 (calix[4]arenes C-715, C-716, C-772, C-956, C-957, C-975) on PMCA activity.

All calix[4]arenes were synthesized and described with NMR and infrared spectroccopy in the Department of Phosphorane Chemistry, Institute of Organic Chemistry of National Academy of Sciences of Ukraine (chief – prof. V. I. Kalchenko). All enzymatic activities were assayed in plasma membrane fraction or in myometrium cell suspension perforated with 0.1% digitonin.

In the concentration of 100 μM, the most inhibition of PMCA was in the presence of calix[4]arene C-956, which inhibited enzyme activity to 20.8 ± 0.4% relative to control. It should be emphasized that in this concentration, calixarene barely had an effect on ATPase activity of Mg^{2+}-ATPase, Ca^{2+}-ATPase, and Na^{+},K^{+}-ATPase. The calculated inhibition coefficient $I_{0.5}$ was 15.0 ± 0.5 μM that was the evidence of higher inhibition efficiency for calix[4]arene C-956 than for calixarene C-90 ($I_{0.5}$ = 20.2 ± 0.5 μM). The Hill coefficient was 0.55 ± 0.01 (n = 5).

It was also shown that application of 20 μM calix[4]arene C-956 into uterine myocytes caused a temporary increase of intracellular Ca^{2+} concentration. Interestingly, during 2.5 minutes $[Ca^{2+}]_i$ decreased that could be explained by involvement of compensatory mechanisms which regulate calcium homeostasis. In addition, 50 μM calix[4]arene C-956 induced a decrease in myocyte's hydrodynamic diameter to 45% that could be explaned by contraction of myocytes.

Thus, calix[4]arene C-956 is selective inhibitor of PMCA, which is more effective than other calixarenes and therefore it is promising compound for modulation of smooth muscle contractility.

We are thankful to the academician of NASU V.I. Kalchenko for scientific cooperation.

DIAMIDE-INDUCED MODIFICATIONS OF PROTEINS IN MEMBRANE-CYTOSKELETON COMPLEX OF CRYOPRESERVED ERYTHROCYTES

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Space-conformation modifications of the proteins in the membrane-cytoskeleton complex of human erythrocytes under the cryopreservation in the presence of different cryoprotective agents (CPAs) were studied using a protein-cross-linking reagent diamide, which oxidizes the sulfhydryl groups of proteins with the development of disulfide cross-links between individual macromolecules and the formation of high-molecular aggregates. Disturbances in spatial orientation of proteins within the membrane-cytoskeleton network or changes in the structural packing of molecules can change the availability of SH-groups to the oxidizing action of the reagent. Analysis of the protein profile of the erythrocyte ghosts (membrane-cytoskeleton complex) with Ds-Na-PAGE can distinguish the changes in proteins involved in the modification during the cell cryopreservation with different CPAs.

The level of diamide-induced aggregation of proteins in human erythrocytes frozen under the protection of glycerol, dimethylsulfoxide (Me_2SO), sucrose, dextran and polyethylene glycol (PEG), largely reflected the effectiveness of CPA protective action. Low levels of protein aggregates in erythrocytes after freeze-thawing in the presence of glycerol, PEG and $M_{e2}SO$ correlated with high safety cells. Meanwhile, a high hemolysis after freeze-thawing with sucrose and dextran, which gives a measure of a degree of cell destruction, was consistent with the formation of a significant amount of diamide-induced aggregates. Consequently freezing erythrocytes in the presence of sucrose and dextran was accompanied by an increase in availability of reactive sulfhydryl groups due to disturbances in the structure of protein macromolecules. The rise in aggregate amount was patently caused by structural disorders of the two main proteins, spectrin and band 3.

Nevertheless, after removal of CPAs only in erythrocytes frozen under glycerol protection, the distribution of protein fractions and diamide-induced aggregation were close to the control cells. In erythrocytes frozen in the presence of Me_2SO and PEG, after removal of the CPAs, the contents of the spectrin and band 3 were decreased.

These facts confirm the possibility of a nonspecific modification of the proteins in the membrane-cytoskeleton complex under the impact of CPAs and freezing, which can affect the physical properties of membranes and the erythrocytes stability.

POSTER SESSION II. BIOTECHNOLOGY

INFLUENCE OF MELANINS OF *CLADOSPORIUM CLADOSPORIOIDES* ON THERMAL STABILITY OF HUMAN INTERFERON-B

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Interferon (IFN) is a key protein produced by the immune system and plays a profound role in determining the outcome of viral infection in mammals. Medicinal properties of melanins revealed such beneficial effects as lowering of blood pressure, modulation of the immune system, reduction of blood lipid concentrations, inhibition of tumors, and prevention of inflammation or anti-microbial activity. Given the ongoing necessity of new immunoprotectors suitable for treatment, ingestion of melanin from *Cladosporium cladosporioides* could represent a new inexpensive and safe protective drug.

In our research, we focused on the investigation of the thermal stability of protein INF in the presence of melanins of *Cladosporium cladosporioides* standard producing strain 396 and strain 10 from radioactively contaminated territory. Also, we cultivated its strain of C.Cl under the influence of a nanoparticle of a colloidal iron solution. The method of fluorescence spectroscopy was used in our study. The fluorescence melting curves were obtained from fluorescence spectra of protein- melanins solutions measured at different temperatures (from 30 to 75 °C). For estimating the dissociation constant (K_d) next formula was used $K_d = \frac{(1-\theta)\left(D-(\theta P_0)\right)}{\theta}$.

It was shown that addition of melanins to INF leads to the shift of protein melting temperature in higher temperature region. For example, the melting point of INF is 62 °C. After adding melanins of C.Cl 396 and C.Cl 10 to the solution the temperature of protein melting point is shifted to 68 and 65 °C. After adding melanins of C.Cl 396 and C.Cl 10 after cultivated with iron of protein melting point is shifted to 64 and 63 °C. The magnitude of the structural changes is given relatively weak (micromolar) dissociation constant for the interaction between these molecules. For example, the dissociation constant between INF and C. Cl 10 was $K_d = 0.22 \pm 0.42$ μM, and when the fungus was treated with a nanoparticle, the pigment allocated gave $K_d = 2.25 \pm 0.32$ μM. At the same time, for a strain 396 $K_d = 0.45 \pm 0.75$ μM, and for a strain of 396 under the action of colloidal iron $K_d = 0.12 \pm 0.16$ μM. We can suppose that thermal stabilization of protein indicates the interaction between melanins and INF that leads to changing of its conformation. So, we assume that protein INF becomes more stable in the presence of melanins of soil micromycetes *Cladosporium cladosporioides.*

β-KETOENOLE DYE AS AN AMYLOID-SENSITIVE FLUORESCENT PROBE FOR BACTERIAL BIOFILM INVESTIGATION

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Amyloid fibrils formed by various proteins are involved in the pathogenesis of neurodegenerative diseases and amyloidoses. Moreover, formation of amyloid fibrils is also observed in non-pathological conditions to make some advantage for living organisms. *E. coli* and other *Enterobacteriaceae* form functional amyloid aggregates called curli at the bacterial cell surface involved in biofilm formation. Since a dye-based fluorescence assay is an effective technique for detection of β-pleated structure of amyloid fibrils there is a need in new specific amyloid-sensitive dyes for visualization of amyloids in biofilms. Here we report that alkylamino β-ketoenole dye is able to detect both pathological (*in vitro*) and functional amyloid fibrils (*in situ*) as a component of biofilms. β-ketoenole has shown the sensitivity to pathological insulin and lysozyme fibrils. The sensitivity of β-ketoenole to these fibrils was comparable or higher to that of commonly used Thioflavin T (fluorescence response ratio 51 and 54 times in the case of insulin, 75 and 33 times in the case of lysozyme, respectively). β-ketoenole possesses no fluorescent response upon the presence of dsDNA and RNA, while Thioflavin T shows strong fluorescent response on RNA presence (in 61 times). The range of insulin fibrils detection by β-ketoenole is 0.8-100 μg/ml, that is higher than that for Thioflavin T (0.5-40 mg/ml). Thus, β-ketoenole turned out to be more selective to amyloid fibrils than Thioflavin T. Confocal Laser Scanning Microscopy was used to assess an applicability of the dye as a probe for fluorescence imaging of *E. coli* DH 10B biofilms. It was shown, that both dyes (β-ketoenole and Thioflavin T) were able to stain *E. coli* biofilms, however, the staining behavior of these dyes was different – β-ketoenole showed more intensive and brighter biofilm staining than Thioflavin T. Staining by β-ketoenole allowed to visualize the structural elements of biofilms with a higher resolution. β-ketoenole binding specificity was evaluated using human mesenchymal stem cells – dye did not stain cells and their components. Hence, we have suggested that staining of *E. coli* biofilms with β-ketoenole visualized amyloid components of biofilms specifically. In conclusion, we demonstrated that β-ketoenole was an amyloid-specific fluorescent dye and proposed it as a probe for amyloid detection and visualization in bacterial biofilms.

The study was supported by NASU Program of Fundamental Research "New Functional Substances and Materials for Chemical Engineering" №8-17 and H2020-MSCA-RISE-2014-RISE-645628 - METCOPH project.

FLUORESCENT PENTAMETHINE BENZOTHIAZOLE CYANINE DYES FOR SENSING OF SERUM ALBUMINS

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Development and investigation of high efficient extrinsic fluorescent probes for detection and visualization of proteins, DNA, cell organelles etc are an active approach in biomedical studies and cyanine dyes are widely used for these applications. Thus, development of fluorophores with new required spectral properties and sensitivity to biomolecules is of interest.

Here the series of functionalized pentamethine benzothiazole cyanine dyes bearing bridge group in polymethine chain were studied for their fluorescent sensitivity to serum albumins (bovine and human BSA, HSA). The dyes were found to be weakly to moderate fluorescent (11-107 a.u.) in the free state. Their fluorescence excitation and emission maxima are located in red spectral region - the ranges are 649-681 and 665-695, respectively. Upon the binding to BSA and HSA, a notable enhancement in the fluorescence of the dyes was observed (in 16-110 and 28-150 times, respectively). The most effective dye 1756Sl (bearing alkyl sulfo group) showed the highest increase in fluorescence intensity with both serum albumins (110 times for BSA and 150 times for HSA).

Quantum yield values of most efficient dyes bound to bovine and human serum albumins were obtained. Upon binding to albumins, the increasing of quantum yield value achieves the in dozens of times, resulting in 0.16-0.37 values (while 0.002-0.005 for free dyes). The linear detection range of BSA by dye 1550Sl is 0.003-0.2 mg/ml ($R^2 = 0.90$).

For effective albumin-sensing dyes, the fluorescence response to other globular proteins (trypsin, beta-lactoglobulin and lysozyme) was explored. In the case of lysozyme and trypsin, these dyes demonstrated no fluorescent response. However, studied dyes in the presence of beta-lactoglobulin increased their emission in 10-43 times. These dyes gave no significant fluorescent response upon addition of dsDNA and RNA.

Thus, we proposed bridged pentamethine cyanine dyes for further study as high-efficient red emitting dyes for sensing of proteins, particularly albumins. Due to variation of the substituents in structure of such dyes, the significant variation of their affinity to proteins could be achieved.

INTERACTION OF POLYVALENT T7-LIKE BACTERIOPHAGES WITH NATURALLY OCCURRING *E. COLI* STRAINS

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Due to the rapid emergence of multi-drug resistant bacteria, a phage therapy has reappeared in the spotlight recently. Convenient components of therapeutic phage cocktails are T7-like phages, since they feature small genomes, are strictly lytic and escape various bacterial defense mechanisms. Nevertheless, an application of such biological agents can be associated with unexpected threats and anticipates comprehensive studies of bacteria-phage interaction.

We previously showed a set of T7-like phages to be polyvalent and able to infect various laboratory strains of *E. coli*. Three of them are well-known phages T7, T3, BA14; phage FE44 was isolated and sequenced in our previous research; isolation and DNA sequencing of phage ZeVs was performed in the course of this work.

The main goal of the presented research was to evaluate sensitivity of naturally occurring *E. coli* strains to this phage set and trace its relation to host autonomous genetic elements. 28 *E. coli* strains isolated from patients with dysbiosis were used.

Combined use of five T7-like phages, as well phage lambda allowed the identification of type I and II restriction-modification (RM) systems in the set of clinical *E. coli* strains. In addition, resistance to phages was shown to be related to the presence of plasmids that cause the restriction or abortion of phage infection.

Thus, human-originated *E. coli* strains naturally possess a variety of adaptive mechanisms including resistance to phages provided by a repertoire of autonomous genetic elements. Moreover, they act against phages of T7 supergroup that are popular agents in phage therapy mixes. Use of T7-like phages as components of therapeutic phage cocktails is being discussed.

DEVELOPING OF NEW MODEL FOR STUDY OF HYPERGLYCEMIA EFFECT UTILIZING *CARASSIUS AURATUS* STRESS RESPONSES AND DIFFERENT SOURCES OF GLYCANS

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Piscine insulins are structurally close to higher vertebrate insulins stipulating the utilizing of the Cyprinidae fish for the investigation of the diabetes mellitus. Current approaches explore mostly *Danio rerio* for the detection of the morphological changes of diabetic complications (retinopathy) or for genetic manipulations. With the aim to detect the early biochemical responses to hyperglycemia in fish and their corrections, we initiated firstly the utilizing of crucian carp, *Carassius auratus*, as a model organism. *C. auratus* was treated by glucose in low (5.55 mM, LCG), middle (55.5 mM, MCG) or high (111 mM, HCG) concentrations and low (5.55 mM, LCC) and high (55.5 mM, HCC) sucrose waterborne concentrations for 21 days. The responses of Zn-buffering proteins metallothioneins (MT), oxidative stress, DNA instability in the hepatopancreas, as well as erythrocytes indices and cholinesterase (ChE) activity in brain were evaluated.

The hyperglycemia was confirmed at HCG due to elevated level of glycated hemoglobin by 54.9%. However, the decrease in the total hemoglobin concentration, and the increase in the methemoglobin level was detected at MCG and HCG. Two-three times increased level of the cells with the micronuclei and decreased lysosomal membrane stability in erythrocytes were detected after all treatments. However, the responses of oxidative stress, ChE and metallothioneins were distinct. All treatments caused the increase in the oxyradical level in the hepatopancreas (up to 1.8 times by the LCC) except HCC that caused its down-regulation by 5.3 times. The level of lipid peroxidation decreased in LCG and HCC groups and elevated in other exposed groups (up to 1.6 times in HGC). The depletion of ChE (by 3.3 times in LGC) attested the neurotoxicity in all groups except HCC. The striking difference between the effect of glucose and sucrose was reflected by the evaluation of MT concentration (detected from their thiol groups, that represent about 33% of the composition of these low weight thermostable intracellular proteins): the loading by glucose caused the decrease of MT concentration by 1.7-3.9 times, whereas sucrose provoked its increase by 2.7 times in both exposures. We speculate that this opposite effect is corresponding to the reductive properties of glucose and nonreductive nature of sucrose and the limited ability to hydrolyze sucrose during digestion in fish. Most effective glucose concentration (MCG) was three times lower than recommended for *D. rerio.* These circumstances make the *C. auratus* the promising model organism.

This work has been granted by the Ministry of Education and Science of Ukraine (Project # 133B).

FATTY ACID PROFILES IN PLANKTONIC AND BIOFILM *P. AERUGINOSA* CELLS WITH DIFFERENT LEVEL OF C-DI-GMP

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Pseudomonas aeruginosa is a Gram-negative pathogen responsible for a variety of opportunistic infections, including chronic airway infections in patients with cystic fibrosis (CF). During the course of chronic infection, *P. aeruginosa* forms matrix-encased, surface-associated communities called biofilms. Biofilms are thought to contribute to persistence in the CF airway by contributing to evasion of the host immune response and antimicrobial therapy. Biofilm communities differ from planktonic bacterial cultures not only in terms of their metabolic activity but they also display stage-specific phenotypes during development and considerable spatial heterogeneity of physiological condition. Cells alter the fatty acid composition of their lipids to maintain membrane fluidity with varying environmental conditions. In this study, fatty acid profiles of biofilms versus planktonic cells of the *P. aeruginosa* with different level of intracellular c-di-GMP were compared.

Wilde-type strain *P. aeruginosa* PA01 and strains with low (PA01 pJN2133) and high (PA01 ΔwspF1) level of c-di-GMP were used as test-organisms. We identified that not all fatty acids are included in each experimental *P. aeruginosa* strains. For example, undecanoic acid is part of the cellular wall of PA01 pJN2133 at a level of 0.11%. Tridecanoic acid is part of the cellular wall of PA01 ΔwspF1 at a level of 0.10%. Palmitic acid is present in each strain at high level, however, N alcohol (1-Hexadecanol) is present in only two strains PA01 (0.78%) and PA01 pJN2133 (0.61%). 12:1 3OH acid is present only in the mutant strains PA01 pJN2133 (0.79%) and PA01 ΔwspF1 (0.11%). γ-Linolenic acid present in wild-type PA01 (0.49%) and one mutant strain PA01 pJN2133 (0.38%). The levels of saturated fatty acids in planktonic cells of *P. aeruginosa* strains were: PA01 – 59.62%; PA01 pJN2133 – 59.42%; PA01 ΔwspF1 – 77.97%. Biofilm cells of this strains containing respectively 66.22%; 64.37% and 49.31%. The levels of unsaturated fatty acids in planktonic cells of *P. aeruginosa* strains were: PA01 – 40.3%; PA01 pJN2133 – 40.58%; PA01 ΔwspF1 – 22.01%. Biofilm cells of this strains containing respectively 33.78%; 35.62% and 50.78%. The fatty acid composition of different strains of *P. aeruginosa* was compared with the mobility of their cells.

DETECTION OF MARKERS IN THE FAT-ACID PROFILE OF *BACILLUS SUBTILIS* ONU551 - DESTRUCTOR OF DISINFECTANTS

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To increase the efficiency of the work of medical institutions and pharmaceutical production facilities, where a large number of phenolic and other hardly oxidizable compounds are found in the sewage waters, we recommend using strain *Bacillus subtilis* ONU551 (F13) to purify this kind of effluent. The strain was isolated in 2017 from wastewater produced by Ukrainian pharmaceutical products, and does not lose destructive properties with respect to aromatic xenobiotics in the presence of pathogenic microbiota - *E. coli, K. pneumoniae, S. moscow, P. aeruginosa*. The ability of the strain *B. subtilis* ONU551 to oxidize widely used disinfectants (phenol, N-cetylpyridinium chloride, etc.), as well as its lack of virulent and toxigenic properties, makes it possible to use it in the biotechnology of wastewater treatment from aromatic xenobiotics. An assessment of the pathogenicity of *B. subtilis* ONU551 was carried out in vitro - on the model of human cell culture lines - Hep2 and RD and animals - L20B, and in vivo - in white laboratory mice. According to the fatty acid composition, the spectrum of which was obtained on the gas chromatograph Agilent 7890, and deciphered using the RTSBA6 6.21 library database of the Sherlock MIDI program, the investigated strain with a high similarity index was identified as *Bacillus subtilis* ONU551.

In the present work, a study of the details of the fatty acid composition at a level below 5% is undertaken to find specific chemicals for this non-pathogenic microorganism (markers) for possible detection in other sources of the environment (contaminated soil, sea water), and also for its subsequent use in the biopreparations on the basis of a synergistically active association of strains-destructors. A feature of the fatty acid profile of *B. subtilis* ONU551 is the presence on the chromatogram of a 16: 1 biomarker fatty acid strain w7c alcohol (with a molar fraction w = 1.1% of the total area of the peaks). To the biomarker fatty acids detected in a minor amount, also unsaturated long chain fatty acids of normal structure - 15: 1 w5c (1.9%), 16: 1 w11c (1.2%); and branched structure - 17: 1 iso w10c (3.2%). Biomarker should be considered as the ratio of fatty acids [15: 0 iso (34.7%) / 15: 0 anteiso (33.7%)] and [17: 0 anteiso (10.2%) / 17: 0 iso (7.1%)] in comparison with the content of fatty acids of normal structure: 12: 0 (0.4%), 14: 0 (0.3%), 16: 0 (1.3%). Analysis of the obtained chromatogram allowed to state that the strain of *B. subtilis* ONU551 is characterized by the saturation coefficient, which is 10.

PECULIARITIES OF CHEMICAL COMPOSITION OF PHENOL-OXIDIZING STRAIN CELLS *AEROMONAS ICHTHIOSMIA* ONU552

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The chemical composition of cells of strains of microorganisms is necessary for their chemodifferentiation. This information can be used to determine the generic (species) belonging to a strain in ecological, biotechnological and infectious communities of microorganisms for medical diagnosis or for further use in the biotechnological direction. Of particular interest is the introduction of advances in biotechnology techniques based on the use of non-pathogenic strains of microorganisms immobilized on synthetic carriers, in the practice of treating effluent from medical institutions with a high content of phenolic contaminants.

This paper presents the results of the chemical composition of the cells of the phenol-oxidizing strain *Aeromonas* sp. F2. The fatty acid analysis of the strain was carried out by gas chromatography using the Sherlock micro-organism identification system. 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 is identified as Aeromonas ichthiosmia ONU552.

Analysis of the results of chromatographic studies showed that hexadecane (16: 0, 21.84%) and hexadecene (16: 1 w7c / 16: 1 w6c, 36.89%) were detected in the profile of the strain under study. The total content of unsaturated fatty acids, including biomarker, detected in a minor amount, was 50% of the total fatty acid pool: 16: 1 w7c / 16: 1 w6c (36.89%), 18: 1 w7c (8.53%), 16: 1 w7c alcohol (3.45%), 17: 1 w8c (0.60%), 17: 1 w6c (0.26%), 16: 1 w5c (0.12%). A distinctive feature of the strain *A. ichthiosmia* ONU552 from microorganisms belonging to another genus (or species) is the presence of monounsaturated re-dicenic acids 16: 1 with double bonds at positions Δ6, Δ7, and the absence of 12-methyltetradecanoic acid 15: 0 anteiso and negligible the amount of 13-methyltetradecanoic acid 15: 0 iso (0.97%), which in the lipid composition of cells of other microorganisms dominate and in the sum varies from 30 to 85% of the total fatty acid pool. Biomarkers for the species-specific detection of the strain *A. ichthiosmia* ONU552 should be considered saturated fatty acids of the branched structure in the form -is with an odd number of carbon atoms, recorded in an amount of less than 2% of the sum of the areas of all peaks in the chromatogram: 13: 0 iso (0.20%), 15: 0 iso (0.97%), 13: 0 iso (1.49%). The chemical markers of the 3-hydroxy acid are used to classify this strain as gram-negative bacteria of a sliding type: 12: 0 3OH (0.23%), 15: 0 3OH (0.35%) and 15: 0 iso 3OH (3.85%). The total amount of hydroxy acids is 11.02% of the total fatty acid pool of strain *A. ichthiosmia* ONU552.

COMPUTATIONAL MODELING OF SIRTUIN7

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The family of sirtuins includes 7 members (SIRT1-SIRT7) and all of them are NAD^+-dependent histone deacetylases. The activity of SIRT7 is associated with cardiovascular diseases, diabetes and different types of cancer. SIRT7 is currently considered as a promising therapeutic target.
All of the family members have crystal structure apart from SIRT4 and SIRT7. Moreover, crystal structures of sirtuins include mainly the core of proteins, while N- and C-termini are cut. However, these termini could have an important role in sirtuins activity.
The aim of this study was to create in-silico model of SIRT7 with its N-terminus, which is known to affect the enzyme's catalytic activity, and establish binding modes of its binding partners such as NAD^+ cofactor and acetyl-lysine. Modeling of SIRT7 is necessary for searching databases and looking for potential inhibitors for SIRT7. Virtual screening allows to screen commercial and non-commercial compounds libraries and select compounds for in vitro analysis.
Methods: Three-dimensional model of SIRT7 structure was prepared using X-ray structures of SIRT1, SIRT2, SIRT3, SIRT5, SIRT6 and a resolved fragment of the N-terminus of SIRT7 as templates. All of them are available in Protein Data Bank database (PDB ID's: 4ZZH, 1J8F, 3GLS, 3RIG, 3K35 and 5IQZ, respectively). Sequence alignment was prepared with MAFFT. Spatial orientation of NAD^+ and acetyl-lysine inside the SIRT7 were established by molecular docking using Surflex and validated by comparison with ligand positions in X-ray structures of other sirtuins.
SIRT7 was modeled with its cofactor and acetyl-lysine. Ligands were calculated using Hartree-Fock 6-31g* quantum mechanics method. The complex was subjected to molecular dynamics (MD) simulations in GROMACS program with AMBER 03 force field. RESP charges for the cofactor and the non-standard residue were obtained with RED Server Development, and topologies were created with ACPYPE.
Results: Our investigation has proven resemblance of catalytic core of SIRT7 to the rest of family members. Moreover, our investigation showed that -terminus of SIRT7 remains in spatial proximity of the catalytic core, and therefore, it may affect its catalytic activity. We managed to find the preferred orientations of NAD^+ and acetyl-lysine inside SIRT7, with all components forming a stable complex. MD simulations yielded a stable structure that can be used for structure-based drug design methods.
Conclusion: Our study allowed to model reliable SIRT7 *in silico* structure. It will be a useful tool in searching for its inhibitors, which can be potential drugs in cancer treatment.

IDENTIFYING NEW PATHOGENIC GENETIC FACTORS OF SEX DEVELOPMENT DISORDERS USING WHOLE-EXOME SEQUENCING

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Aim: Disorders of sex development (DSD) include clinical phenotypes such as ambiguous genitalia (1 in 4500 live births), complete XX or XY sex reversal (1 in 20,000 births) (Baskin LS, 2001) and represent a major pediatric concern. There are more than 80 known genes involved in DSD pathogenesis and about 960 possibly implicated candidates. The aim of the research was to identify new DSD gene candidates using whole exome sequencing in trios (DSD-affected child and both parents).
Methods: Samples were collected from 7 DSD-patients (3 patients 46, XX, SRY-negative; 4 patients 46, XY, *SRY*-positive) from Ukraine and Poland, selected after genealogical, clinical, biochemical and cytogenetic investigation. Sequencing was performed on an Illumina HiSeq 4000 System. Read alignment was carried out using DRAGEN Germline Pipeline (Edico Genome). Filtering was performed using VarSeq (Golden Helix).
After the first step of filtering, we have obtained approximately from 340 to 780 variants for each trio with the population frequency less than 0.01% (1000 Genome Project Database). The genotypes were classified as homozygotes, compound heterozygotes, X-linked and *de novo* variants. After segregation analysis, variants were annotated according to DSD-associated gene panel (Fan, 2017) and their implication in gonadal development inferred from previously published animal models, RNA-seq studies, and known molecular pathways.
Results: In the 46, XY, *SRY*-positive patient, a new missense SNP (X:139586222 Gly/Asp) was annotated in *SOX3.* An interesting combination of paternal SNP (15:41862801) and *de novo* 12 bp insertion (15:41865665) was revealed in *TYRO3*, which both may disrupt the splice sites. In the 46, XX, *SRY*-negative patient we found *de novo* duplication in NR5A1 and SNP in *WT1* gene (11:32413528 T>C).
Conclusions: The novel variant in *SOX3* identified for the 46, XY, *SRY*-positive patient can be added to update the DSD diagnostic panels. The further investigation of *SOX3* and *TYRO3* mutant protein structure and functions will be important for better understanding of DSD-mechanisms.

ANALYSIS OF APOPTOSIS/NECROSIS STAGES OF CORD BLOOD NUCLEATED CELLS AFTER CRYOPRESERVATION WITH DMSO AND GLUTATHIONE

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Cord blood nucleated cells (CBNCs) were subjected to physico-chemical stresses during cryopreservation, which causes the development of apoptotic processes both immediately after freeze-thawing and delayed in time. Antioxidants are largely able to prevent the development of pathological processes. In connection with this, we assessed the development of CBNCs apoptosis/necrosis traits immediately after cryopreservation in presence of DMSO and glutathione, as well as after cell transfer under the conditions close to physiological ones.

The CBNCs fractions isolated by polyglycine were cryopreserved in solutions containing DMSO (5, 7.5 and 10%) and glutathione (1 and 3 mM). They were frozen at a rate of 1-3 °C per min to -80 °C, followed by immersion in liquid nitrogen. The apoptosis/necrosis stages of cells were determined by cytofluorimetry using combinations AnnexinV and 7-AAD. Transfusion modeling was performed by moving a portion of frozen-thawed cells to Hanks solution (37 °C, 1 hour).

After cryopreservation with DMSO the different stages of apoptosis/necrosis covered up to 30% of CBNCs, with predominance of necrosis (AnnexinV$^-$7AAD$^+$). In the samples frozen with 7.5% DMSO and 1 or 3 mM glutathione, the number of necrotic cells was lower in 2 and 2.5 times respectively, and the number of intact cells was 11-12% higher than without antioxidant.

Incubation of cells in Hanks solution for 1 h led to an increase in AnnexinV$^-$7AAD$^+$-cells among all the samples by 2 times compared with those obtained immediately after freeze-thawing. The number of intact cells (AnnexinV$^-$/7AAD$^-$) in all experimental groups was lower by 10-30% than immediately after freeze-warming. The largest number of intact cells was observed in samples frozen with 7.5 and 10% DMSO. In samples cryopreserved with glutathione adding, the amount of AnnexinV$^-$7AAD$^+$-cells was lower by 18-32%, and AnnexinV$^-$7AAD$^-$-cells increased by 17-22%. Introduction of glutathione into the cryoprotective solution 5% DMSO allows preserving up to 62% of AnnexinV$^-$/7AAD$^-$-cells after 1 h incubation in the Hanks solution. These results exceed the analogous indices after application of DMSO at 7.5 and 10% concentrations without antioxidant.

Adding glutathione to a cryoprotective solution can prevent the development of apoptotic processes in the cells and may be a prerequisite for the development of cryoprotective media with a lower content of DMSO.

BACTERIAL DIVERSITY IN SOILS, CONTAMINATED AFTER THE CHORNOBYL ACCIDENT: THE FIRST ANALYSIS ATTEMPT

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With a fairly small and easy genome organization, each bacterial cell is surprisingly resistant to adverse environmental factors. At the same time, the microbiome as an integrated community of individuals is sensitive to the slightest changes in the environment and can be used to examine processes occurring in the ecosystem. Incredible radioresistance of bacteria and fungi is well known. However, radiation can alter bacteria functions, what may lead to differences in the compositions of the communities.

In the presented research, we used fragments of the 16S rRNA gene encompassing the V3 and V4 variable regions for sequencing using amplified universal and bacteria-specific primers. DNA was collected, purified and sent for sequencing. It was performed the internal home-brewed methodology of the company, targeting 2 x 300 (Paired-End) with target coverage 10 000 reeds using MiSeq device and MiSeq Reagent Kit v3 (Illumina, San Diego, USA). The data processing has been based on proprietary analysis pipeline (www.mrdnalab.com, MR DNA) with open-source bioinformatic pipeline QIIME (http://qiime.org/) (Caporaso et al, 2010).

As for now, we got the database on the general bacterial diversity of 6 samples, collected from Chornobyl exclusion zone in summer 2017. Data were analyzed of the Order taxonomic level. Data evidence, that in all samples Actinobacteria and Alphaproteobacteria are dominating.

Actinobacteria inhabit soil, as well as fresh- and seawater ecosystems. This taxon may play an important role in the decomposition of organic substances such as cellulose and chitin and, thus, participate in the cycle of organic substances and in the carbon cycle. It replenishes the supply of nutrients in the soil, which is important for the formation of humus.

Alphaproteobacteria, due to their specific form, may increase their absorbance surface and, thus, better survive in the poor environment. This group can have a various form of nutrition, including chemoheterotrophy and chemoautotrophy.

Given the obtained data, it can be hypothesized, that in all samples of soils analyzed, intensive organic matter decomposition occurs – of both easily available forms of carbon and cellulose.

NANOPARTICLES AFFECT THE EXPRESSION OF IMMUNE-RESPONSE ASSOSIATED GENES IN NORMAL HUMAN ASTROCYTES

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Nanomaterials have numerous applications in biotechnology and medicine. At the same time, experimental evidence on adverse effects of various nanomaterials on genetic, cellular and organism levels is being accumulated. In this study we demonstrated that such nanomaterials as single-walled carbon nanotubes (SWCNTs), fullerene C_{60}, titanium nitride (TiN), and chromium disilicide (CrSi2) nanoparticles alter the expression levels of the key immune-response associated genes in normal human astrocytes (line NHA/TS).

For instance, in cells, treated with SWCNTs in concentrations 2, 10 and 50 ng/ml for 24 h we observed a marked, dose-dependent down-regulation of the mRNA levels of HLA-DRA (major histocompatibility complex, class II, DR alpha) – 2.6-fold, 8.4-fold and 10-fold respectively. The expression levels of HLA-G (major histocompatibility complex, class I, G) gene in human astrocytes were also prominently decreased – 5.8 fold, 22 fold and 25 fold (at SWCNTs concentrations 2, 10 and 50 ng/ml respectively). Moreover, treatment with SWCNTs at different concentrations led to down-regulation of HLA-DRB1 (HLA class II histocompatibility antigen, DRB1-15 beta chain) and up-regulation of HLA-F (major histocompatibility complex, class I, F) in human astrocytes.

Fullerenes C_{60} were added to NHA/TS cells at final concentration of 1 μg/ml and cells were incubated with nanoparticles for 24 and 48 h. We observed a time-dependent down-regulation of HLA-DRA in astrocytes treated with fullerene C_{60}.

Incubation of NHA/TS cells with TiN nanoparticles for 24 h at final concentrations of 0.2 and 1 μg/ml resulted in significant dose-dependent down-regulation of HLA-DRA expression levels in these cells. Very similar results were obtained when cells were incubated with 0.2 and 1 μg/ml of CrSi2 for the same length of time.

The HLA-DRA and HLA-DRB1 genes encode the alpha and beta1 subunits of HLA-DR, which primary role in immune response is presenting peptides derived from extracellular proteins (Marsh S. G. et al., 2005). Notably, incubation of human astrocytes with all four types of studied nanoparticles led to a significant down-regulation of HLA-DRA expression in these cells. Such uniform and prominent effect allows us to suggest, that nanomaterials in question elicit a genotoxic effect on normal human astrocytes *in vitro.* Whether nanomaterials are capable of altering immune functions of astrocytes *in vivo* is, therefore, a question of great importance.

ADAPTIVE LABORATORY EVOLUTION AS A TOOL TO INCREASE THE ABILITY OF MICROORGANISMS FOR BIODEGRADATION OF BIOPLASTICS

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The accumulation of plastic waste in the environment creates an increasing ecological problem. Due to the constantly growing production of plastic, it is necessary to search for new methods of waste degradation. It was shown that microorganisms have a potential in biodegradation of plastic. Hence, methods of increasing these abilities should be taken into account. Here, we present a result of isolation and identification the microorganisms from Antarctic soil samples able to degrade bioplastics such as PLA (polylactic acid), PCL (polycaprolactone), PBS (polybutylene succinate), PBSA (polybutylene succinate adipate) and PHB (polyhydroxybutyrate). Moreover, due to increased leverage of natural selection in laboratory settings for basic science applications, we show application of Adaptive Laboratory Evolution (ALE) as an approach letting for increase of natural properties of isolated microorganisms to degradation bioplastics. The obtained results are a good starting point for further process optimization in efficient plastic degradation.

This work was realized as the Ph.D. research program 'Innowacyjny Doktorat' (no. D220/0006/18) financially supported by Wrocław University of Environmental and Life Sciences.

PRODUCTION OF IL7-HIS RECOMBINANT PROTEIN FOR PURIFICATION OF POLYCLONAL ANTIBODIES

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Recent studies of physiological functions of pleiotropic cytokine interleukin-7 (IL-7) have shown that this cytokine plays an essential role in modulating T- and B-cell development and T-cell homeostasis. IL-7 is a type I glycoprotein produced primarily by stromal cells and exerts its effects through a receptor complex consisting of IL-7 R alpha and common gamma-chain/IL-2 R gamma. Investigations of IL-7 have shown that there is a complex mutual influence of IL-7 concentration on T-cell proliferation. IL-7 serum availability has a modulating effect on the immune system and significantly affects the course of such diseases as viral infections (HIV, CMV, HCV), autoimmune diseases (multiple sclerosis, type I diabetes, rheumatoid arthritis) and others. Monitoring of IL-7R concentrations appears to play an important prognostic value. IL-7-His can be a perspective component of test systems for IL-7R detection. Also, IL-7-His can be used as a ligand for purification of polyclonal antibodies specific to IL-7. The aim of the study was to obtain IL-7-His fusion proteins in Escherichia coli, to prove its functionality and to receive stable immunosorbent with it.

The DNA sequences encoding human IL-7 was subcloned into pET24a(+) expression vector under control of T7 promoter and upstream of the vector-derived 6xHis-tag. *pET24-IL7-His* was transformed into *E. coli*, protein synthesis was induced with IPTG and with auto-induction protocol. Use of the auto-induction protocol provided significantly higher protein yield as compared to IPTG induction. Protein of interest was obtained in the form of inclusion bodies. Immobilized-metal affinity chromatography was used for purification of solubilized protein with subsequent renaturation of IL-7-His, which was tested in ELISA. Purification and immobilization of IL-7-His were essentially a one step that significantly reduced the cost of production. Resulted affinity medium – IL-7-His on the Ni^{2+}-sepharose – was successfully used for purification of polyclonal antibodies.

Fully functional IL-7-His protein was obtained and applied for purification of high specific polyclonal antibodies. IL-7-His and purified polyclonal antibodies can be used in capture sandwich immunoassays. In addition, our results may potentially contribute to the development of diagnostic tools for IL-7R detection.

NANOBASED ZEARALENONE SENSING SYSTEM FOR HEALTHCARE APPLICATIONS

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Zearalenone is an estrogenic mycotoxin, which people can be exposed to through the diet. It has been reported that zearalenone and its metabolites were associated with the development of hormone-dependent tumors and breast cancer. To evaluate the risk of breast cancer related to zearalenone exposure, levels of mycotoxins can be determined by direct detection in urine.
Therefore, fluorescent sensor system based on nanostructured molecularly imprinted polymer (MIP) membrane was developed and used for determination of zearalenone and its metabolite – zearalenol. The MIP membranes capable of recognizing zearalenone were synthesized using a fragment-based approach with cyclododecyl 2,4-dihydroxybenzoate as a dummy template and 1-allyl piperazine as the functional monomer. Triethyleneglycoldimethacrylate was used as a cross-linker. Sensor responses of the fluorescent system were investigated as a function of both the type and concentration of the functional monomer in the initial monomer mixture used for the membranes' synthesis, as well as the sample composition. The influence of pH, ionic strength, and buffer concentration on sensor responses were investigated. The fluorescent sensor system based on the optimized MIP membranes provided a possibility of zearalenone detection within the range 1-25 $\mu g ml^{-1}$ demonstrating detection limit (3Б) of 1 $\mu g m^{l-1}$. Proposed sensor system is highly selective for zearalenone and its metabolite detection. The sensor system could be used for the detection of target analytes in urine samples. Overall, the brand new biosensor system based on nanostructured MIP membranes showed great potential for rapid and accurate zearalenone and its metabolite determination in urine. It could be used for food safety and environmental monitoring as well as for clinical diagnostics and healthcare.

Acknowledgment. Financial support from Ministry of Education and Sciences of Ukraine and National Academy of Sciences of Ukraine is gratefully acknowledged.

ESTABLISHMENT AND CHARACTERIZATION OF S6K1 ISOFORM-SPECIFIC KNOCKOUT HEK-293 CELLS

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Ribosomal protein S6 kinase 1 (S6K1) represents an executioner kinase of the Akt/mTOR signaling pathway and regulates a cell size, protein translation, cell proliferation and migration. To exert its functions the S6K1 gene encodes several protein isoforms, including alternatively translated p85 and p70 (from the first and second AUG, respectively), as well as hypothetical p60. Despite well-studied roles of p85- and p70-S6K1, the origin and function of p60-S6K1 remain unclear. However, the p60-S6K1 isoform was suggested to be a product of alternative mRNA translation as well, but from the third in-frame AUG. The aim of the current study was to establish S6K1 isoform-specific knockout HEK-293 cells ($p85^-p70^-p60^-$HEK-293 and $p85^-p70^-p60^+$HEK-293) and assess their functional characteristics in the context of cell proliferation, migration, and regulation of the main S6K1 substrates involved in protein synthesis control.

The $p85^-p70^-p60^+$HEK-293 and $p85^-p70^-p60^-$HEK-293 cells were established via the application of the CRISPR/Cas9 gene editing technology by targeting the DNA sequence located either after the second or hypothetical third alternative translation start. The specificity of S6K1 gene targeting was further confirmed by the detection of S6K1 isoforms expression by western blot and immunofluorescent analyses. In the assessment of functional properties of the generated cells, both cell models revealed decreased rates of cell proliferation and migration (estimated by the MTT assay and *in vitro* scratch assay, respectively), as well as a reduction in Akt Ser-473 phosphorylation levels compared to wild-type HEK-293 cells. Meanwhile, the results demonstrated that the $p85^-p70^-p60^+$HEK-293 cells proliferated and migrated faster than the $p85^-p70^-p60^-$HEK-293 cells. Interestingly, both S6K1-mutated cell lines generally revealed unaffected control of protein translation, as evidenced by phosphorylation levels of rpS6 (S235/236/240/244), eIF4B (S422), and eEF-2K (S366).

In summary, we demonstrated that the p60-S6K1 isoform originates from the common for p85- and p70-S6K1 transcript by means of alternative translation initiated from the third AUG. Moreover, given increased rates of $p85^-p70^-p60^+$HEK-293 cell proliferation and migration over that of the $p85^-p70^-p60^-$HEK-293 cells, the p60-S6K1 isoform could participate in the regulation of cell growth and motility, function attributed to p85- and p70-S6K1.

POSTER SESSION III. CANCER STUDIES

MOLECULAR CHARACTERISTICS OF AN INTERACTION BETWEEN PROSTATE TUMOR CELLS AND THE HOST ORGANISM

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Background: Prostate cancer is one of the most heterogeneous epithelial tumors. Cancerous cells possess several specific molecular aberrations, for example, the appearance of the *ETS* fusion transcripts, loss of *PTEN*, expression of *NKX3-1*, increased levels of *PSA* and *PCA3*, altered expression of a set of lncRNAs during cancer progression, etc. It is known that tumor initiation and progression depends on the intake of nutrients, a state of the extracellular matrix and cell microenvironment, and the immune surveillance escape. These parameters are regulated by the host organism. The developing tumors adjust them to their own needs.

The aim of our work was to reveal clinically significant parameters, characterizing the prostate cancer – host organism interaction at the gene expression level. We aimed to detect specific changes in both, tumors and stromal cells, namely fibroblasts, macrophages, different immune cells, etc.

Methods: relative gene expression (RE) of 30 prostate cancer-associated genes (PCAG) and 25 genes – markers of the organism response (GOR) were analyzed by quantitative PCR in 37 prostate cancer tissues (T) of different tumor stages and Gleason scores (GL), 37 corresponding (paired) conventionally normal prostate tissues (N) and in 20 samples of prostate adenomas (A).

Results: We found the differentially expressed PCAG in A and T, namely *AR, PTEN, VIM, MMP9, KRT18, PCA3, HOTAIR, SCHLAP1, ESR1, GCR, PRLR, SRD5A2, VDR*; and differentially expressed GOR in A and T, such as *THY1, CXCL12, CXCL14, CTGF, HIF1A, FAP, IFNB1, PDL1, CTLA4, IL-1RL1, IL1R1, CD163, CCR4, CCL17, CCL22, NOS2A.* Moreover, most of these genes showed significant RE differences in T of the different GL and stages. Significant changes in RE in GOP genes *CXCL12, CXCL14, CTGF, CD163, CCR4* and *CCL17* were found in tumors depending on the presence or absence of the *EST* fusion transcript and different *PTEN* levels. This may indicate different types of interactions between prostate tumors with different molecular characteristics with the host organism, i.e. the tumor microenvironment. We found that RE of 29 out of 55 genes in tumors correlated with the GL, stage, *PSA* level and age. Also, we found correlations in expression of PCAG and GOR.

Conclusions: The revealed expression profile can be used for molecular profiling of prostate tumors. The specific features of the interaction between the tumor and the host organism require more detailed analysis of prostate tumors in a larger cohort.

FIRST STEP TO CHAOS: TUMOR-ASSOCIATED ABNORMALITIES IN MICRORNA EXPRESSION PATTERN CAN AFFECT EPITHELIAL CELL ADHESION, POLARITY AND MITOTIC SPINDLE ORIENTATION

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Normal epithelial cells form a well-ordered sheet where the cells are tightly bound to each other as well as to basement membrane and have apical-basal polarity – significant asymmetry between luminal (apical) and basal cell areas. The proper spatial positioning of the mitotic spindle is necessary for epithelial tissue homeostasis during growth and wound repair. On the contrary, impaired adhesion, detachment from the neighboring environment, aberrant orientation of mitotic spindle and loss of apical-basal polarity are typical features of cancer cells, leading to disruption of epithelial tissue integrity. As overexpression and downregulation of microRNAs (miRNAs) are necessary for cancer cells to grow and survive, this research aims to identify how the shifts in miRNA expression pattern can also promote the alterations in cell adhesion, polarity and mitotic spindle orientation. MiRNA targets within gene transcripts were predicted *in silico* using the TargetScan software.

Overexpressed miRNAs miR-18, miR-19, miR-21, miR-23, miR-27, miR-29, miR-153, miR-155, miR-181 and miR-375 can silence *CRB1, MPP5* and *INADL, DLGL1* and *PARD3* genes encoding CRB1/PALS1/PATJ, DLG and PAR3 – main components of, respectively, CRB, SCRIB and PAR complexes responsible for the establishment of epithelial cell polarity. In addition, some overexpressed miRNAs can also silence genes involved in mitotic spindle orientation – *VHL, APC* and *PROX1.* Adherens and tight junctions also participate in the establishment and maintenance of cell polarity as well as in normal orientation of the mitotic spindle. Therefore, overexpressed miRNAs miR-18, miR-19, miR-21, miR-23, miR-27, miR-29, miR-155, miR-181, miR-210, miR-221/222 and miR-375 can affect cell polarity also indirectly, due to silencing of genes that encode key molecules responsible for cell-cell adhesion – E-cadherin (encoded by *CDH1* gene), claudin 1 (*CLDN1*), junctional adhesion molecules JAM-A and JAM-C (*F11R* and *JAM3*), tight junction proteins ZO-1 and ZO-2 (*TJP1/2*), occludin (*OCLN*), cingulin (*CGN*), nectins 1 and 3 (*PVRL1/3*), nectin-like molecules 1 and 2 (*CADM3/1*), alpha-catenin (*CTNNA1*), p120-catenin (*CTNND1*), alpha-actinins (*ACTN1/2*), tropomyosin 1 (*TPM1*) and vinculin (*VCL*).

Therefore, tumor-associated abnormalities in miRNA expression can contribute to the silencing of numerous epithelial junction and polarity genes. This impairs cell-cell adhesion and facilitates detachment, movement and invasiveness of the tumor cells. Loss of cell polarity complexes leads to randomized mitotic spindle orientation and to the symmetric division of cells, despite the normal asymmetric division. As a result, transformed cells evade further differentiation, maintain stem-like properties, form irregular multi-layer conglomerates and accumulate in tissue.

ANTICANCER PROPERTIES OF MAGNOFLORINE AGAINST CANCER CELLS

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Introduction. Magnoflorine, one of the most common aporphine alkaloids, exists widely in herbs including Annonaceae, Aristolochiaceae, Berberidaceae, and Euphorbiaceae, and shows a number of biological activities such as antioxidant, antidiabetic, anti-inflammatory, antifungal, and antiviral properties.

The aim of this study was to evaluate the anticancer activity of magnoflorine *in vitro* on six cancer cell lines: rhabdomyosarcoma (TE671), larynx cancer (RK33), two estrogen receptor-positive breast cancer lines (T47D and MCF7), non-small cell lung cancer cell line (NCI-H1299) and cervical cancer cell line (HeLa). Experiments were also executed on normal human skin fibroblasts (HSF) primary cultures regarded as control cells.

Methods. Magnoflorine was isolated from Berberis vulgaris and Berberis siberica MeOH root extractsing hydrostatic counter-current chromatography (hCCC) methods. The inhibition of cancer cells viability was analyzed using MTT colorimetric assay.

Results. Magnoflorine demonstrated dose-dependent activity to all cancer cell lines with different sensitivity. NCI H1299 adenocarcinoma lung cell line proved to be the most sensitive of all cancer cell lines tested. HSF were not affected by magnoflorine treatment, showing the specific activity against cancer cells.

Conclusion. Magnoflorine could be considered as a potential anticancer agent.

THE ROLE OF THE *FBXO11* GENE IN EPITHELIAL-MESENCHYMAL TRANSITION (EMT)

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The FBXO11 protein is a ubiquitin ligase that regulates Snail transcription factor, the level of the latter is significantly increased during epithelial-mesenchymal transition (EMT) process. EMT plays a key role in the development as well as the progression of tumors. As a result of this biological process, epithelial cells lose their characteristic properties due to the disappearance of cell-to-cell interactions and polarity, changing their phenotype and resembling mesenchymal cells with a tendency of increased mobility and invasiveness. Snail inhibits the expression of genes involved in epithelial phenotype such as *E-cadherin* causing a reduced level of these proteins in cells undergoing EMT.

The aim of the present study was to evaluate the role of FBXO11 in regulating the EMT process by Snail, as well as to analyze its effect on tumor progression and metastasis in *in vitro* models. Using the CRISPR interference (CRISPRi) technique, *FBXO11* expression was repressed at the transcriptional level in lung cancer (NCI-H1299), two lines of colon cancer (stage I SW480 and stage IV SW620) and human embryonic kidney cells (HEK293). As a result, reversible epigenetic modifications of genomic DNA, leading to gene silencing, were obtained cells with repressed *FBXO11.* The expression of the FBXO11 gene was further analyzed by real-time PCR (qPCR). An assessment of cell viability in *in vitro* cultures was performed by the MTT test. The analysis of EMT markers and migration assays are on the way.

The obtained results indicate an important role of FBXO11 in the proliferation of the above-mentioned cells and probably in EMT.

L-CANAVANINE INHIBITS CELL GROWTH UNDER ARGININE DEPLETION BY INDUCING CELL DEATH IN HUMAN GLIOBLASTOMA CELL LINES

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Arginine deprivation-based combinational treatment with the plant origin arginine analog L-canavanine can enhance the anticancer effect of arginine deprivation. In this study, the anti-proliferative effect of L-canavanine was investigated in U251MG and U87MG human glioblastoma cell lines. It was shown, that under lack of arginine, L-canavanine effectively suppressed the growth of human glioblastoma cells in a concentration- and time-dependent manner. We decided to analyze the effect of 50 and 100 μM L-canavanine on cell death.

We noticed that 50 μM canavanine in the absence of arginine after 24 h induced pro-apoptotic response as observed by an increase of phosphorylation of stress-activated protein kinase SAPK/JNK. Also, an increase in activation of the mitogen-activated protein kinase p38 (known to mediate proapoptotic effects as well) was observed. Moreover, elevation of the level of the transcription factor CHOP was observed but this increase seemed to be independent of the presence of canavanine, at least in U87MG cells.

The number of dead cells was noticeably increased after the treatment of 100 μM canavanine. A search for hallmarks of several apoptotic markers such as cleaved PARP1, cleaved caspase-9, and caspase-3, Bcl-2 and AIF was performed. In addition, we looked for the presence of markers of necroptosis such as the mixed lineage kinase domain-like protein (MLKL), receptor-interacting protein kinase 1 and 3 (RIP1, and RIP3, respectively). All of them were detectable after 24, 48, and 72 h of the treatment indicating that L-canavanine under arginine deprivation induced apoptotic cell death. These findings, therefore, support our idea that L-canavanine can be a potential candidate in the development of anticancer agent.

This work was supported by European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No.665735 granted to the Nencki Institute.

INFLUENCE OF TRANSPLANTED ALLOGENIC BONE MARROW AND ADIPOSE-DERIVED MESENCHYMAL STROMAL CELLS ON THE BIOCHEMICAL PARAMETERS OF C57BL/6 MICE BLOOD

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Previously we established that mesenchymal stromal cells (MSCs) from bone marrow and adipose tissue differ in expression of cytoplasmic, nuclear and membrane proteins. These parameters play role in the proliferative activity, migration properties, apoptosis level of mesenchymal stem cells during long-term cultivation *in vitro*. Therefore, the purpose of our work was to determine the changes in some biochemical parameters of blood under influence of allogenic bone marrow and adipose-derived MSCs.

The studies were conducted on 2-3-months-old males of C57BL/6 mice weighing 20-24 g. Obtaining and cultivating of allogenic bone marrow and adipose-derived MSCs were carried out in a sterile laminar box in compliance with conditions of asepsis and antiseptics in a CO_2 incubator at 37 °C and 5% CO_2 in DMEM with 10-15% of fetal bovine serum, 1% of antibiotic-antimycotic solution (Sigma-Aldrich, USA). The following groups of animals were formed: 1 group – intact (control group); 2 group – animals, to whom 0.5 ml of 0.9% NaCl solution (placebo) were injected into the caudal vein; 3 group – animals, to whom 10^4 of allogenic bone marrow MSCs (BM MSCs) in 0.5 ml of phosphate buffer solution were injected into the caudal vein; 4 group – animals, to whom 10^4 of allogenic adipose-derived MSCs (AD MSCs) in 0.5 ml of phosphate buffer solution were injected into the caudal vein. On the 18th day after MSCs transplantation were the biochemical parameters of blood determined. The most significant changes in the biochemical parameters were observed in the serum level of proteins, the ratio of globulin and albumin fractions, the metabolism of the end-product protein, as well as some enzymes. The level of total protein was not significantly different in the control and experimental group animals and ranged from 69.7 ± 1.7 to 72.4 ± 7.5 g/l. The index of albumin content was significantly lowered to 35.3 ± 0.6 g/l ($P < 0.05$) compared with the control and placebo groups. On the contrary, the content of globulins increased significantly and amounted to 37.1 ± 2.3 g/l ($P < 0.05$). According to the results of the study, the bilirubin level decreased by almost 5-fold, in the groups that were administered BM MSC and AD MSCs, as compared to control and placebo groups and was 2.2 ± 1.0 ($P < 0.05$) 2.9 ± 1.3 μmol/l ($P < 0.05$), respectively. The creatinine content increased to 26.8 ± 1.7 and 23.1 ± 1.5 ($P < 0.05$) mmol/l, and the urea content decreased to 8.4 ± 1.0 and 9.2± 1.7 ($P < 0.05$) mmol/l significantly. The ALT indicator did not significantly change. The AST amount was significantly lowered and amounted to 312.0 ± 42.4 and 293 ± 31.3 ($P < 0.05$) U/l. So, such changes in indicators are evidence of the modulatory effect on the systemic effects of MSCs on the recipient's organism.

HOW TO CATCH THE CHEATING CANCER CELLS? PD-1/PD-L1 IMMUNE CHECKPOINT BLOCKERS IN ACTION: SMALL-MOLECULES, MACROCYCLIC PEPTIDES AND ANTIBODIES

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PD-1 (programmed cell death protein) is a cell surface receptor that plays an important role in suppressing T cell inflammatory activity. Its ligand, PD-L1, is expressed mainly on antigen-presenting cells. Furthermore, PD-L1 is commonly overexpressed in tumor cells. Blockade of PD-1/PD-L1 interaction has achieved a spectacular success as an anticancer therapy. When PD-1 or PD-L1 protein is blocked, the "brakes" on the immune system are released and the ability of T cells to recognize and kill cancer cells is rising. So far, several anti-PD-1 and anti-PD-L1 antibodies have been approved, but no small-molecules have been reported to be active. In our study, we revealed the potency of new inhibitors of PD-1/PD-L1 pathway. We focused on small-molecules (BMS-1001; -1166) and bioactive macrocyclic peptides (51 and 71) from Bristol-Myers Squibb patents. We identified PD-L1 protein as a target of patented compounds with DSF and NMR method. We also used the X-ray crystallography to obtain a structural insight into an interaction between the compounds and PD-L1. In the cell-based assay - with Jurkat T cells, which overexpress PD-1 and carry a reporter luciferase gene under the control of NFAT promoter and CHO cells overexpressing PD-L1 - we demonstrated that each of the compounds in a dose-dependent manner restores the activation of PD-1 Effector Cells. We also characterized the immunomodulatory effects by calculating EC_{50} values. The compounds' ability to restore the activation of Jurkat T cells by soluble PD-1 was also confirmed by a luminescence measurement.

The macrocyclic peptides are the most promising PD-1/PD-L1 inhibitors among the tested compounds. Their activity is not as spectacular as for monoclonal antibodies, but on the other hand, their different structure and biology eliminate a number of side effects and disadvantages of antibodies. We hope that our results can be used for the preclinical development of the compounds and also for design new promising PD-1/PD-L1 non-monoclonal antibodies inhibitors.

K.J. acknowledges the support of InterDokMed project no. POWR.03.02.00-00-I013/16.

THE EFFECTS OF MDM2-p53 ANTAGONIST RG7388 ON THE PROLIFERATION OF HUMAN OSTEOSARCOMA CELLS

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Nuclear transcription factor p53 is a well-known tumor suppressor and it critically regulates cellular pathways which oppose malignant transformation, such as induction of cell cycle arrest and DNA repair, or apoptosis. It is negatively regulated by the interaction with oncoprotein MDM2. Over 50% of human cancers carry mutations in p53 gene. In the remaining cases, p53 function is most commonly inhibited by high expression levels of its natural inhibitor, MDM2, which leads to downregulation of tumor-suppressive pathways. The inhibition of MDM2-p53 binding restores the activity of p53 and is a promising strategy in the treatment of p53-wt cancers.
RG7388 (Idasanutlin) is a potent and selective small-molecule MDM-2 inhibitor. This inhibitor blocks p53-MDM2 binding and activates the p53 pathway. As a result, it leads to cell cycle arrest and/or apoptosis in wild-type p53 cell lines and tumor growth inhibition or regression of osteosarcoma xenografts in nude mice. In our studies, we examined the effect of this novel inhibitor on human osteosarcoma cell lines: U-2 OS ($p53^{wt}$), Saos-2 ($p53^{-/-}$) and U-2 OS cell populations with acquired resistance to RG7388 ($p53^{mut}$). The MTT assay was used to evaluate cell viability under the treatment with RG7388. Western blot analysis was performed to verify the potency of the compound. Most importantly, the effect of RG7388 treatment on cell proliferation in co-culture (especially resistant in combination with non-resistant) was analyzed with EdU staining by flow cytometry. Our results indicate that resistant Saos-2 cells ($p53^{-/-}$) do not proliferate under the treatment with RG7388 when co-cultured with U-2 OS ($p53^{wt}$). This observation may provide valuable information for preclinical studies with promising compound RG7388.

This study was supported by Sonata grant UMO-2016/21/D/NZ7/00596 from the National Science Centre, Poland.

THE TGF-BETA-SMAD PATHWAY AND IL2-STAT5 PATHWAYS ARE INACTIVATED IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Chronic lymphocytic leukemia (CLL) is the most common form of leucosis in Europe and the USA (about 30%). Most of the cases of CLL, if not all, are preceded by the monoclonal B-cell lymphocytosis (MBC), which occurs in 5-10% of people over the age of 40 and progresses to CLL with a frequency of about 1% per year. Cells of the chronic lymphocytic leukemia (CLL), despite being non-proliferating, express a set of cytokine receptors. Among the most important, there are receptors for IL2 and TGFB. In lymphoid cells, the active canonical TGFB pathway leads to apoptosis. IL2 is one of the main inductors of T-cell activation and differentiation.

We asked a question what is the status of the IL2 and TGFB pathways in CLL cells, with an aim to uncover the molecular details of the appearance of these cells.

We showed that the TGFB-SMAD canonical pathway is not active in CLL cells. SMAD-responsive genes, such as BCL2L1 (BCL-XL), CCND2 (Cyclin D2) and MYC are down-regulated in CLL-cells, compared with B-cells of peripheral blood of healthy donors. Also, the IL2-induced JAK-STAT5 pathway is largely inactivated in CLL cells. Despite elevated expression of STAT2 and STAT5 genes at the mRNA level, STAT5-responsive genes, such as BCL2L1 (BCL-XL), CCND2 (Cyclin D2), HIF1A, ID1, MCL1 and MYC are downregulated in CLL-cells, compared to peripheral blood B-cells of healthy individuals. Moreover, we found that SMAD2 is almost not expressed in CLL cells. No nuclear heterodimers of SMAD4 and SMAD3 (-2) were detected. Importantly, a phosphorylated form of the STAT5 protein was detected; however, no nuclear signal of this form was observed.

The TGFB-mediated signaling is not active in CLL cells, due to low (or absent) expression of SMAD4. The phosphorylation status of SMAD2 and -3 should be further elucidated. The inactivation of the JAK-STAT5 pathway could be explained by the high levels of soluble IL2RA, as was reported earlier. Another possibility could be inhibition of STAT2 phosphorylation, leading to inability to form the active transcriptionally protein heterodimers. The phosphorylation status of STAT proteins in CLL cells should be further illuminated. Also, the expression of proteins, regulating nuclear export/import should be studied.

GENETIC MODEL OF ESR1 Y537S MUTATION IN BREAST CANCER MCF7 CELLS

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Over 70% of breast cancer cells are estrogen receptor-alfa (*ESR1*) positive. Estrogen is primarily and directly responsible for inducing the ductal component of breast development, as well as for causing fat deposition and connective tissue growth (Lin et al., 2004). The actions of estrogen are mediated by the estrogen receptors (*ESR1* and *ESR2*), a dimeric nuclear protein that binds to DNA and controls gene expression. Like other steroid hormones, estrogens enter passively into the cell where they bind to and activates the ESRs. The estrogen: *ESRs* complex binds to specific DNA sequences called a hormone response element to activate the transcription of target genes (Kleinberg, 1998). ESRs are also involved in pathological processes including breast cancer, endometrial cancer, and osteoporosis. *ESR1* has several domains important for hormone binding, DNA binding, and activation of transcription. Mutations in these domains are often found in breast cancer cells because it is ER ligand binding domain (LBD), with a 'hotspot' at the consecutive amino acids L536, Y537 and D538, which map to the loop connecting α-helices 11 and 12.
We used CRISPR-Cas9 genome editing tool to generate MCF7 breast cancer cells carrying the Y537S mutation, the most common mutation in the *ESR1* in aggressive breast cancer. We designed *in silico* the DNA template and gRNA targeting exon 10 of the *ESR1* gene codon near amino acid Y537. These cells will be used to study the effects of this mutation in the survival, resistance, growth and proliferation of these cells.

MANNOSE-CONTAINING GLYCOTOPES OF LEUKOCYTES IN NEURO-ONCOLOGICAL DISEASE

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There is a tendency to an increase in the incidence of tumors of the central nervous system in Ukraine. Among the primary brain tumors in adults, glioblastomas predominate. With the growth, differentiation and malignization of cells, the structure of membrane glycoproteins changes. In the oncologic process, there is a change in the expression of the number of glycoconjugates on the membranes of malignant cells. Therefore, the change in glycobiological indicators in cancers can be a priority direction of the research, namely, the development and improvement of existing methods of treatment.

The aim of the work was to establish quantitative changes in the glycosylation of leukocytes and separately in their lymphocyte fraction upon neuro-oncological diseases.

The subject of the study were blood leukocytes of patients with brain tumors (n = 10). To isolate leukocytes from human heparinized blood, OptiLyse C lysis solution (Beckman Coulter, USA) was used. Separation of the lymphocytes fraction from blood was carried out in a gradient of ficoll-urografin density according to the modified Boyum A. The control group consisted of 10 conditionally healthy donors. The number of blood cells was determined by flow cytometry using mannose-specific lectin of the *Canavalia ensiformis* - Concanavalin A (ConA). The analysis was carried out using a flow cytometer Coulter Epics XL. Calculation of changes in exposure density was carried out in accordance with the FCS Express 3 program.

The study found that the level of lymphocytes interacting with ConA was practically the same in normal and pathological processes, being 92.9 and 94.8%, respectively. However, the fraction of leukocyte cells that contain the α-mannose residues on their surface in neuro-oncological diseases was 90.2%, which is 1.2-folf higher than normal. This is ensured by the granulocyte and monocyte fraction.

Our results and published data allow us to confirm that the development of neuro-oncological diseases causes a change in the degree of glycosylation of leukocyte membranes, and can be used as an additional diagnostic criterion.

ASSESSMENT OF THE CLINICOPATHOLOGICAL RELEVANCE OF MESOTHELIN LEVEL IN PLASMA, PERITONEAL FLUID AND TUMOR TISSUE OF EPITHELIAL OVARIAN CANCER PATIENTS

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Background: Ovarian cancer remains the most lethal gynecologic malignancy. This is due to lack of effective screening, diagnosis predominance in the late stage of disease, a high recurrence rate after primary therapy and poor treatment response in platinum-resistant tumor. Unique biomarkers, predictive of individual disease course, and prognosis are urgently needed. The aim of our study was to assess the clinicopathological significance of plasma, peritoneal fluid (PF) and tumor tissue (TT) levels of mesothelin (MSLN) in epithelial ovarian cancer (EOC) patients.
Methods: Plasma and PF levels of MSLN were measured by ELISA in patients with EOC and benign tumors. Plasma concentrations of MSLN were also assessed in healthy women. The expression levels of the MSLN gene in TT and benign masses were evaluated using qPCR. Clinicopathological characteristics were used to assess the relevance of MSLN in EOC patients.
Results: Preoperative plasma MSLN levels were significantly higher in EOC patients in comparison to the patients with benign tumor and controls. There were noticed significant differences in the plasma MSLN levels based on FIGO stage, grade and histology type. No significant changes were observed between Kurman and Shih (K-S) type I versus type II EOC. Interestingly, PF MSLN levels revealed significant differences based on both grade and K-S type EOC. There were no relevant changes in the MSLN level in PF between different stages and histology types, respectively compared to benign tumor. MSLN expression level in TT was significantly higher based on stage, grade, and K-S type EOC than in the benign masses. Plasma, PF and TT levels of MSLN positively correlated with the level of CA125. Low MSLN concentrations in plasma were associated with prolonged patient survival.
Conclusions: Our current results highlight the differences in the clinicopathological relevance of plasma, PF and TT levels of MSLN in EOC patients.

GENETIC AND EPIGENETIC CHANGES IN PLASMA CELL-FREE DNA AS MARKERS OF RENAL CELL CARCINOMA

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Aim: The genetic and epigenetic material originating from a tumor that can be detected in the body fluids of cancer patients has tumor-specific alterations and is an attractive target for the biomarker discovery. Since these changes can constitute a signature of specific exposure to certain risk factors, they can serve as highly specific biomarkers for risk assessment. Therefore, we tested the markers based on epigenetic and genetic changes in order to investigate the aberrant methylation of DNA and loss of heterozygosity (LOH) of different genomic regions.
Methods: Blood plasma and tumor tissue were obtained from 89 clear cell RCC (ccRCC) patients who received a nephrectomy. The concentrations of cfDNA in blood plasma were determined by quantitative real-time PCR (qPCR) of the b-actin gene. For the LOH analysis tumor DNA was amplified by PCR using FAM-labeled primer and analyzed by capillary electrophoresis. The methylation status of different promoters of the tumor-suppressor genes was determined by the methylation-specific polymerase chain reaction (MS-PCR), by using primers to modified sequences of genes CpG islands.
Results: qPCR revealed statistically significant increases in cfDNA concentrations in cancer patients compared to non-tumor donors. Receiver operating characteristic (ROC) analysis and AUC (0.8049, p=0.0012) obtained for qPCR analysis confirmed that the concentration of cfDNA can be used as a diagnostic feature for the detection of renal tumors. The frequency of LOH in tumors DNA was found at 15% at D3S966 and 21% at D3S1568, which corresponded to the RASSF1 gene (locus 3p21.3), 32% at D3S1038, corresponding to the VHL gene (locus 3p25–26). Simultaneous deletions were detected for two markers in 15% of the samples. MS-PCR showed methylation of the investigated CpG-islands (*RASSF1A, RASSF1C, LRRC3B, GPX3, PCDH8, RUNX3, APC*) of tumor genomic DNA and plasma cfDNA of patients with ccRCC (72%, 48%, 56%, 74%, 79%, 57%, 81% and 68%, 20%, 42%, 46%, 57%, 41%, 73% respectively). At the same time, the methylation of the analyzed genes in nontumor donors' cfDNA was either very low (0–12%). The use of the combined analysis of methylation status of four genes (*GPX3, PCDH8, RUNX3* and *APC*) increased the sensitivity up to 98%, while the specificity remained high (96%).
Conclusions. In summary, microsatellite markers with the high level of LOH must be verified on cfDNA in future studies. The concentration of cell-free DNA in plasma and methylation of specific genes can be a significant addition to serological tumor markers in the identification of patients with ccRCC. However, further studies need to be performed to evaluate their diagnostic value.

This work was supported by Grant 115U002951 of the NAS of Ukraine and 0117U002802 of SFFR of Ukraine.

ALKBH OVEREXPRESSION IN NECK AND HEAD CANCER AS A NEW FIELD FOR ANTICANCER THERAPY

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It has been accepted that individual proteins of the ALKBH family are overexpressed in various human cancers. ALKBH proteins are homologs of *E. coli* 2-oxoglutarate (2-OG) and Fe(II)-dependent AlkB dioxygenase, a DNA repair enzyme. In humans, nine homologs of *E. coli* AlkB protein are identified: ALKBH1-8 and FTO, but only two of them, ALKBH2 and 3, repair DNA *in vivo,* while the others show different substrate specificities and diverse biological functions. The aim of the presented study was to estimate the ALKBH levels in head and neck squamous cell carcinomas (HNSCC) and to correlate them with the cancer development. Here, Western blot and immunofluorescent analysis with the use of available anti-ALKBH antibodies allowed proving that the levels of seven ALKBH dioxygenases: ALKBH1, 2, 3, 4, 5, 8 and FTO were highly increased in HNSCC. Moreover, we discovered that in cancer cells nuclear proteins ALKBH 2, 5, and FTO are also present in the cytoplasm, which might be assigned to the formation of oligomers and/or aggregates that are not able to cross the cytoplasm-nucleus barrier. Simultaneously, we identified an increased level of N6meA in RNA from HNSCC relative to the normal surrounding. The expression level of FTO was shown to be connected with primary tumor size (T), but not related to the degree of spread to regional lymph nodes (N). Statistical analysis also pointed to a high correlation between ALKBH5 levels in relation to FTO and ALKBH1 compared to ALKBH3, respectively. Since the obtained results strongly imply an involvement of several ALKBHs in tumor development, we screened for ALKBH inhibitors using both *in silico* and *in vitro* methods. Taking into consideration the already known anti-FTO activity of natural anthraquinone, rhein, we designed and further synthesized new derivatives of this compound, and proved their anti-ALKBH activity. In the light of newly established ALKBH overexpression in HNSCC and FTO involvement in the elevation of primary tumor size, a development of ALKBH inhibitors from anthraquinone derivatives seems to be the appropriate way to improve the anticancer therapy with alkylating agents.

HISTONE DEACETYLASE INHIBITORS MODULATE EXPRESSION OF EPITHELIAL-MESENCHYMAL TRANSITION (EMT) PROTEIN MARKERS IN BREAST CANCER CELL LINES

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Background: Histone deacetylase inhibitors (HDIs) are a group of targeted anticancer drugs which exhibit pro-apoptotic, cell-cycle arrest and anti-metastatic activity in many types of cancer cells, including breast carcinoma. Currently, four HDIs (vorinostat (SAHA), belinostat (PXD-101), romidepsin (FK-228), and panobinostat (LBH-589)) have been approved for T-cell lymphoma and multiple myeloma treatment. Although HDIs have been shown to effectively induce the suppression of migration and proliferation of breast cancer cells, the anti-metastatic mechanism of HDIs still remains poorly understood. Nevertheless, since both anti-invasive and pro-invasive properties have been described, there is some disputation about the effect of HDIs on invasion of breast cancer cells. EMT is a series of cellular and molecular alternations by which epithelial cells reduce their epithelial character and acquire a mesenchymal phenotype. During this process, cells lose their cell-cell adhesion and polarity and transform to cells with increased migratory and invasive abilities and drug resistance. However, the molecular mechanisms underlying this transition are poorly understood.
Methods: HDI treatment-induced expression of E- and N-cadherin at the mRNA and protein levels was evaluated by means of qPCR technique, western blotting and immunocytochemistry (confocal microscopy), respectively. Inhibition of cells migration after HDIs treatment was assessed by the xCELLigence RTCA impedance monitoring.
Results: Our study revealed that two histone deacetylase inhibitors - valproic acid (VPA) and vorinostat (SAHA), significantly increase the mRNA and protein expression of E-cadherin (epithelial marker) in T47D, MCF-7 and MDA-MB-231 breast cancer cell lines. Interestingly, in MDA-MB-468 cells, we observed that the expression of N-cadherin was upregulated after VPA and SAHA treatment. Moreover, VPA and SAHA treatment resulted in morphological changes of cancer cells and inhibition of migration in all analyzed breast cancer cell lines in a time-dependent manner.
Conclusions: HDI treatment changes the expressions of structural proteins connected with specific (epithelial or mesenchymal) phenotype of breast cancer cells. Yet, such effect is cell line-specific probably due to the heterogeneous epigenetic mechanisms of action of HDIs.

ASSESSMENT OF INTERACTIONS BETWEEN HISTONE DEACETYLASE INHIBITORS AND CISPLATIN IN BREAST CANCER CELLS WITH ALTERED NOTCH 1 ACTIVITY

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Background: The aberrant activation of Notch 1 signaling is associated with the development and progression of many cancers, including breast cancer (BC). Although, the molecular components of Notch signaling have been well defined, the specific mechanisms regulating Notch activity are poorly understood. The aim of this study was to investigate the type of pharmacological interaction between cisplatin (CDDP) and two histone deacetylase inhibitors (HDIs) – valproic acid (VPA) and vorinostat (SAHA) in the BC cells with increased and decreased activity of Notch 1.
Methods: The type of interaction between CDDP and HDIs was determined using isobolographic analysis. The isobolography is a rigorous pharmacodynamic method to establish the type of interaction between different active agents, which exhibit a broad range of concentration. The isobolography permits to accurately classify the observed interactions of drugs used in the mixture at the fixed drug dose ratio. Theoretically, four types of interaction can be discerned: supra-additivity (synergy), additivity, sub-additivity (relative antagonism) and infra-additivity (absolute antagonism).
Results: Our experiments showed that the CDDP/VPA combination at a fixed ratio of 1:1 exerted tendency towards synergy in MCF7 cells with increased and decreased Notch 1 activity, while CDDP/SAHA showed additive interaction in the same BC cells. In contrast, tendency towards antagonism was observed for the combination of CDDP/SAHA in MDA-MB-231 "triple-negative" BC cells with decreased activity of Notch 1, whereas combination of CDDP/SAHA and CDDP/VPA in the MDA-MB-231 cells with increased activity of Notch 1, as well as CDDP/VPA in the MDA-MB-231 cells with decreased activity of Notch 1 yielded additive interaction. CDDP and VPA applied together augmented their anticancer activities in the MCF-7 and MDA-MB-231 cells both with decreased and increased activity of Notch 1, comparing to the Notch 1 naive cells.
Conclusions: Our findings indicate that combined therapy of HDIs and CDDP may be a promising therapeutic tool in the treatment of BC with altered Notch 1 activity.

POSTER SESSION IV. CLINICAL STUDIES

USP1 PROTEIN COLOCALIZES WITH BCR-ABL ONCOPROTEIN IN K562 CELLS AND ACTS AS POTENTIAL TARGET FOR CML THERAPY

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Chronic myeloid leukemia (CML) is the result of reciprocal translocation between chromosomes 9 and 22 and emergence of Philadelphia chromosome. The product of this abberation is Bcr-Abl oncoprotein. According to preliminary results of the mass spectrometric analysis 23 proteins were identified as potential candidates for interaction with the PH domain of Bcr-Abl oncoprotein. One such protein is an ubiquitin specific protease 1 (USP1). The main function of USP1 protein is deubiquitination of proteins in the cell. As a result of deubiquitination, USP1 protein can prevent proteasomal degradation of Bcr-Abl oncoprotein in a cell and, consequently, contribute to its accumulation and progression of the disease. In this work we analyzed colocalization of USP1 and Bcr-Abl proteins in K562 cell line after staining with primary antibodies to native USP1 and Bcr-Abl proteins combined with fluorescently labeled secondary antibodies. Prior to colocalization analysis, K562 cells were fixed in 4% paraformaldehyde in PBS and embedded into non-hardening antifading/anti-bleaching mounting medium – Citifluor AF1. We found regions of overlapping signals between USP1 protein and Bcr-Abl oncoprotein in K562 cells. Colocalization between USP1 and Bcr-Abl oncoprotein indicates their mutual distribution and/or interaction with similar molecular complexes. Obtained results can help in understanding of molecular signalling pathways during the CML and show that USP1 may act as potential target during CML treatment along with traditional kinase inhibitors.

CORRELATION BETWEEN METABOLIC MARKERS AND BONE MINERAL DENSITY IN DIAGNOSIS OF OSTEOPOROSIS

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The aim of investigation: to study correlation between concentration of metabolic markers and bone mineral density in men and women with age-related osteoporosis.

Introduction: Osteoporosis (OP) is a systemic disease that causes a decrease in strength and fractures of bones. Quantitative measurements of mineral density and ultrasonic methods for determining the quality of bone tissue have clinical value for generalized osteopenia (Engelke K. et al., 2006). Biochemical indices can determine the balance between bone tissue formation and resorption (Masheiko I.V., 2017; Cohen-Solal M.E. et al. 2006).

Materials and methods of investigation. We have examined 28 women (66.1 ± 5.7 years) and 26 men (67.3 ± 6.2 years) with age-related OP. Control group consisted of 24 women (30.3 ± 5.1 years) and 23 men (32.8 ± 4.6 years) without osteoporotic changes. We investigated levels of osteocalcin (OC, ng/ml) and procollagen I C-terminal propeptide (PICP, ng/ml), tartrate-resistant acid phosphatase activity (TRAP, U/l) and alkaline phosphatase activity (ALP, U/l) in serum, concentration of β-C-terminal telopeptides of type I collagen (β-CTx, ng/ml) in urine. Projection bone mineral density (BMD, g/cm^2) was measured by dual-energy X-ray absorptiometry (DXA) Lunar Prodigy (GE Medical Systems) of lumbar spine ($L_{I–IV}$) (Pisani P. et al., 2013). Statistical analysis was performed using the Statistica 6.0 (Statsoft Inc., USA).

Results and discussion. The most significant decrease of BMD in comparison with control group was found in men with age-related OP (40.45 ± 3.96%, $P < 0.05$). Increased TRAP activity (6.02 ± 0.97, $P < 0.05$) and β-CTx concentration (1.32 ± 0.17, $P < 0.001$) in women with age-related OP testifies to active bone resorption. However, the concentration of OC (18.21 ± 2.53, $P < 0.05$) and ALP activity (49.74 ± 5.76, $P > 0.05$) are close to normal and indicate sufficient activity of bone tissue formation. We also established low and moderate negative correlation of BMD with TRAP concentration (-0.176, $P < 0.05$) and β-CTx (-0.328, $P < 0.01$) in men with OP and low negative correlation of these parameters in women with OP: TRAP (-0.192, $P < 0.05$), β-CTx (-0.221, $P < 0.05$). In men with OP decrease of OC concentration (12.51 ± 1.92, $P < 0.001$) and low positive correlation of BMD with OC concentration (0.157, $P < 0.05$) was revealed, this a indicates decrease of osteoregenerative activity.

Conclusions: In women with age-related osteoporosis the osteoregenerative activity of bone tissue is normal. In men with age-related osteoporosis the high activity of bone tissue resorption and decreased osteoregenerative activity are detected.

15q11.2-q13 ALTERATIONS IN PATIENTS WITH PRADER-WILLI AND ANGELMAN SYNDROMES

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Prader-Willi (PWS) and Angelman (AS) syndromes are 2 clinically distinct disorders associated with multiple anomalies and intellectual disability (ID). Angelman syndrome affects an estimated 1 in 12,000 to 20,000 people. Prader-Willi syndrome affects an estimated 1 in 10,000 to 30,000 people worldwide. There are 4 known genetic mechanisms of PWS and AS: de novo maternal or paternal deletions involving chromosome region 15q11.2-q13, uniparental disomy (UPD) of 15q11.2-q13, mutations in the imprinting center (IC) and point mutations of *UBE3A* gene. PWS and AS should be differentiated from disorders caused by other microdeletions, duplications and point mutations in genes associated with ID.

The aim of the study was an estimation of 15q11.2-q13 rearrangements and epigenetic alterations frequency in the group of patients with PWS and AS clinical phenotype.

Groups of 60 patients with PWS phenotype (18-females and 42- males) and 32 patients with AS phenotype (21 and 11, respectively) have been collected. To identify methylation status of the *SNRPN* locus all collected samples were analyzed by methylation-specific PCR (MS-PCR).

Based on the published data the analysis of *SRNPN* gene region by MS-PCR could detect about 100% alterations associated with PWS and about 65% – in AS. In the analyzed group of 60 patients with PWS phenotype paternal deletions/maternal UPDs/IC alterations of *SNRPN* locus were detected in 25 patients (42%). In AS group the frequency of *SNRPN* maternal deletions/paternal UPDs/IC alterations were 28% (9 of 32). The lower than expected detection rate in PWS group can be explained by the presence of patients with PWS-like syndromes. Moreover, in PWS group we observed the significant prevalence of males – 70% (42 of 60), but the frequency of detected alterations in females was higher than in males - 56% (10 of 18 patients) and 36% (15 of 42) correspondently. This can indicate that less strict diagnostic criteria for males lead to incorrect overdiagnosis of PWS in the current group. The low detection rate in AS could be due to both clinical misdiagnosis and limitations of the used method of molecular analysis.

Analysis of *SRNPN* gene region by MS-PCR could be implemented for PWS and AS molecular diagnostic. Our results revealed that the proposed molecular test could exclude the clinical diagnosis of PWS in approximately 60% of Ukrainian patients with suspected clinical diagnosis of PWS. To improve the detection rate of molecular causes of AS further molecular analysis of AS associated genes, such as *UBE3A* and *MECP2* should be done.

STUDY OF ADENOSINE DEAMINASE IN ERYTHROCYTES OF PATIENTS WITH BURN SHOCK

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This study is aimed to investigate the activity of adenosine deaminase (ADA) in RBC under hypoxic conditions in patients with burn shock.

Adenosine is released by endothelial cells in response to lack of oxygen and oxidative stress. It binds with ADOR2B receptor and promotes adaptive changes of metabolism in cells during hypoxia.

Most of adenosine is taken up by erythrocytes and is converted to inosine by ADA. Increased level of inosine activates pro-oxidant enzyme xanthine oxidase.

The 70 heavily burned patients of 18 to 56 were included in the research. The blood was collected during the 1st 24 h after trauma and on the 3rd 24 h upon termination of the stage of burn shock. Depending on their state, patients were divided into two groups. Group I includes 21 patients with severe burn shock (BS), Group II includes 49 patients with extremely severe BS.

The degree of hypoxia was estimated by lactate level. The progress of oxidative stress is represented by the content of thio-barbituric acid reactive substance (TBARS). In hemolysate of RBC the activity of ADA and SOD were measured spectophotometrically.

Significant hypoxic state in all burnt patients before treatment was indicated by a two-folf increase of the level of lactate. In group I it was 4.2 ± 0.4 μmol/l and in group II – 4.30 ± 0.44 μmol/l (normal 1.90 ± 0.06).

Activity of ADA elevated depending on severity of BS. In group I it was 2 times higher than normal, but in group II it was statistically higher 1.2 times than in group I. In the 1st 24 h of BS the content of TBARS was increased 1.5 times in all patients that demonstrated the evident activation of lipid peroxidation. In response the adaptive rising of SOD activity by 10% was detected but in group II only.

The antihypoxant and antioxidant therapy was administrated to all patients. In the 3rd 24 h after trauma at the end of stage of BS in group I the level of lactate decreased significantly by 24% in group I and by 26% in group II. But activity of ADA, SOD and concentration of TBARS remained without changes. We suppose that increased activity of ADA in patients with BS was due to adenosine release in response to hypoxia.

But high intensity of adenosine deamination decreases adaptive capacity of cells which should be promoted by adenosine. Product of inosine deamination stimulates lipid peroxidation.

So study of ADA activity in hypoxic state may give clinical information about adaptive capacity.

THE *ADRB2* GENE A46G AND C79G POLYMORPHISMS IN THE BRONCHIAL ASTHMA CHILDREN FROM UKRAINE

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Bronchial asthma (BA) is a common complex long term inflammatory disease of the lung airways. It is an urgent medical and social problem in Ukraine and all over the world in different countries 4-20% of population (including 5-15% of children) suffer from this disease. Recent increase in environmental pollution and appearance of new allergens raised the number of BA patients, while their treatment is not always efficient. Current data indicate that the interaction of genetic and environmental factors lead to BA clinical expression. These factors influence both BA severity and its responsiveness to treatment. The polymorphisms of the β2-adrenergic receptor (*ADRB2*) gene are approved genetic factors that are associated with the BA onset, severity and responsiveness to treatment, but the results of association studies are inconsistent across each other. However, it is unknown whether *ADRB2* genetic variants and its combination (haplotypes) are associated with asthma in Ukrainians.

The present study primary objective was to investigate whether *ADRB2* gene A46G (rs2400707) and C79G (rs1042713) polymorphisms and its haplotypes were associated with BA severity. We examined these polymorphisms in BA patients with different disease severity. A new multiplex assay based on allele specific PCR followed by RFLP was elaborated and approved in order to determine patients' haplotypes. The data concerning *ADRB2* A46G and C79G genotypes, alleles and haplotypes distribution in patients with different BA severity were obtained. Our results revealed that in the severe persistent asthma patients' group 46AG (38.7%) and 79CC (45.2%) genotypes were the major ones. Whereas in the group of patients with moderate and mild phenotypes 46AG (57.1%) and 79CG (60.7%) were major genotypes. The major haplotype of ADRB2 gene in the severe BA patients' group is AC (46A+79C) (48.4%) whereas in the group of patients with moderate and mild persistent asthma - GG (46G+79G) haplotype (44.6%). Moreover, statistical analysis showed a significant association of 46AA genotype and combined AA+CC (46AA+79CC) genotype (OR=5,32; CI95%: 1,04-27,25) with the greater asthma severity ($P < 0.05$).

We have elaborated the routine multiplex PCR based assay for *ADRB2* A46G and C79G polymorphisms' genotypes and haplotypes screening. The AC haplotype, 46AA and combined AA+CC genotypes could be proposed as a genetic susceptibility markers of BA severety prognosis.

OXIDATIVE-MODIFIED LIPOPROTEINS AS BIOMARKERS OF CARDIOVASCULAR DAMAGE IN PATIENTS WITH ARTERIAL HYPERTENSION

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Investigation of the recent years have shown that low and high density lipoproteins (LDL and HDL) play a complex role in the functioning of the cardiovascular system in norm and with pathology. The different effects of LDL and HDL are connected with lipid and protein molecules associated with them. HDL particle enriched in paraoxonase-1 (PON1), which has been shown to help HDL prevent the accumulation of lipid peroxides in oxidized LDL, inactivate bioactive oxidized phospholipids, stimulate HDL-mediated eNOS-dependent NO production, and enhance cholesterol efflux from cholesterol-laden macrophages. Myeloperoxidase (MPO), like PON1, both binds to HDL and is mechanistically linked to oxidative stress and atherosclerosis. The work aim is to investigate the degree of oxidation modification of lipoproteins in patients with arterial hypertension (AH) as the prediction markers of complication development.

The study included 63 patients (mean age 61 years) with AH 2 grade. As control group we enrolled 24 healthy persons (mean age 59 years). Level of carbonyl oxidation protein products in serum, HDL and LDL+VLDL fractions, activities of PON1 and MPO, degree of oxidative modification of LDL, and level of C-reactive protein were evaluated in all subjects. Lipid parameters were measured in serum, such as total cholesterol, triglycerides, LDL-Cholesterol, HDL-Cholesterol. The differences were considered to be statistically significant at $P < 0.05$.

AH subjects demonstrated a higher level of carbonyl oxidation protein products in serum, HDL and LDL+VLDL fractions in comparison with healthy persons. The degree of oxidative modification of LDL was also higher in patients with AH. The decrease of PON1 activity and increase of MPO activity were observed in patients with AH. The levels of total cholesterol, LDL- and HDL-Cholesterol were within the normal range in patients with AH. C-reactive protein was also within the values characteristic of healthy individuals.

The accumulation of carbonyl oxidation protein products in blood, HDL and LDL eventually results in oxidative modification of HDL and LDL, which can lead to a change in their functional properties. Our evaluation showed a significant decrease of PON1 activity and increase of MPO activity that may contribute to the HDL and LDL oxidation, irrespective of HDL- and LDL-Cholesterol levels. Changes of MPO and PON1 activity may serve as a useful marker of dysfunctional HDL. A more sensitive marker of inflammation can serve as MPO activity, while the level of C-reactive protein would remain within the normal range. Demonstrated changes may create a predisposition to development and progression of atherosclerosis in patients with AH.

EXPRESION OF *BRAF, YWHAS, EGF* AND AURORA KINASES' GENES IN URINE SEDIMENTS FROM PATIENTS WITH PROSTATE CANCER

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Aurora kinase family is a family of serine/threonine kinases, which participate in cell cycle regulation. This family includes Aurora A, B and C kinases, each of them is involved in different events during the cell cycle. Aurora A takes part in centrosome maturation/separation, mitotic entry, microtubule nucleation, spindle assembly, bipolar spindle microtubule formation, cytokinesis and mitosis exit. Aurora B is an important player in chromosome condensation, microtubule-kinetochore attachment, chromosomal alignment, chromosomal segregation, regulation of SAC cytokinesis. Aurora C is the less studied member of this family, plays the similar role as Aurora B in meiosis and some studies showed it can overlap the function of Aurora B. All of Aurora kinases are the potential targets for prostate cancer therapy, but not only their overexpression, but also the loss of expression can lead to malignization. Moreover, they can function through interaction with oncogenes, which share the cell pathways with them. For better understanding of the role of Aurora kinases in tumorigenesis and their potential as tumor markers and/or targets for therapy additional research is essential. As in the last decades the method of liquid biopsies in tumor diagnostics was reported to be one of the most promising, we suppose that the identification of expression of Aurora kinases' genes and some common oncogenes as *BRAF, EGF* and *YWHAZ* in the urine from patient with prostate cancer can be advisable and potential of both theoretic and practical importance.

Methods. Samples of urine had been taken from patients with diagnosed prostate cancer after digital rectal examination. RNA was isolated from cells' sediment according to standard method using TRIzol reagent. cDNA was synthesized from 1 μg of isolated RNA. Genes' expression were detected by RT-PCR analysis. The data was statistically analyzed.

Results. The results of relative expression of oncogenes demonstrated that differential expression of all five genes could be identified. Among gene expression inside Aurora kinase family, the expression level of *AurB* and *AurC* genes was significantly higher than the expression of *AurA.* The cumulative expression of *AurB* and *AurC* genes was higher than the expression of *AurA* in 17 out of 22 prostate cancer samples. We observed positive correlation between expression of *AurC* and *BRAF* genes (rs=0.688, p=0.01), *BRAF* and *EGF* genes (rs=0.719, p=0.01) and *YWHAZ* and *AurC* genes (rs=0.591, p=0.01).

Conclusions. Differential expression of Aurora kinases' genes which was detected in the samples of urine can have several explanations. We can conclude, that even if Aurora kinases participate in prostate cancer development the impact of each of those kinases can vary from sample to sample. In addition, as we cannot control whether the cells consist of our samples, our results can represent the characteristics of the cells which occur in the sample, and their proportion and composition can differ from tumor tissue. Correlations between expression of the *AurC* and *BRAF* genes and between the *YWHAZ* and *AurC* genes lead to the conclusion that there is joint and/or mutual regulation of expression of these genes. The further research should be directed to examination of the expression of Aurora kinases' genes in tissues from prostate cancer patients, normal prostate tissues and hyperplasia.

AUTOPHAGY INDUCTION AS A STRATEGY FOR TREATMENT OF VARIOUS DISEASES CAUSED BY ACCUMULATION OF PATHOGENIC MACROMOLECULES

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There are many metabolic diseases which are caused by accumulation of various macromolecules in cells. If occurring at highly elevated levels, such compounds become toxic to cells which leads to dysfunction of tissues, organs and organisms. In many such diseases, central nervous system is affected and neurodegeneration is observed along with severe symptoms. No treatment for these disorders is currently available. On the other hand, it was postulated that stimulation of autophagy might be a possible therapeutic option. However, most of known autophagy activators provoke severe adverse effectcs which perhaps could be acceptable for a short-time treatment, but not in the life-long therapy which is required in inherited and/or neurodegenerative diseases. We have demonstrated that genistein (trihydroxyisoflavone) is an efficient stimulator of autophagy while being safe and not inducing adverse effects. This isoflavone can cross the blood-brain-barrier which is particularly important in treatment of neurdegenerative diseases. We have demonstrated efficiency of genistein in decreasing levels of various macromolecules in three different diseases: mucopolysaccharidosis (MPS), Huntington's disease and Alzheimer's disease. In MPS, treatment with genistein caused a clearance of glycosaminoglycan storage in cellular models and correction of animal behavior in MPS IIIB mouse model. Efficienct degradation of mutant huntingtin was evident in the cellular model of Huntington's disease. Significant decease in levels of beta-amylodid and hyperphosphorylated tau protein was observed in rat and cellular models of Alzheimer's disease, which was accompanied with correction of animal behavior. In all these models, genistein-mediated induction of autophagy was effective. These results indicate that genistein can be considered as a potential therapeutic agent in treatmant of various neurodegenerative diseases due to its activity as autophagy stimulator.

DGGE-BASED STRATEGY FOR THE SCREENING OF *EEF1A2* GENE MUTATIONS

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Intellectual disability (ID) is clinically and genetically highly heterogeneous disorder with a frequency of 1-3%. Due to whole genome screening, numerous ID associated genes have been identified. In recent studies the correlation of numerous de novo mutations in *EEF1A2* gene with ID, epilepsy and psychoemotional disorders was shown. This gene encodes an isoform of the alpha subunit of the elongation factor-1 complex, which is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome. Various methods of mutation detection have been developed, but a few are suited for flexible and low-cost applications. The denaturing gradient gel electrophoresis (DGGE) is a technique of choice for known and novel mutations screening.

The presented study is aimed to develop the PCR-DGGE for mutations screening of 3rd and 4th exons of the *EEF1A2* gene.

Specific primers with GC-clamps for mutations screening of the 3rd and 4th exons of the *EEF1A2* have been designed and synthesized. PCR conditions for the 3rd and 4th exons of *EEF1A2* gene amplification were optimized. DGGE duration was 4 hours in 10-60% urea-formamide gradient. Using developed methodics the group of patients (n = 21) with ID combined with epilepsy and psychoemotional disorders were analyzed; 3 different abnormal migration profiles (1 in 3rd exon and 2 in 4th) were detected. Further restriction fragment length polymorphism analysis of probes with abnormal migration profiles was performed. rs3818681 (p.Arg69Arg) were detected by RFLP analysis of the 3rd exon after *Hin6I* digestion, rs2274860 (p.Pro160Pro) and rs310617 (p.Gly198Gly) were detected by RFLP of the 4th exon after *MspI* and *MwoI* digestion, respectively.

Summarizing our results we propose new routine DGGE based assay order to detect a wide range of mutations in the 3rd and 4th exons of the *EEF1A2* gene. The current technique could help to identify new mutations in the *EEF1A2* gene and improve diagnostic rate of intellectual disability in Ukraine.

MOLECULAR-BIOLOGICAL AND CHEMOTAXONOMIC INDICATIONS OF SUPERFOODS PROPERTIES AS INNOVATIVE COMPONENTS OF PREVENTIVE MEDICINE

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Modern views on the issues of innovative medicine insert significant corrections in the range of diagnostic studies, associated with involvement of new developments in related fields of science: molecular biology, genetics, nutriciology and microbiology. Special attention is payed to superfoods, as biocorrectors of metabolism.

Aim of the work is a complex bio- and chemoindication of superfoods safety and their ability to correct the impact of negative factors on the organism.

The priority methods, developed by us, were used to study the potential presence of toxic compounds of organic and inorganic origin. Procedure of determination is based on the study of anomalies in the genome of apical meristem cells of *Allium cepa* L. under the influence of different environmental factors and pollutants, which can be manifested in chromosome divergence disorders, chromosomal aberrations, chromosome gluing, the onset of giant cells, etc.

Quantitative characteristics of the integral effect of toxic factors was established by the computer program "Automated System for Express Analysis of Food Safety" using organism's biotests (in particular, *Stylonichia mytilus*).

The results obtained on 10 types of superfoods, showed no disorders of the genome of apical meristem cells of *Allium cepa* L. and nontoxicity of biotested samples on the content of pollutants in concentrations, not exceeding 0.15 MPC.

The conducted chemotaxonomic indication of the fatty acid composition of microbial contaminants of the studied products via the Sherlok MIDI system showed the presence of microorganisms of the order of *Bacillales*, among which microorganisms belonging to three genera (*Bacillus, Paenibacillus, Lysinibacillus*) were detected.

The priority multiplex PCR methods, developed by us for diagnostics of toxigenic *B. cereus* representatives, based on the presence of amplification products of specific genes *nheA, cytK, hblD*, etc., have shown the absence of *B. cereus* strains, responsible for the emetic and diarrheal syndromes. Similarly, the presence of *C. perfringens* and potential causative agents of spoilage was investigated in the samples of superfoods, studied by PCR methods, developed by us. Pathogenic microorganisms were not detected, and the number of saprophytic microorganisms according to MAFAnM was significantly lower than the regulated levels.

The therapeutic and prophylactic action of the products was tested on models of induced mutagenesis and antioxidant activity (aoa).

On the models induced by N-methyl-N′-nitro-N-nitrosoguanidine, 5-bromouracil and sodium nitrite mutagenesis using histidine-auxotrophic strain of *Salmonella typhimurium* TA98 and TA100 shows a decrease in mutagenic activity by the studied superfoods from 16 to 44%, an increase in aoa compared with control.

Thus, molecular-genetic, biological and chemotaxonomic diagnostics of superfood characteristics showed their ability to position themselves in the system of methods of preventing diseases as nutritiological components of preventive medicine.

MOLECULAR-GENETIC DIAGNOSTICS OF BACTERIAL MICROBIOTA FOR PREDICTING ALIMENTARY IMPACT ON THE HUMAN ORGANISM

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Genetic diagnostics of biological toxigenic and beneficial contaminants can be regarded in the system of innovative medicine methods as a modern method of preventing diseases which allows turning from the disease treatment to the preservation of human health. One of the most important innovative aspects of such diagnostics is its rapidity, accuracy and predictability of biocorrection of alimentary disorders by means of alimentary impacts.

The target of the research is to create a system of methods for rapid molecular-genetic diagnostics of biological contaminants to predict the biocorrecting capabilities of products.

The indication of the potential ability of bacterial biological contaminants to cause food poisoings and diseases was performed by PCR as the most effective according to preliminary screening of classical immunological and chemotaxonomic methods. In the investigations, to identify *B. cereus*, we used group-specific primers BCGSH-1F and BCGSH-1R encoding for the target gene groEL with the corresponding nucleotide sequences: 5′-GTGGGAACCCAATGGGTCTTC-3′ and 5′-CCTTGTTGTACCACTTGCTC-3′, forming an amplification product of 400 bp. We found that not all strains of *B. cereus* were toxigenic. The contaminants were studied with specific primers (nheA-F and nheA-R, hblB-F and hblD-R, cytK-F and cytK-R, cesB-F and cesB-R) encoded by the corresponding sequences 5′-GTTAGGATCACAATCACCGC-3′ and 5′-ACGAATGTAATTTGAGTCGC-3′, 5′-ACCGGTAACACTATTCATGC-3′ and 5′-GAGTCCATATGCTTAGATGC-3′; 5′-GTAACTTTCATTGATGATCC-3′ and 5′-GAATACTAAATAATTGGTTTCC-3′; 5′-ACCCATCTTGCGTCATT-3' and 5′-CAGCCAAGTGAAGAATACC-3′ to identify the target genes *nhe-A* (617 bp), *hblD* (465 bp), *cytK* (800 bp), *cesB* (154 bp).

We conducted further studies (priority of which was proven by 1 patent and 3 utility models) that demonstrated the sufficiency of identification of a number of specific genes responsible for the manifestation of diarrhea and emetic syndromes. The PCR-based method was also developed to identify *Clostridia*.

The probiotics are considered by innovative medicine as biocorrectors of a new generation with polyfunctional properties. To identify the *Bifidobacterium* and *Lactobacillus* genera, the 16S rRNA gene sequence is used. The following primers were used to detect the most active and common in Ukraine bacterial metabolic biocorrectors *Bifidobacterium bifidum* and *Lactobacillus acidophilus*: for *Bifidobacterium bifidum* BiBIF-1F 5′-CCACATGATCGCATGTGATTG-3′ and for BiBIF-2R 5′-CCGAAGGCTTGCTCCCAAA-3′ with 278 bp product and for *Lactobacillus acidophilus* 8f 5′-AGAGTTTGATCCTGGCTCAG-3′ and for 806R 5-GGACTACCAGGGTATCTAAT-3, with 312 bp product.

The genetic molecular diagnostics as a modern method of innovative medicine makes a significant contribution to the spectrum of diagnostic studies of the capability of alimentary correction of the organism's metabolic properties and is a promising branch of medicine of the 21-st century.

POSTER SESSION V. MEDICINAL BIOCHEMISTRY

THE EFFECTS OF MELANIN ON THE LEVEL OF PRO- AND ANTI-INFLAMMATORY CYTOKINES IN SERUM OF RATS WITH PROGESTERONE-INDUCED OBESITY

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Inflammation is the hallmark of obesity. Low grade local inflammation leads to systemic inflammation associated with increased cytokine production by blood monocytes. Progesterone (Pg) used for contraception and hormone replacement therapy enhances food intake and thus causes significant weight gain by increasing the fat deposition. Our previous studies demonstrated the rise of body weight and adiposity index of Pg-treated rats.

Dietary antioxidants may have a potential to recover chronic inflammatory process in obesity; they therefore could have beneficial effects in obesity treatment. Melanin (Mel) produced by Antarctic black yeast *Nadsoniella nigra* strain X-1 acts as scavenger of free radicals thus having antioxidant and anti-inflammatory activity. The present study was designed to investigate the production of pro-inflammatory (IL-1 and IFN-γ) and antiinflammatory (IL-10, IL-4, TGF-β) cytokines in blood of rats with Pg-induced obesity on the "Mel prophylaxis" and "Mel treatment" experimental models. Rats were randomly divided into 4 groups: 1) control; 2) obesity – injected with Pg daily for 28 days (10 mg/kg); 3) "Mel prophylaxix" – simultaneously injected with Pg and received per os Mel solution (1 mg/kg) daily for 28 days; 4) "Mel treatment" - injected with Pg daily for 28 days and then received per os Mel solution daily for 28 days. Serum cytokine levels were estimated by ELISA.

The level of pro-inflammatory cytokines IL-1 and IFN-γ in blood serum of Pr-treated rats was elevated 2.5 times and by 90%, respectively, in comparison with control group, suggesting the development of systemic inflammation in response to Pg-induced weight gain. Mel decreased IL-1 and IFN-γ levels 2.6 times and by 31%, respectively, in "Mel prophylaxis" model, as compared with the group, received Pg only. Data from "Mel treatment" model indicates the decrease in their levels by 73 and 92%. Notably, Mel administration restored their levels to control values in both cases.

Pg treatment decreased the circulation level of anti-inflammatory cytokines IL-4, IL-10 and TGF-β 2.8 and 2.2 and 2 times, respectively, as compared to the control. Mel slightly increased the IL-4 levels in both experimental models and augmented IL-10 levels (25 and 38%) and TGF-β levels (60 and 44%) in "Mel prophylaxis" and "Mel treatment" models, respectively, though they were lower than control. These data indicate that Pg-induced obesity is associated with systemic inflammatory response likely due to chronic inflammation in adipose tissue via altering the pro-inflammatory and anti-inflammatory cytokine balance. Mel alleviates inflammatory conditions due to its antioxidant properties thus restoring the circulation level of pro-inflammatory cytokines and improving the level of anti-inflammatory cytokines.

THE REACTION OF THE MICROGLIA DUE TO ACUTE WATER-IMMOBILIZATION STRESS AND UNDER INFLUENCE OF A-KETOGLUTARATE

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The biological function of stress is adaptation, a special psychophysiological state, which provides protection of the organism from threatening and destructive effects.

Most of studies show that stress contributes to significant structural remodeling of the microglia to contribute the release of proinflammatory cytokines from this cell. The release of stress-mediators has a short-term protective function and prepares the organism for external challenges. However, maladaptation in stress responses can result in mental illness, such as posttraumatic stress disorder, anxiety et. al. The purpose of this study was to investigate the effect of short-term water-immobilization stress to the level of microglial protein Iba-1 in the rat's brain.

The Wistar rats, which weight 180-230 g, were used in the experiment. Animals were kept under standard vivarium conditions. All experiments were conducted according to the guidelines established by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986). The model of water-immobilization stress (Weiner, 1996) was used. Animals were divided into 3 experimental groups (6 animals per each): group 1 – control rats; 2 – rats undergoing water- immobilization stress; 3 – rats exposed to water-immobilization stress and treatment with α-ketoglutaric acid (AKG) 2% in drinking water for 14 days (SGPlus, Sweden). In the end of the experiment, animals were decapitated with anesthesia (thiopental 60 mg/kg). The cerebral cortex, hippocampus and cerebellum were isolated from the rat's brain, which were later used for extraction of cytosolic proteins.

Obtained data showed not a notable change in amount of Iba-1 protein in the studied brain areas just after water immobilization for 3-5 hour. However, the level of Iba-1 in the thalamus and hippocampus tended to decrease to 8-10% just after stress procedure. Long lasting stress procedure for 3 days lead to elevation of microglial reaction (previous data presented). The adaptation time after stress with AKG 2% in drinking water for 14 days resulted normalization of microglial reaction.

Experimental data allow us to conclude that exogenous AKG can be useful for effective rehabilitation after stress.

DEVELOPMENT OF OXIDATIVE STRESS IN RATS BRAIN IN CONDITIONS OF MELATONIN DEFICIENCY

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The oxidative stress is one of the mechanisms of development of pathological states of the organism. Oxidative stress occurs upon excessive free radical production resulting from an insufficiency of the counteracting antioxidant response system. The brain, with its high oxygen consumption and lipid-rich content, is highly susceptible to oxidative stress.

The aim of this work was investigation of the endogenous circadian clock and the relationship between oxidative stress, brain in conditions of melatonin deficiency.

The subject of the study in this work were biochemical parameters of the development of oxidative stress and antioxidant enzymes in the cerebellum, hippocampus and the cerebral cortex of the brain rats under conditions of chronic melatonin deficiency. Experiments were conducted on Wistar rats. Old rats were randomly divided into two groups, each with 6 females and 6 males. Group 1 was a 1 control group – kept at constant temperature (24 °C) and controlled light-day length (12 h/12 h – lighting/darkness). Group 2 (experimental) – kept in constant lighting 750 lux/m^2 for 30 days. The experiment duration was 30 days. We determine the intensity of lipid peroxidation processes by the concentration lipoperoxidation product – malondialdehyde (MDA) (Volchegorsky et al., 2002) and diene conjugates (Gavrilov et al., 1988). Superoxide dismutase (SOD) and catalase enzymic activity in brain homogenates was determined by the direct spectrophotometric method (Greenwald et al., 1985; Koroluk et al., 1988),

Chronic desynchronosis (30 days) leads to an increase in the lipid peroxidation rate in the brain of rats. Diene conjugates increased in the cerebral cortex by 24.5%, in the hippocampus by 25.6%, cerebellum by 23.1%. It was established that there was an increase in secondary (MDA) LPO products in the brain of rats by 39.2% in the cerebral cortex, by 41.1% in hippocampus, by 31.3% in cerebellum compared to the control group. Superoxide dismutase is a key enzyme of the antioxidant system, the activity of SOD was probably lower in all parts of the brain of experimental animals in comparison with the tissues of control animals, by at average 47% in the brain old animals with constant light (30 days). A significant decrease the activity of catalase in different parts of the brain are observed in mature animals in the experimental group by 38.8% at a average compared with control animals.

The results will be shown significant differences in SOD activity in different parts of the brain - the highest in the cerebral cortex and the lowest cerebellum. This shows a different one the intensity of the pro-oxidant and antioxidant processes in conditions of chronic melatonin deficiency.

CANINE ERYTHROCYTE MEMBRANE PERMEABILITY: QUANTITIVE EFFECTS OF INITIAL CRENATION ON FRAGILITY

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Insofar as cryoprotectants must enter erythrocytes to be effective, quantification of the membrane permeability coefficient *P* for these molecules has long been key to the mechanistic understanding of their function. This abstract describes a novel revisitation of permeability determinations by osmotic shock, in which: (i) crenation occurs essentially instantaneously upon immersion of erythrocytes in a concentrated cryoprotector solution, followed by slow membrane penetration of the cryoprotector over a specific period Δt, and (ii) swelling occurs upon subsequent removal of the erythrocytes and immersion in fresh saline. Hemolysis results if Δt was long enough for so much cryoprotector to have entered that the final osmotically equilibrated cell volume exceeds the mechanical threshold of the membrane. Data on percent hemolysis as a function of Δt ultimately yields *P* as a fitted parameter in a supporting theoretical model.

Classical approaches rely on a separate osmotic fragility experiment to determine the critical (threshold) volume at which 50% of erythrocytes hemolyze. However, cells that have first undergone crenation in the osmotic shock experiment would likely to be more fragile than the fresh cells used to assess fragility. Indeed, it is difficult to guess a priori the consequences of so heavily wrinkling the membrane and its supporting cellular structures. We quantify this phenomenon, and find that the initial crenation renders erythrocytes more fragile, making the critical volume applicable to the actual osmotic shock experiment smaller than usually determined separately.

Our presentation details a new data analysis taking the increased fragility into account, yielding *P* smaller than classical determinations, and potentially resolving differences among different literature results. We demonstrate our procedure with reference to glycerol and DMSO permeation into bovine and equine erythrocytes at 0-37 ºC. Mechanistic conclusions are suggested by comparing our results with a new critical assessment of the intrinsic permeability properties of gel-phase bilayers.

STRESS INDUCED CHANGES OF THE GLIAL FIBRILARY ACIDIC PROTEIN LEVEL IN THE RAT'S BRAIN AND 2-OXOGLUTARATE IMPACT

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During the life people get into stressful situations. However, many physical and mental illnesses could be the results of stress. The brain is the central organ that regulates biochemical and behavioral responses to the stress. Restraint water-immersion stress (RWIS) is a compound stress model that includes both psychological and physical stimulation. Most research in the field of the study of stress are focused on neurons. It is important to show the sensitivity of astrocytes, associated with morphological and functional changes in response to stress. The aim of our study was to investigate the changes in the level of glial fibrillary acidic protein (GFAP) level in different rat's brain areas under three-days RWIS condition, during physiological adaptation and 2-oxoglutarate treatment.

A group of 24 rats was used for investigation and divided into 4 groups (n = 6): 1 group – control; 2 – rats with RWIS for 3 days; 3 – 14-days of physiological recovery after stress; 4 – rats with 2-oxoglutarate after stress for 14 days (2% in drinking water). All animals was decapitated under mild anesthesia after the end of the experiment. The different areas (hippocampus, thalamus, cerebellum, visual cortex) isolated from the rats brains were used for differential ultracentrifugation, and fractions with cytoskeletal proteins were obtained. The level of filamentous GFAP was determined due to competitive ELISA using monospecific polyclonal antibody (Santa Cruz Biotechnology Inc., USA).

It is shown that under immobilization stress procedure the level of filamentous GFAP was redistributed in the brain: it increased by 23% and 18% in the visual cortex and thalamus, respectively, compared to the control group. Nevertheless, the level of filamentous GFAP was decreased by 31% in the hippocampus under immobilization stress. No changes of GFAP level in the cerebellum were observed under studied condition. The physiological recovery after stress for 14 days was enough for normalization of GFAP level in the studied brain areas. However, the application of 2-oxoglutarate for 14 days (2% in drinking water) after stress support more effective recovery as astrocytes reaction and behavior of rats too.

Obtained data showed that application of 2-oxoglutarate after stress could demonstrate neuroprotective effect and support more effective astrocytes recovery.

INTEGRIN α2β1 IS INVOLVED IN REGULATION OF COLLAGEN DEPOSITION IN CARDIAC FIBROBLAST CULTURED ON BOTH SOFT OR HARD GELLS

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Cardiac fibroblasts are subjected to various physical stimuli. Both hypertrophy or fibrosis of the heart change stiffness and compliance of the fibroblasts environment and may modify the cell function. Strain, induced by physical stimuli, is distributed by collagen fibers in the extracellular space. Collagen molecules are bound by integrins, transmembrane receptors, that transmit information from environment to the cell. The aim of this study was to determine whether changes within surface stiffness are involved in regulation of collagen metabolism. Moreover, we examined the regulatory role of α2β1 integrin (known also as mechanotransducer) on collagen deposition.

Cardiac fibroblast (ABM, Canada), were cultured on polyacrylamide gels with different hardness (soft gel 15kNt/m^2, hard gel 28kNt/m^2).

Augmentation of collagen content as well as increase in gene expression of type I procollagen α1 chain were detected in cells cultured on soft gels compared with hard gels. The expression of type III procollagen α1 chain was not changed by experimental conditions. We observed that lower hardness of the surface is connected with the increase in levels of matrix metaloproteinase-1 (MMP-1) as well as with the decrease in the levels of tissue inhibitor of matrix metaloproteinase-3 (TIMP-3) and TIMP-4. MMP-2, MMP-9,

TIMP-1 and TIMP2 levels were similar in two investigated models of the experiment. On the fibroblast surface, cultured on soft gels, increased densities of both α2 and β1 integrin subunits were reported. Additionally the density of α2β1 integrin receptor was also augmented. This phenomenon was linked with elevated level of α2 integrin subunit mRNA. Overexpression of the integrin receptor was linked with lower phosphorylation of integrin dependent signaling molecules Src and focal adhesion kinase (FAK). Inhibition of α2β1 integrin by TCI-15 applied at 10^{-7} and 10^{-8} M, augmented collagen level within the culture on both soft and hard gel. Similar effect was observed in fibroblasts subjected to α2 gene silencing by siRNA. The effect of silencing was confirmed by flow cytometry, Western Blot and qPCR.

Both synthesis and catabolism of collagen is regulated by stiffness of the cardiac fibroblasts environment. The expression of α2β1 integrin is dependent on physical properties of environment. Integrin α2β1 is involved in regulation of collagen deposition and may decrease collagen accumulation.

MELATONIN EFFECT ON THE HEPATIC HYDROGEN SULFIDE SYSTEM OF RATS UNDER CONDITIONS OF EXPERIMENTAL NEPHROPATHY

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Activation of free radical oxidation and exhaustion of the antioxidant system is a considerable risk factor of different pathologic conditions of the body and kidney diseases in particular. It can cause metabolic disorders of sulfur-containing amino acid metabolism, one of the metabolic products of which is a biologically active molecule – hydrogen sulfide (H_2S). Hydrogen sulfide is known as a gas transmitter possessing antioxidant, antiinflammatory and other regulating effects within the limits of physiological concentrations. Its deficiency can promote progressing nephropathy and complications associated with it.

Recently physiological effects of melatonin have been studied actively. Melatonin is known to be one of the most effective antioxidants able to conjugate not only toxic radicals, but to increase the activity of antioxidant enzymes as well.

Objective of the study was to investigate melatonin effect on H_2S-producing enzyme system, concentration and production of H_2S in the liver of rats under conditions of experimental nephropathy.

The experiment was conducted on mature albino male rats with the body weight of 160-180 g.The animals in experimental group were administered a single intraperitoneal dose of folic acid (250 mg/kg). Melatonin was introduced intragastrally (10 mg/kg) during 3 days after intoxication. The data were processed statistically by means of Wilcoxon criterion.

Under conditions of nephropathy hydrogen sulfide concentration decreased by 37.92% and its production decreased on 34.84% as compared to the control group. Activity of H2S-producing enzymes cystathionine-γ-lyase (CSE), cystathionine-β-synthase (CBS) and cysteine aminotransferase (CAT) in the liver of rats with nephropathy reduced to 38.98, 33.73 and 26.76% in comparison with the animals from the control group. At the same time, melatonin introduction resulted in the increase of these indices – to 16.59 and 21.30% as compared to the indices of animals with nephropathy. Melatonin increased hydrogen sulfide level by means of increasing activity of CSE and CBS to 24.15 and 32.52% in comparison with the group of animals with nephropathy. Moreover, melatonin introduction increase CAT activity by 28.62%.

Therefore, melatonin introduction increased H_2S content and activities of H_2S-producing enzymes in the liver of rats with nephropathy, promoting oxidative stress weakening with a direct participation of melatonin in the neutralization of active oxygen forms and by increasing H_2S, possessing antioxidant and anti-inflammatory properties.

THE INFLUENCE OF 14 DAYS INTRODUCTION OF MELATONIN ON THE CYSTATHIONIN-β-SYNTHASE AND CYSTATHIONINE-γ-LYASE ACTIVITY OF HYDROGEN SULFIDE SYNTHESIS IN THE LIVER OF ALLOXAN DIABETIC RATS

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A major risk factor for various pathological conditions, including diabetes mellitus, is a disorder of sulfur-containing amino acids metabolism. Hydrogen sulfide belongs to biologically important metabolites of these amino acids. It is known that the liver is an important organ that provides sulfur-containing amino acids splitting and hydrogen sulfide formation. In diabetes mellitus there is also activation free radical oxidation. Melatonin has established itself as one of the most effective antioxidants, which not only binds toxic radicals, but also increases the activity of antioxidant enzymes.
Therefore, the aim of our research was to determine the influence of melatonin on blood glucose level, the cystathionin-β-synthase (EC 4.2.1.22) and cystathionine-γ-lyase (EC 4.4.1.1) activity in the liver rats.
The experiments were carried out on 105 sexually mature male albino rats with the body weight – 0.16-0.18 kg. Alloxan diabetes was evoked via single injecting the rats with 5% alloxan monohydrate solution in the dose of 150 mg/kg. The animals were divided into subgroups: 1) control rats; 2) alloxan diabetic rats (basal glycaemia 11.4-26.3 mmol/l); 3) animals with diabetes were introduced the melatonin intragastrically in the dose of 10 mg/kg at 8 a. m. daily during 14 days.
Alloxan diabetes contributes to changes of the investigating parameters. In the liver of alloxan diabetic rats the cystathionine-β-synthase and cystathionine-γ-lyase activity increased by 90 and 23%, respectively, compared with the control animals. The introduction of melatonin promoted the normalization of basal glycaemia level in diabetic animals. In the liver of alloxan diabetic rats treated with melatonin the cystathionine-β-synthase and cystathionine-γ-lyase activity decreased by 24 and 21%, respectively, compared with parameters of diabetes mellitus animals. Reducing the effective action of the antioxidant system leads to endothelial dysfunction, which is known to produce hydrogen sulfide.
Several mechanisms can serve as the basis of the antidiabetic action of melatonin. At the cellular level, the main role is the antioxidant activity of melatonin.
In case of evident alloxan diabetes the introduction of exogenous melatonin normalizes enzyme activity of hydrogen sulfide synthesis in the liver of rats possibly due to the protection antioxidant enzymes, especially glutathione system that depends on sulfur-containing amino acids metabolism.

CHANGE OF CYTOSKELETON ACTIN ISOFORMS SPECTRUM IN AGING SKIN FIBROBLASTS AT MECHANICAL DEFORMATION

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In the experimental work a comparative analysis of changes in the intensity of the Actb, Actg1 and Acta1 genes expression and the content of their products – marker cytoskeleton proteins – β-, γ-, and α-actin in deformed skin of rats aged 3 and 24 months has been carried out.
The isolated skin was fixed in ventilated culture flasks in a DMEM medium and cultured at 37 °C. The skin was single-time deformed by 0.18 MH/m^2 during 6 hours. The gene expression analysis was performed on DNA–microchips (Arrayit) on Affymetrix 428 Scanner. The total RNA from the cells was isolated on spin columns with the RNeasy Mini Kit (Qiagen). Synthesis of cDNA by reverse transcription was performed using QIAGEN OneStep RT-PCR Kit (Qiagen). 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.).
It has been shown that the mechanical tension enhances the expression and the content of β-, γ-, and α-actin within the deformed skin. But the dynamics of expression intensity of the investigated genes and the content of their products has both age and stress-strain dependence – the skin fibroblasts of rats aged 3 months more intensively produce and collect alpha smooth muscle actin (αSMA) isoform unlike cells of rats aged 24 months. αSMA isoform is not specific for fibroblasts but is typical for myofibroblasts. As known, the manner in which wound fibroblasts respond to strain influences the wound repair. The compaction of granulation tissue pulls on the surrounding dermis, generating tension. The fibroblasts transform into myofibroblasts, characterized by prominent cytoplasmic stress fibers with the alpha smooth muscle actin (αSMA) isoform of actin. More intensive formation of stress fibers influences the level of strain-dependent responses of cells and increases production of structural extracellular matrix proteins leading to faster wound healing in young age.

We are heartily thankful to our supervisor, Prof., Dr.Sc. Ye. Persky for his guidance during the development of this experimental work.

AGING IMPACT ON THE MITOCHONDRIAL PROOXIDANT/ANTIOXIDANT BALANCE IN THE GERBIL'S LIVER

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Problems of aging have always been a concern of scientists. There are many scientific data dedicated to searching the ways to overcome processes that cause aging. The most widespread and generally accepted theory of aging is mitochondrial. Given this theory, the cause of aging is the excessive formation of free radicals and inhibition of processes of transformation of not completely oxidized products (Ikeda, 2014; Potapenko, 2017; Zhao, 2014). Such free radicals and metabolites accumulate in the cell, which results in the displacement of the oxidation-reducing balance. This, in turn, leads to aging of the cell and its death. The purpose of our work was investigation of status of gerbil's liver mitochondria under aging.

With physiological aging the inhibition of the main functions of the gerbil`s liver mitochondria occur. There is a shift in the oxidation-reducing balance in the direction of the formation of peroxide products, whose concentrations are constantly increasing with age, and after reaching 39-months-old gerbils, the concentration of TBA-active products was 4.5 times higher than in 6-month-old group. At the same time, inhibition of the protective antioxidant system by inhibiting activity in the oldest gerbils 39-month-old the superoxide dismutase was observed at 40%. However, the activity of the catalase of these animals was at the 6-month-old level. Correlation analysis of the parameters of prooxidant/antioxidant indices showed the formation of an excellent connection between the number of TBA-active products and the activity of catalase and SOD in the liver of 36-month-old gerbils of ($r = – 0.70$, $P < 0.05$). This fact indicates the inhibitory effect of peroxide products on enzymes that form and transform them. Also, at the same age, the decrease of catalase activity leads to the accumulation of peroxide metabolites that inhibit SOD activity with a positive relationship with high significance ($r = 0.93$, $P < 0.001$). The intensification of peroxidation processes led to permeability of the mitochondrial membrane, which was accompanied by decrease of cytochrome *C* concentration in mitochondria of 36-months-animals by 25% compared to 6-month-old animals, that can be a marker of activation of apoptotic processes in the liver of aging animals. On the other hand, the increase of cytochrome *C* concentration in mitochondria in the liver of 39-month-old gerbils by 80% may be additional mechanism for protecting mitochondria from the excessive amount of damaging factors and inhibiting the functioning of the antioxidant system, for the possibility of implementing the underlying processes.

BIOCHEMICAL STATUS OF LIVER UNDER WATER-IMMOBILIZATION STRESS AND α-KETOGLUTARATE IMPACT

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Stress is a protective reaction of the body that stimulates the adaptation processes in the body to changing environmental conditions. Due to this phenomenon, the mobilization of individual protective mechanisms, which allows the biological system to normal function. The liver is one of the organs that actively reacts to adaptation in the body due to stress (Potapenko et al., 2017; Serova et al., 2016). Therefore, the purpose of this work was to investigate the changes of biochemical parameters of oxidative-reducing balance and functional state of the liver of rats under water-immobilization stress (WIS) and α-ketoglutarate impact.

The influence of the combined action of WIS and exogenous α-ketoglutarate 2% in drinking water for 14 days on the level of oxidative-reducing balance, the effectiveness of the antioxidant system, and the functional status of the rat's liver have been determined. The WIS for 5 hours lead to the substantial shift of oxidative-reducing balance in the rat`s liver under conditions of stress factors towards the formation of oxidative TBA-active products (increased 2 times) and not fully oxidized metabolites as lactate (increased 2.5 times) and pyruvate (decreased 4 times) has been established. Such changes are indicative of a decrease of liver ability to convert the oxidized products to glucose under the influence of physical stress factor. Elevated oxidative stress in the liver was coincident with changes of lactate dehydrogenase (LDH) activity. The activity of LDH related to the pyruvate increased 2.5 times and LDH related to the lactate decreased 3 times compared to the control group under the WIS. Application of 2% α-ketoglutarate in drinking water for 14 days after WIS normalized the activity of LDH related to pyruvate. At the same time the activity of alanine aminotransferase (ALT) was still elevated 2 times that indicate the involvement of ALT in the pyruvate metabolism. The use of α-ketoglutarate after WIS also resulted in normalization of AST activity owing to the use of keto-glutaric acid in the transamination reaction.

THE POTENTIAL PROTECTIVE EFFECT OF OLIGORIBONUCLEOTIDES-D-MANNITOL COMPLEXES AGAINST THIOACETAMIDE-INDUCED LIVER FIBROSIS

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Aim: Hepatic fibrosis is a common consequence of chronic liver injury caused by a variety of etiological factors. It is associated with inflammation, oxidative stress, necrosis and ends with cirrhosis, liver cancer, or liver failure. Oligoribonucleotides-D-mannitol complexes (ORNs-D-mannitol) display a vast spectrum of biological effects, including cellular metabolism stimulation with activation of endogenous synthesis of regulatory proteins, stimulation of reparation processes. Therefore, the aim of this study was to investigate the protective effect of the ORNs-D-mannitol on liver fibrosis.

Materials and Methods: Mice received thioacetamide (TAA) (200 mg/kg, intraperitoneal) thrice weekly, for 8 successive weeks to induce liver fibrosis. The ORNs-D-mannitol (200 mg/kg, *per os*) was administered orally during TAA intoxication. Body weights and mortality of mice were assessed during the experiment. At the end of the experiment, oxidative stress, inflammatory and profibrogenic markers were evaluated.

Results: The results of the research showed that treatment with the ORNs-D-mannitol attenuated TAA-induced liver fibrosis in mice. The ORNs-D-mannitol prevented TAA oxidative stress. The ORNs-D-mannitol decreased TBA-reactive products, carbonyl derivatives levels and myeloperoxidase activity by 60.6, 35 and 52%, respectively, in comparison to control thioacetamide in the liver cells. In addition, these complexes increased protein and non-protein thiol groups levels, and glutathione-S-transferase and glutathione peroxidase activities compared to the TAA-treated mice. During TAA-induced liver fibrosis was investigated that the ORNs-D-mannitol reduced the expression mRNA level of pro-inflammatory (*Il-6, Tnf-α*) genes by 70 and 76%, respectively, compared to the mice with TAA. Furthermore, the ORNs-D-mannitol suppress the HSCs/myofibroblasts activation by reduced expression of markers *α-SMA, Col-1,* and *Tgf-β1* in the liver.

Conclusion: The ORNs-D-mannitol could ameliorate the effects of TAA-induced liver fibrosis in mice by inhibiting oxidative stress, expressions of pro-inflammatory cytokines and profibrotic markers.

INFLUENCE OF VITAMIN E ON ASTROCYTES IN BRAIN OF OLD DIABETIC RATS

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Chronic hyperglycemia can cause oxidative stress in the nervous system which, in turn, can promote the development of diabetic encephalopathy. Recent studies have found the increased expression of glial fibrillary acidic protein (GFAP) and S100B, both of which are indicators of glial reactivity, in the brain of diabetic rats.

Diabetes in rats was induced by a single intraperitoneal injection of streptozotocine (50 mg/kg body weight). Rats in one diabetic group received vitamin E (10 mg·kg^{-}1) The levels of S100β and GFAP were determined by immunoblotting in the hippocampus, cortex, and cerebellum 1.5 months after intraperitoneal injection. Lipid peroxidation (LPO as malondialdehyde+4-hydroxyalkenals) and glutathione (GSH) levels were also determined in the same brain parts.

In the brain of the old diabetic rats, the increased levels of lipid peroxidation were detected in all investigated brain region in comparison with the control group. Introduction of vitamin E to diabetic old rats has led to a statistically significant reduction in the level of MDA + 4-hydroxyalkenes. Injections of vitamin E, in addition, caused a significant increase in the level of GSH in the brain of diabetic rats. The results of immunoblotting showed a significant increase in the content of GFAP in the hippocampus, cerebellum and cortex of the diabetic rats compared with the control group. The increase in the content of intact as 49 kDa polypeptide and degraded GFAP products in the range of 48 - 45 kDa was revealed. The content of the S-100β protein was also significantly higher in the rat brain with STD-induced diabetes compared with the control group. Introduction of vitamin E to diabetic rats has contributed to a statistically significant reduction in the content of GFAP and its degraded polypeptides. The content of the S-100β protein was significantly reduced in the brain of the rat group receiving vitamin E injections. A significant decrease in the protein content of S-100β was detected in the hippocampus and cerebellar cortex. In the cerebellum, the introduction of vitamin E caused a decrease, but statistically unreliable. A significant positive correlation was found with the level of peroxidation products in the groups of STZ-diabetic rats and dietetic rats receiving injections of vitamin E. A slight correlation was found between the content of S-100β and the level of lipid peroxidation in the cerebellum alone ($r = 0.24$; $P > 0.05$).

The development of oxidative stress, astrogliosis causes a disturbance in the supply of neurons, which negatively affects neuroplasticity.

SOME BIOCHEMICAL PARAMETERS OF RAT HEPATOCYTES UNDER INCUBATION WITH ORTHOVANADATE NANOPARTICLES

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The significant increase in production, technology and research of nanoparticles (NPs) has recently been reached. Orthovanadate gadolinium and europium – $GdYVO_4:Eu^{3+}$(-) spherical NPs are of 2-3 nm in diameter and externally covered with citrate ions which create their negative charge. Optical, photophysical and other properties of this kind of NPs are well described as well as redox activity. The purpose of this study was to investigate the pro-antioxidant balance and the activity of the glutathione cycle enzymes in rat hepatocytes in the presence of $GdYVO_4:Eu^{3+}$(-).

NPs were kindly provided by Dr. Yu. V. Malyukin, Institute for Scintillation Materials, NAS of Ukraine. Hepatocytes were isolated by non-enzymatic dispersion of rat liver. Cell viability was determined using the trypan blue. The concentration of cells in the suspension was $(1.5\text{-}2.2)\times10^6$ cells/ml; viability was 81-89%. Hepatocytes were incubated for 2 or 14 h at +37 °C in McCoy 5A culture medium without or in the presence of NPs. After incubation the cells were lysed by 1% Triton X-100. The activities of alanine and aspartate aminotransferases (AlAT and AsAT) and lactate dehydrogenase (LDH) were determined in the incubation medium using standard test systems in order to evaluate the integrity of plasma membranes. The content of TBA-reacting species (TBARS) and the activities of the glutathione cycle enzymes were determined spectrophotometrically in the lysate of hepatocytes. Glutathione peroxidase (GP) and glutathione reductase (GR) activities were measured by the level of NADPH (340 nm); glutathione-S-transferase (GST) – by the level of 2,4-dinitrochlorbenzene (340 nm); the activity of catalase – by the level of hydrogen peroxide (240 nm).

It was shown that the content of TBARS and the activities of enzymatic markers of cellular damage (AlAT, AsAT and LDH) did not change in both terms of investigation, that testified to the absence of NPs effect on the cell membranes. Contact of rat hepatocytes with NPs resulted in the increase of GST activity in 2 h and GP activity in 14 h of incubation, while it did not affect GR and catalase activities. The effect of NPs on the glutathione cycle enzymes in rat hepatocytes could be due to the changes of GSH/GSSG ratio, which primarily lead to the activation of GST.

Thus, the data indicate the activation of some components of hepatocytes glutathione antioxidant system in the presence of $GdYVO_4:Eu^{3+}$(-) NPs.

ANTI-INFLAMMATORY EFFECT OF LIPOSOMAL CURCUMIN IN THE NEOCORTEX AND HIPPOCAMPUS OF RAT BRAIN WITH ALZHEIMER'S DISEASE MODEL

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Amyloidosis in the pathogenesis of Alzheimer's disease (AD) is associated with toxicity of β-amyloid peptide aggregates for neocortex and hippocampus neurons of the brain, which is accompanied by activation of the cytokine inflammatory response. Natural polyphenols, in particular, curcumin (CUR), are excellent anti-inflammatory agents because they are able to inhibit induction of pro-inflammatory cytokines (IL-1β, TNFα). The task is only to deliver CUR to the inflammatory focus – to the neocortex and hippocampus neurons, where β-amyloid peptide aggregates predominantly form that leads to disruption of the neuronal network, cognitive deficits and dementia. However, one of the significant disadvantages of curcumin is its low bioavailability. The aim of the study was to research the efficacy of nasal therapy with CUR in liposomes in rats with AD model.

The study was performed on 30 sexually mature, male rats, weighing 250 g. An experimental AD model in animals was reproduced by disposing of intra-hippocampal administration of 15 mM β-amyloid peptide 42 (Aβ42) aggregates (Amyloid β Protein Fragment 1-42 (Sigma-Aldrich, USA)). Nasal therapy with liposomal CUR (3.5 μg/animal) was performed within 30 days after administration of Aβ42. In the supernatants of neocortex and hippocampus, the concentrations of IL-1β, IL-6, IL-10 and TNF-α were determined by immune-enzyme analysis on a microtiter plate (GBG Stat FAX 2100, USA) using the appropriate Rat ELISA Kits Invitrogen BCM DIAGNOSTICS kits, USA. The results were expressed per gram of total protein, which was determined by Lowry's method. Statistical analysis of the differences was performed using Student's *t*-test, $P < 0.05$ were considered significant. It was shown that nasal therapy with liposomal CUR within 1 month of rats with AD model leads to a significant suppression of inflammation caused by Aβ42-aggregates. Namely, it is the decrease in the concentration of TNFα by 71%, IL-6 by 67%, IL-10 by 41% in the neocortex, and TNFα by 56%, IL-6 by 39%, IL-10 by 52% in the hippocampus. These data indicate a high anti-inflammatory potential of the liposome form of curcumin. It is known that this polyphenol interferes with the activation of the nuclear transcription factor NFκB by inhibiting phosphorylation and degradation of IκBα (NFκB inhibitor). Since the effect of CUR is to inhibit the activation of IB kinase (IKK) required for NFκB activation, this can be explained by the detected anti-cytokine effect of CUR in experimental animals. The phospholipid-cholesterol container for CUR in the form of liposomes, used in our work, provided its target delivery without losses during active metabolism and rapid elimination, whereas nasal application of this compound contributed to its successful overcoming of the hemato-encephalic barrier.

ABOUT SOME MECHANISMS OF DERMATOTROPIC ACTION OF MELANIN

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Formerly we have shown that pharmcological composition based on melanin promotes rapid healing of various ethiology wounds without rough scar. But mechanisms of dermatotropic action of melanin are not completely studied.

The aim of the study was to investigate antibacterial properties of pharmacological composition method of application of the drug (1 g) to the surface of the nutrient medium was used (NA – Nutrientagar, manufacturer Sigma-Aldrich, Spain). The prooxidant-antioxidant balance in skin gomogenate and serum blood in dynamics on the 3^{th}, 6^{th}, 9^{th}, 14^{th} day and day of full epithelization was estimated using the spectrophotometric biochemical method. Pharmacological composition consists of 0.1% melanin (produced by Antarctic black yeast-like fungi *Pseudonadsoniella brunnea* received by us microbiologically) dissolved in 0.5% carbopol for wounds' healing.

It is established that the pharmacological composition (PC) comprising 0.1% melanin dissolved in 0.5% Carbopol produces bactericidal effect of the test cultures *Staphylococcus aureus* and *Pseudomonas aeruginosa* and a fungistatic effect on the test culture of *Candida* fungi. The use of a new PC promoted the healing of the full-thickness wounds without a gross tissue scarring, as confirmed by our studies (the percentage of collagen and melted gelatin in the skin was less, and the moisture content was greater than in animals with untreated wounds). It is shown that upon daily application of a melanin-based PC on the wound, starting from the sixth day of the wound healing, the content of superoxide anion radical and hydrogen peroxide is reduced in skin homogenate and serum, indicating the antioxidant properties of melanin. The decrease of lipid peroxidation products upon skin pathologies simulated by them under the influence of the melanin-based PC was observed. Upon a full-thickness wound, glutathione peroxidase and glutathione transferase activity is increased, superoxide dismutase activity is reduced and catalase activity is increased in skin homogenate and serum. When the melanin-based pharmaceutical composition is topically applied to the wounds, the activity of these enzymes is normalized.

Thus, the mechanism of dermatotropic action of the melanin-based PC has antibacterial, antioxidant properties, indicating the effectiveness of this composition to accelerate the wound healing without the formation of a rough keloid scar.

HEMOGLOBIN SYSTEM AND ENZYMATIC ACTIVITY OF ERYTHROCYTES IN CHOLELITHIASIS

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Cholelithiasis is one of the most common diseases, which is inferior to the rate of growth of atherosclerosis and diabetes (Ashburn D., 2010; Conte D., 2011). In Ukraine, the proportion of patients suffering from cholelithiasis, according to different authors varies from 17 to 22%. Every ten years this number doubles.

The aim of this study was to examine the changes in the individual forms of hemoglobin (hemoglobin, glycosylated hemoglobin and methemoglobin) and glutathione reductase and catalase activity of red blood cells (RBC) in the development of cholelithiasis.

The material of our investigation was RBC of conditionally healthy people (1st group) and patients with cholelithiasis (2nd group). Study population was classified according to age (50-55 years) and gender (male). To study the state of the hemoglobin system of RBC in the development of cholelithiasis, were used RBC separated from the plasma. The hemoglobin (Hb), glycosylated hemoglobin (GlHb) and methemoglobin (MetHb), glutathione reductase (GR) and catalase (Cat) activity were determined spectrophotometrically using standart test kits.

The 10.6% increase in quantity of the Hb level in the development of gallstone disease was shown in comparison with a control group. A significant 1.42-fold increase of MetHb level compared with the control group was observed in group 1, that may indicate an intensification of oxidative reactions in RBC. A 1.5-folf increase in GlHb as compared with group 1. The increase in the quantity of GlHb can possibly affect hydroxy-transport function. Also it was shown a decrease of GR activity by 25% and 3-folf increase of Cat compared with the control group.

So, it was shown that with the development of gallstone disease, there are changes in the state of the hemoglobin system, exactly: the amount of production methemoglobin is increasing, with a simultaneous increase in the amount of glycosylated hemoglobin and a slight increase in hemoglobin. Some changes were also registered in the system of antioxidant protection: a decrease of glutathione reductase activity (by 25%) and an increase of catalase activity (3 times) compared with the control group. Apparently, RBC are responsible for a decrease of glutathione reductase activity by an increase of catalase activity, launching a special compensatory mechanism for this.

EFFECT OF PROBIOTIC ON PARAMETERS OF GLUTATHIONE SYSTEM AND LIPID OXIDATION IN SERUM OF RATS WITH MONOIODOACETATE-INDUCED OSTEOARTHRITIS

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The processes of glutathione and lipid oxidation are permanent in normal physiology of cells but changing in balance between substrate and product caused the development of many pathologies. The positive effect of single strain lactobacteria composition on antioxidant status in autoimmune osteoarthritis was recently shown. The purpose of this study was to investigate parameters of the glutathione system and lipid oxidation during monoiodoacetate-induced model of osteoarthritis with the administration of multistrain probiotic.

The study was carried out on Wistar male rats weighing 180-240 g. The model of osteoarthritis conducted by single infrapatellar injection of monoiodoacetate to right hind knee of kindly anesthetized rats. Control groups received saline under the same condition. Administration of probiotic (O.D.Prolisok, Ukraine) run daily by intragastrical feeding for 14 days. Sampling was made on the 30th day of the experiment. The level of reduced and oxidized glutathione (GSH and GSSG), the activity of glutathione transferase (GT), glutathione reductase (GR), glutathione peroxidase (GP), catalase, superoxide dismutase (SOD), and thiobarbituric acid (TBA) reactive substances were measured spectrophotometrically.

Monoiodoacetate-induced osteoarthritis has caused the disbalance of glutathione system. The level of GSSG and activity of GP, GT increased while the level of GSH and activity of GR decreased in pathology group, compared with the control group. Administration of PB had a positive influence on the parameters of glutathione system, but they did not reach control values. The parameters of lipid oxidation showed similar changing: the level of catalase, SOD and TBA active compound increased in pathology group and were near to control values in a group with PB administration. Thus, therapeutic feeding of PB has positive influence on glutathione system and lipid peroxidation in blood. The possibility to use PB feeding with classical treatment is interesting for further investigation.

THE FUNCTIONING OF DUODENUM MUCOSA SEROTONIN SYSTEM UNDER CONSUMPTION OF KIDNEY BEAN (*PHASEOLUS VULGARIS*) PODS EXTRACT IN THE OBESE MODEL OF RATS

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Obesity is a complex disorder that involves an excessive amount of body fat formation. Obesity is not just a cosmetic concern. It increases the risk of health problems, such as heart disease and diabetes. It is known that the dysfunction of serotonergic system is one of the indicators of obesity development. So it is relevant to study this system as for the consensus concerning the prevention and treatment of the obesity has not been done yet. Recently, the scientific interest has been focused on the studying of various plant extracts. A perspective and available raw for the drugs development is kidney beans (*Phaseolus vulgaris*) that are known in folk medicine as an effective hypoglycemic agent. Kidney bean pods are believed to be helpful in obesity and weight loss programs but the complex study of the effects of *P. vulgaris* extract on the obesity development has not been yet carried out.

Materials and Methods. Experiments were conducted on white nonlinear male rats with initial weigh of 195-205 g. During the first seven days, all rats received standard food "Purina rodent chow" and water ad libitum. On the 8th day the animals were randomly divided into 3 groups. Animals from the first group ("Control") were fed with a standard food during the experiment. Animals from the second group ("HCD") as well as the animals from the third group ("HCD+Ex") were on high-calorie diet. After 4 weeks of experiment they started receiving the extract of *P. vulgaris* (200 mg/kg).

Results. It was established that the tryptophan and serotonin levels in the group that was on high-calorie diet were by 45 and 62% lower compared to the control group. There were no changes of tryptophan and serotonin levels in animals that received the *P. vulgaris* extract compared to those of the control group. The level of 5-hydroxytryptophan was by 32% lower in "HCD" group than in "Control" group. In "HCD+Ex" group no changes were observed compared to the "Control" group. Also it was found that the activity of tryptophan hydroxylase in groups that were on high-calorie diet and alongside received the *P. vulgaris* extract were by 29% and 10% lower compared to the control group. The monoamine oxidase activity was by 126% higher in rats from "HCD" group and only by 8% higher in rats from "HCD+Ex" group compared to the "Control".

Conclusion. In the results of this research we have shown statistically significant changes of the duodenum mucosa serotonergic system in rats under high-calorie diet consumption. It is worth mentioning there were no statistically significant changes in rats that have received the *P. vulgaris* extract. The highlighted effects suggest that this extract may be used as a functional ingredient in addition to the conventional therapy of obesity.

POSTER SESSION VI. METABOLISM REGULATION AND PATHOLOGY

BIOCHEMICAL PROPERTIES OF NO-SYNTHASE ACTIVITY IN UTERUS SMOOTH MUSCLE MITOCHONDRIA

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The information about the catalytic and kinetic properties of the mitochondria NO-synthase from uterus smooth muscle is missing currently. To date, it has been found that NO modulates energy, metabolic and transport processes in mitochondria. It is a well-known point of view that mitochondria are the primary cell targets of NO. The possibility and biochemical properties of nitric oxide formation in smooth muscles mitochondria remain to be clarified, and the problem of biosynthesis of NO in myometrium mitochondria is not considered.

The presence of NO in uterine smooth muscle cells, in particular in mitochondria, has been visualized, involving laser confocal microscopy, NO-sensitive DAF-FM probe and the specific marker of functionally active (energized) mitochondria MitoTracker Orange CM-H2TMRos. NO-synthase activity in the isolated myometrium mitochondria has been identified in the presence of appropriate cofactors and substrates in the reaction medium using DAF-FM fluorescence probe and the flow cytometry method. The analysis of the results suggests that the optimal work of mtNOS in myometrium mitochondria requires the presence of respiration substrates, L-arginine, Ca^{2+} and NADPH. It is established that the dependence of nitric oxide production on the concentration of L-arginine has a bell-shaped character with a maximum of 75 μM, the dependence of nitric oxide production on Ca^{2+} concentration has a maximum at 100-250 μM. The inhibitors of Ca^{2+} transport in mitochondria 10 μM ruthenium red, 1-10 mM Mg^{2+}, as well as the inhibitor of NO-synthase 25 μM NG-nitro-L-arginine, reduced NO production. Calmodulin antagonists, 1-100 μM trifluoperazine and 0.1-10 μM calmidazolium, significantly inhibited NO-synthase activity. Collapse of the membrane potential in the presence of protonophore 10 μM carbonyl-cyanide 3-chlorophenylhydrazone, the use of inhibitors of the electron transport chain 5 μM rotenone or 1 μg/ml antimycin A resulted in inhibition of NO synthesis in mitochondria.

Thus, NO can be synthesized in uterine smooth muscle cells mitochondria. The high activity of NO synthase requires the presence of substrates of respiration, L-arginine, Ca^{2+} and NADPH. The biosynthesis of nitric oxide by mitochondria depends on its energized level.

Acknowledgement: authors thank Academician of NAS of Ukraine, Prof. Sergiy O. Kosterin for advices and discussion.

EFFECT OF 3 DAYS GLUTATHIONE INTRODUCTION ON ENERGY METABOLISM IN THE LIVER MITOCHONDRIA OF RATS WITH NEPHROPATHY

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Kidney disease often leads to the general state of oxidative stress and can co-exist with different liver diseases, or stimulate their development. Mitochondria play an important role in regulating energy metabolism, while disorders of energy metabolism often promote the disease progression. But the link between the development of nephropathy and energy metabolism in the liver are incompletely understood. The understanding of energy metabolism under physiological and pathophysiological conditions will help improve therapeutic strategies for patients with kidney disease or other organ injuries. So the aim of our study is to determine the activity of enzymes of the mitochondrial respiratory chain in the liver of rats with experimental nephropathy.

The experiment was carried out on male albino rats with the body weight 0.16-0.18 kg. The animals in experimental group were administered a single intraperitoneal dose of folic acid (250 mg/kg). Glutathione was introduced intragastral (100 mg/kg) during 3 days after intoxication. Mitochondria were isolated by differential centrifugation. The statistical data processing was performed using the method of Mann-Wilcoxon. Results were considered significant at $P < 0.05$

There was a decrease of NADH-dehydrogenase activity by 10% and succinate dehydrogenase activity by 26% in the mitochondria of hepatocytes of rats with nephropathy compared to control group. At the same time, activities of cytochrome oxidase and H^+-ATP-phase in the mitochondria of rats with nephropathy were decreased by 50 and by 37% compared to animals of the control group. The glutathione increased the production of ATP by upregulating the activity of NADH-dehydrogenase, succinate dehydrogenase, H^+-ATP-phase by 10, 23.6 and 17% compared to rats with nephropathy accordingly. The activity of cytochrome oxidase after the introduction of exogenous glutathione was without changes.

So, the expression of enzymes of energy metabolism in the liver under conditions of nephropathy decreased, thereby exacerbating renal failure. The introduction of glutathione increases activities of enzymes of I, II, V Complexes of the mitochondrial respiratory chain and is not effective for the activity of cytochrome oxidase in mitochondria isolated from the liver of rats with nephropathy. In our opinion this is due to the fact, that glutathione has antioxidant properties and promotes the energy metabolism of the liver. These findings may help widen our understanding of the fundamental metabolism of the organism and the state of mitochondrial energy metabolism under conditions of development of kidney disease. Our result suggests that glutathione play the important role in protection of the mitochondrial membrane against oxidative damage and for the control of ATP production.

CHANGES IN THE INFLAMMATION STATE OF WHITE ADIPOSE TISSUE IN RATS WITH DIET-INDUCED OBESITY AT THE DIFFERENT TIMES OF MELATONIN ADMINISTRATION

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The obesity development is closely associated with inflammatory processes in the white adipose tissue [Caër C., 2017]. In addition to the main function – regulation of circadian rhythm, melatonin has pronounced immunomodulatory and antioxidant properties (Tordjman S., 2017). The sensitivity of the body to melatonin is known to be different during the day (Dubocovich M., 2005). Therefore, an analysis of the potential effects of the melatonin administration at different times of day (morning and evening) on the inflammation state in rats white adipose tissue with high fat diet-induced obesity is relevant; especially taking into account the rapid development of chronobiological approaches in precision medicine.

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 – HCD ZT01 and 6) HCD ZT11, respectively. Melatonin was administered daily orally by gavage (30 mg/kg) for 7 week with a standard light day (12 hours: 12 hours, with a light on at 7:00 (ZT 00)).The adipocytes area (hematoxylin-eosin stain), the tissue fibrosis level (van Gieson's stain), the mast cells number (toluidine blue stain) was analyzed in the visceral white adipose tissue. The level of pro- and anti-inflammatory cytokines in serum was determined by enzyme-linked immunosorbent assay (ELISA).

The consumption of high-calorie diet (HCD group) causes significant changes in the white adipose tissue morpho-functional state – the adipocytes area increases by 67%, the presence of mast cells by 43%, the fibrosis level by 30%, and pro-inflammatory (IL1b, IL12b, TNFa, IFNg) cytokines content in serum also grow (in comparison with C, $P < 0.05$). After the melatonin use, an improvement was observed: reduced fibrosis and the mast cells number: OM ZT01 – by 27%, OM ZT11 – by 56%, and OM ZT01 by 21%, OM ZT11 – by 47%, respectively (compared with the HCD, $P < 0.05$). In addition, the adipocytes area is also decreased: OM ZT01 – by 34%, OM ZT11 – by 48%, while anti-inflammatory cytokines (IL4, IL6, IL10) content is raised (compared with the HCD, $P < 0.05$).

The melatonin action in the context of the obesity development is manifested in reducing the pro-inflammatory state in the white adipose tissue. The increased efficiency of melatonin in the evening administration is shown.

DIMINISHING OF BISPHENOL A EFFECTS BY NANOPARTICLES TiO_2 IN THE SENTINEL ORGANISM IS NOT RELATED TO ENDOCRINE DISRUPTION

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Recent studies focused on human health emphasized the importance of understanding the integrated response to multiply challengers (so-called exposome) as a mechanistic basis for linking the stressor exposures to health outcomes (Escher et al., 2016). We selected two worldwide-distributed xenobiotics, Bisphenol A known as endocrine disrupter and nanocomposite of TiO_2 (n-TiO_2) that is a prospective photocatalyst of BPA. The aim of this study was to evaluate their combine effect on the sentinel organism and to compare it with the manifestations of complex exposures *in situ*.

Male bivalve mollusks *Unio tumidus* were subjected to 14 days exposure to n-TiO_2 (1.25 μM), BPA (0.88 nM) separately or jointly (n-TiO_2+BPA) or $TiCl_4$ (1.25 μM). *In situ*, bivalve were sampled in four sites, including recreation reservoir of hydro power plant (HPP). Indices of oxidative-reductive stress, and immune-, endocrine- and genotoxicity were studied. The comparison of n-TiO_2 and $TiCl_4$-exposed mollusks has confirmed the particular effect of nanoparticles. Main contradictive effects of n-TiO_2 and BPA were related to the oxidative-reductive stress parameters. Glutathione (GSH&GSSG) level, lactate/pyruvate concentrations and their ratio were increased by n-TiO_2, decreased by BPA and normalized in co-exposure. ROS generation and phenoloxidase activity was oppressed by n-TiO_2, elevated by BPA and correspondent to control in co-exposure. However, DNA instability increased in all exposures except n-TiO2. Up-regulation of vitellogenin-like proteins level in gonads was caused by all exposures, particularly, by n-TiO_2+BPA (by 3.2 times). The same regularity was shown for the CYP450-related activity in digestive gland. All exposures provoked the decrease of the lysosomal membrane stability, increase of cathepsin D activity and its efflux from lysosomes, activated lipid peroxidation. On the other hand, caspase-3 activity decreased by BPA and co-exposure. The level of metal-buffering protein metallothionein was decreased in all exposures (except co-exposure), and its level of metalation increased. These common responses stressed by co-exposure, were similar to the manifestations of mollusks from HPP exposed *in situ* to effluents. Summarizing, this biochemical approach could be the base of the ranking of environmental risk for human welfare.

This work has been granted by the Ministry of Education and Science of Ukraine (Projects M/70-2017, 132B, MV-1).

SUSCEPTIBILITY OF GLUTATHIONE SYSTEM TO MELATONIN INJECTIONS IN BRAIN OF ALLOXAN DIABETIC RATS

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Hyperglycemia can increase the indicators of lipid peroxidation and oxidative stress in which free radicals fulfill the main role in pathogenesis of complications.

The aim was to determine the influence of melatonin on basal levels of glucose (BG), malonic dialdehyde (MDA), reduced glutathione (GSH) levels, glutathione reductase (GR), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G-6-PhD) activities in the brain of alloxan diabetic rats.

Methods. Research performed in compliance with the Rules of the work using experimental animals (1977) and the Council of Europe Convention on the Protection of Vertebrate Animals used in experiments (Strasbourg, 1986), as well as Bioethical Expertise of Preclinical and Other Scientific Researches Conducted on Animals (Kyiv, 2006). 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 control, diabetic and melatonin-diabetic (5 mg/kg, daily and orally for two weeks) groups. Blood was taken from the tail vein to evaluate the BG level with the use of OneTouchUltra (LifeScan, USA). Rats were sacrificed at the 19th day from the beginning of the experiment. The brain tissue was quickly removed, rinsed in saline, blotted, weighed and homogenized. The supernatant of the homogenate, prepared by ultracentrifugation for 10 min at 3000 g was used for measurement of activities of enzymes by standard methods. Statistical analysis was performed using Statistica 10 StatSoft Inc. Given these data, the use of Mann-Whitney test was considered sufficient for valid conclusions. Differences were considered to be statistically significant at $P \leq 0.05$.

Results. The level of BG in diabetic group of rats was found to be higher by 135% than control. Activities of GR, GPx, G-6-PhD and the level of GSH were decreased by 45, 30, 40 and 62% in rats with diabetes mellitus respectively than control. The level of MDA was found to be higher by 97% in this group than in control. So, the lipid peroxidation (MDA) was increased in diabetic brain. Melatonin lowered the BG level by 76% in diabetic rats in comparison to initial levels. It normalized activities of GR, GPx, G-6-PhD and lipid peroxidation, so these indexes did not differ from control. These results are consistent with the degenerative role of hyperglycemia on antioxidant defense, and provide further evidence that pharmacological intervention of antioxidants may have significant implications in the prevention of the prooxidant feature of diabetes and protects redox status of the cells.

Conclusion. Supplementation of melatonin modulated diabetic brain injury and can be potentially used for preventing diabetic neurodegenerative sequelae by improovement of glutathione system.

ACTIVITY OF PYRUVATE KINASE IN THE MUSCLE TISSUE OF ALLOXAN DIABETIC RATS INJECTED BY MELATONIN IN DOSE 5 mg/kg

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Melatonin is equally effective as alpha-lipoic acid; it exhibited marked antioxidant and anti-aging effects at the level of skeletal muscle *in vitro* even when it is given in a much lower dose than alpha-lipoic acid.

The relationship between the effects of melatonin on glycolysis and antioxidant protection in the muscle tissues of rats with diabetes mellitus is poorly understood.

The aim was to determine the influence of melatonin on activity of pyruvate kinase in the muscle tissue of alloxan diabetic rats.

The experiments were carried out on 24 sexually mature male albino rats with the body mass – (0.18-0.20) kg. Alloxan diabetes was evoked via injecting the rats with a 5% solution of alloxan monohydrate intraperitoneally in a dose of 170 mg/kg of body weight (b.w.). The animals were divided into three subgroups: 1) control group; 2) diabetic rats; 3) diabetic animals which were introduced the melatonin preparation intraperitoneally in a dose of 5 mg/kg of b.w. at 8 a. m. daily during 7 days starting with the 5th 24 hour period after the injection of alloxan. Statistical analysis of results was conducted by Student's test. Sufficient level considered probability differences $P \leq 0.05$.

According to obtained results the activity of pyruvate kinase in muscle tissue of alloxan diabetic animals was by 46% less than in control group. It can be explained by low uptake of glucose from the blood to muscle tissue in absence of insulin. In particular in adipose tissue and skeletal muscle, insulin stimulates glucose uptake. This is accomplished by insulin-mediated movement of glucose transporter proteins to the plasma membrane of these tissues. Reduced glucose uptake by peripheral tissues in turn leads to a reduced rate of glucose metabolism.That means now substrate for next catabolic changes in glycolysis. In addition, the activity of pyruvate kinase is regulated by insulin. We found that the activity of pyruvate kinase was increased by 77% in comparison with diabetic rats due to the introducing of melatonin in a dose of 5 mg/kg of b.w. The effects of melatonin on insulin secretion are mediated through the melatonin receptors (MT1 and MT2). It decreases insulin secretion by inhibiting cAMP and cGMP pathways but activates the phospholipase C/IP3 pathway, which mobilizes Ca^{2+} from organelles and, consequently increases insulin secretion. Probably, melatonin enhances the uptake of glucose to muscles and activates pyruvate kinase activity by the influence of insulin secretion or direct.

So, introduction of melatonin may restore reduced activity of pyruvate kinase enzyme in muscle tissue of alloxan diabetic rats even if it is given in a dose of 5 mg/kg.

THE ROLE OF VITAMIN D_3 IN MODULATION OF GLUCOCORTICOID-INDUCED CHANGES IN ANGIOGENESIS/OSTEOBLASTOGENESIS/OSTEOCLASTOGENESIS COUPLING

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Osteoporosis is the most devastating side effect of chronic glucocorticoid (GC) treatment. The main cause of GC-induced bone loss is aberrant bone homeostasis. Despite the crucial role of vitamin D_3 (D_3) and its receptor (VDR) in bone remodeling, the precise molecular mechanisms of their action on GC-induced disturbances, especially on the angiogenesis/osteogenesis/resorption coupling are still controversial. The aim of our study was to elucidate GC-induced changes of the hypoxia-inducible factor 1-α (HIF-1α)/vascular endothelial growth factor (VEGF) axis and receptor activator of NF-κB (RANK)/NF-κB pathway in rat bone tissue (BT) depending on vitamin D status.

Female Wistar rats received prednisolone (5 mg/kg b.w.) with or without 100 IU of D_3 (for 30 days). Biomechanical parameters of rat femurs were assessed by the 3-point bending test. Levels of VDR, VEGF, phosphoNF-κB p65, IκB (inhibitor of NF-κB) and RANK in BT were determined by western blotting. PPARγ (peroxisome proliferator-activated receptor γ), HIF-1α, NF-κB and TNFα (tumor necrosis factor α) mRNAs were measured by qRT-PCR. RANK immunostaining in BT sections was also performed. 25OHD blood serum content was assayed by ELISA.

Chronic GC administration was associated with a decrease in both maximal load and stiffness of rat femurs and was accompanied by a 3-fold reduction of 25OHD level in serum reflecting D-deficiency and osteoporosis development. Prednisolone also caused a decrease in VDR protein level (-36.7 ± 2.3%, $P < 0.05$), however dramatically elevated PPARγ mRNA level (by 10-fold) in BT that may contribute to anti-osteoblastic and pro-osteoclastic effects of GC. Despite elevated mRNA levels of key upstream VEGF regulators: HIF-1α (+82.5 ± 7.2%, $P < 0.05$) and NF-κB (+480.1 ± 10.9%, $P < 0.05$), VEGF protein level dramatically decreased (-89.5 ± 5.4%, $P < 0.05$) after chronic GC administration, reflecting impaired bone angiogenesis. Nevertheless, phosphoNF-κB p65 content declined (-56.6 ± 3.8%, $P < 0.05$) most likely due to reduced levels of NF-κB modulators: RANK (-49.5 ± 4.4%, $P < 0.05$) and TNFα (-30.3 ± 2.5%, $P < 0.05$) and increased IκB (+44.4 ± 2.7%, $P < 0.05$). Prednisolone-induced impairments in D3 metabolism and lowered phosphoNF-κB level resulted in VEGF inhibition and caused impaired osteogenesis/angiogenesis coupling. Vitamin D_3 administration ameliorated GC-evoked changes in BT through a significant decrease in PPAR-γ, elevation of VEGF level and improved coupling of HIF-1α/VEGF and RANK/NF-κB pathways.

Thus, prednisolone-induced imbalance in the interaction of VDR-, HIF-1α/VEGF and RANK/NF-κB, three critical bone signalling pathways, is associated with reduction of D_3 bioavailability and VDR expression and may be partially ameliorated by vitamin D_3 treatment.

HYDROGEN SULFIDE METABOLISM MODULATORS INFLUENCE THIOL/DISULPHIDE STATUS IN STREPTOZOTOCIN-INDUCED DIABETIC RAT'S HEART

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Thiol/disulfide homeostasis is crucially important for antioxidant defense, apoptosis, and protein activity regulation. Diabetes mellitus and its cardiovascular complications is expected to be associated with thiol/disulfide imbalance with the shift towards disulfide forms. Hydrogen sulfide exerts a variety of biological functions and is known for its sulfhydration/desulfhydration activity. The present study was aimed to assess whether H_2S/Cystathionine-y-lyase system participates in thiol/disulfide metabolism and is deteriorated in the myocardium in terms of diabetes mellitus.

32 Healthy male rats 180-250 g were divided into 4 groups. To induct diabetes, rats (group 2-4) were given streptozotocin (STZ, 40 mg/kg/i.p., 0.1 M citrate buffer (pH 4.5). Rats from the 3rd and 4th groups were administered either NaHS (3 mg/ kg/ i.p.) or propargylglycine (PPG, 50 mg/kg/i.p.) once per day starting from the 14th to 28th day after streptozotocin injection. Rats of the first group (control) were administered the equivalent volumes of 0.9% NaCl. Rats' hearts were sampled for the determination of H_2S by reaction with N,N-Dimethyl-p-phenylenediamine.

The activity of thioredoxin-disulfide reductase (EC 1.8.1.9) in the myocardium was determined by the rate of NADPH-dependent reduction of 5,5′-Dithiobis(2-nitro-benzoate)-DTNB. The redox status of myocardium was evaluated by the level of thiol (-SH) and disulfide (-S-S-) groups that were determined by colorimetric method by reaction with 5,5′-Dithiobis(2-nitrobenzoate). The thiol-disulfide coefficient (-SH/-S-S-) was calculated as the ratio of the number of SH-groups to the number of S-S groups.

The development of diabetes was associated with significantly lower H2S content in rat's myocardium (by 39.7%, $P < 0.05$). In diabetic rats, compared to the control group, the activity of thioredoxin-disulfide reductase, levels of thiol (-SH) groups and thiol/disulfide ratio were determined lower (by 39%, $P < 0.05$; 38%, $P < 0.05$ and 68%, $P < 0.05$ respectively), while disulfide levels were much higher (by 68%, $P < 0.05$). Moreover, the administration of CSE inhibitor resulted in more significant H_2S deficiency (by 29.4%, $P < 0.05$), decreased thioredoxin-disulfide reductase activity (by 34%, $P < 0.05$) and deep shift towards disulfide forms compared to STZ-diabetic rats. NaHS treatment significantly ameliorated the alterations in myocardium caused by diabetes. Thus, H_2S/Cystathionine-y-lyase system is embedded in thiol/disulfide homeostasis and is promising treatment target in diabetic heart.

ACTIVE TRANSPORT OF Ca^{2+} IN THE PLASMA MEMBRANE OF ROOTS UNDER SALT STRESS CONDITIONS AND ACTION OF THE PREPARATION METHYURE

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Salting of soils is a strong negative constant factor for plants due to the presence of Na^+ as the main salt cation (Munns, 2008). Its distribution area is rapidly expanding due to global climate change and is intensified under irrigation conditions (Gupta, 2014). This situation is observed, in particular, in the south of Ukraine, forcing to seek ways to reduce its influence.

Therefore, the urgent problem was a search for cheap and safe bioactive preparation to increase the salt tolerance of plants. We established the possibility of increasing the salt tolerance of plants using the bioactive compound of 6-methyl-2-mercapto-4-hydroxypyrimidine (preparation Methyure), LD_{50} > 4000 mg/kg. It was synthesized in IBOPC as a plant growth stimulator, which under stress conditions also exhibited an adaptogenic property (Palladina, 2011).

The active Ca^{2+} transport system plays an important role in the functioning of many biochemical processes in the cytoplasm of plant cells, in particular under salinity conditions (Bose, 2011). In the plasma membrane (PM) of the root cells, this system provides regulation of the activity of Na^+/H^+ antiporter, which removes toxic Na^+ from the cytoplasm through the Ca^{2+}-dependent salt overly sensitive (SOS) signaling pathway (Shi, 2002).

The purpose of our study was to find out the participation of the active Ca^{2+} transport system, represented by Ca^{2+}-ATPase and Ca^{2+}/H^+ antiporter in PM root cells, in the removal of Na^+ from the cytoplasm, and also the influence of these processes on the bioactive compound Methyure. The studies were carried out on seedlings of *Zea mays* L. (hybrid Ostrech SV), grown in a water culture on Hoaglands solution, exposed in the presence of 0.1 M NaCl for 1 day (8-day-old seedlings) and 10 days (17-day-old seedlings).

The seeds were soaked in 10^{-7} M solution of Methyure for 1 day. PMs were obtained by the phase separation method (Larsson, 1994). The protein content in the PM was determined according to the standard method (Bradford, 1976). The activity of Ca^{2+}-ATPase in PM was determined using Fluo-4 AM fluorescence probes (Kolomiets, 2013), and Ca^{2+}/H^+ antiporter using ACMA (9-amino-6-chloro-2-methoxyacridine) (Zhai, 2013). The calculation of the concentration of ionized Ca^{2+} was carried out under Maxchelator (http://maxchelator.stanford.edu/CaMgATPEGTA-TS.htm).

We have found that the presence of 0.1 M NaCl in the growth medium of seedlings intensified the activity of PM Ca^{2+}-ATPase and triggered PM Ca^{2+}/H^+ antiporters. Methyure under salt stress conditions was found to increase the activity of both enzymes, especially Ca^{2+}/H^+ antiporter adaptogenic effect of this preparation. Thus, Methyure is a cheap and safe preparation, which can significantly increase the salt resistance of plants.

PEROXIREDOXIN 2 INTERACTION NETWORK REVEALS ENRICHMENT IN GENES LINKED TO INFLAMMATION-RELATED PATHOLOGIES

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Protein interactions are organized into dynamic networks sensitive to incoming signals and modifications, which may significantly change under stress and pathological conditions. Peroxiredoxins belong to thiol-specific peroxidase family (EC 1.11.1.15) which has antioxidant functions. Human peroxiredoxin 2 (PRDX2) is ubiquitous cytoplasmic protein with the highest expression level in the bone marrow and heart. It is well known to be associated with hemolytic anemia, while the recent data give the direct evidence on the role of PRDX2 in cell cycle regulation and cancer development. PRDX2 interactome functionality has not yet been described, therefore, we analyzed *in silico* PRDX2 partners for their functions, link to pathogenesis and network connectivity.

The data on proteins interacting with PRDX2 were downloaded from STRING (https://string-db.org/) and BioGrid (https://thebiogrid.org) databases. The combined list included 114 proteins having physical interactions proved mostly by affinity capture-MS, two-hybrid system and co-fractionation. Gene set enrichment analysis was performed by two web server tools: Enrichr (http://amp.pharm.mssm.edu/Enrichr/) and DAVID (https://david.ncifcrf.gov/tools.jsp). Protein network was reconstructed by online tool NetworkAnalyst (http://www.networkanalyst.ca/).

Part of our results was in agreement with known data on the role of PRDX2 in oxidative stress response, apoptosis, differentiation and cancerogenesis. Nevertheless, quite new functions of PRDX2-centered network might be supposed from the enrichment of interactome in genes playing a key role in glucose and amino acid metabolism, Ca^{2+}-dependent adhesion, RNA-binding and nucleotide production. Our analysis showed the connection of several PRDX2 partners to progression of Alzheimer disease and encephalopathy. Forty five of 114 genes were revealed to be associated with three microRNAs – hsa-miR-16-5p, hsa-miR-92a-3p and hsa-miR-615-3p – linked to the wide range of diseases, such as cancer, cerebral and myocardial infarction, arthritis, asthma, diabetes. All the mentioned pathologies are known to be associated with inflammation.

The most connectivity in the reconstructed network was found for two groups of proteins: acting in intracellular signaling (APP, YWHAZ, CDK2, ESR1) and linked to cell adhesion (FN1, ITGA4 and VCAM1). Thus, PRDX2 protein interaction network could be tightly involved in the inflammation-related pathologies development.

THE EFFECT OF N-STEAROYLETHANOLAMINE ON CHOLESTEROL CONTENT OF ADIPOCYTES IN DIFFERENT AGE RATS WITH OBESITY-INDUCED INSULIN RESISTANCE

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Introduction: Chronic hypernutrition and high fat diet (HFD) rich in saturated fatty acids leads to obesity and changes in insulin sensitive tissues, impairment in insulin signaling and followed by dyslipidemia. There is a hypothesis that insulin resistance (IR) may originate in adipose tissue. Cholesterol is known as one of the main components of cell membranes and influence significantly on membrane properties. That is why the aim of our study was to investigate the cholesterol content of adipocytes in different age rats with HFD-induced obesity and IR and its changes under N-stearoylethanolamine (NSE) administration.

Methods: The experimental model was induced on 10-month- and 24-month-old rats by the 6-month high-fat diet (58% of fats of the total diet) and confirmed by the oral glucose tolerance test. NSE was administrated as water suspension *per os* in a dose of 50 mg/kg daily during 2 weeks. Adipocytes were isolated from abdominal fat using Type 1 Collagenase solution. Adipocyte lipid extract was separated on the fractions by thin-layer chromatography.The cholesterol level was measured by gas–liquid chromatography. Experimental data were processed statistically using Student's t-test. The statistical significance was determined for $P < 0.05$.

Results and Discussion: The results of this study demonstrated that the total content of cholesterol in adipocytes of 10-month-old rats is significantly higher than in 24-month-old rats. The cholesterol content in fat cells of elder animals was 25-30% of the cholesterol level of adipocytes of younger rats from the same experimental groups. These results may be explained by age-related dysfunction of adipose tissue and the reduction its function as depot as well as changes in membrane lipid composition.

HFD induced statistically significant growth of adipocyte total cholesterol content in both age groups compared to control. NSE administration caused a considerable effect on normalization of the level of cholesterol in fat cells of rats with obesity-induced IR.

Conclusions: It was demonstrated, that prolonged HFD induced IR and leads to the growth of cholesterol content of adipocytes in rats from two age groups. As far as NSE had a positive effect on normalization of cholesterol content of adipocytes, we can consider NSE as a prospective agent for the treatment of obesity-induced complications in patients from different age groups.

POSTER SESSION VII. MOLECULAR BIOLOGY

OF THE PHYSIOLOGICAL AND GENETIC DIFFERENCES BETWEEN THE WHITE COLLARED FLYCATCHER AND PIED FLYCATCHER OF THE GENUS FICEDULA

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The fact that the flycatchers are capable of interspecies hybridization is one of the reasons of particular interest to these species.We studied 30 representatives of the genus Ficedula, including 20 of *Ficedula albicollis* (5 adults and 15 nestlings 10-12 days old) and 10 of *Ficedula hypoleuca* (3 adults and 7 nestlings 10-12 days old) to detect physiological and genetic differences between two species of flycatchers. We took blood samples from the birds eye's sinus in the protected area of Homilshanski Forests, Kharkiv, Ukraine to study their leukogram patterns. The bird feathers were also collected for genetic analysis to identify interspecies differences with the application of the ISSR technique. We did not find any significant difference in the leukogram between the adults of two species. However, the difference in the leukogram distribution was found between the nestlings of white collared and pied flycatchers. The heterophile (granulocyte) indices were higher in the white collared flycatcher nestlings by 2.96% (12.53 ± 0.56) ($P < 0.001$), and those of lymphocytes were higher in the pied flycatcher nestlings by 2.94% (86.0 ± 1.15) ($P < 0.01$). This may indicate difference in the immune system development, although, as mentioned above, there was no particular leukogram difference between the adults of two species. It seems that with age the immune system of two species is levelled and becomes similar. The micronucleus test did not reveal any significant difference between species and age bird groups. Using of the primer (AGC)6G with the DNA of the pied and white collared flycatchers revealed quite high level of genetic polymorphism. Thus, a total sum of amplicons formed in the PCR with the selected primer ranged from 9 in the pied flycatcher to 14 in the white collared flycatcher. The length of amplicons ranged between 270-1400 bp. A majority of PCR-loci (83.33% of the total number of loci) received with this primer were of average size. Four PCR loci (16.67%) were less then 500 bp long. The spectra of amplification products obtained with the primer (AGC)6G showed that the white-collared flycatchers had a more heterogeneous structure. The study of the leukogram, micronucleus test, and the conduction of the ISSR analysis can be especially effective in the study of intraspecies genetic differentiation.

E. COLI AlkB AND ITS HUMAN HOMOLOG ALKBH3 DIOXYGENASES EFFECTIVELY REPAIRED ACROLEIN ADDUCTS TO ADENINE

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1,N6-α-hydroxypropanoadenine (HPA) is six-membered DNA adduct formed in reaction of adenine with acrolein (ACR). ACR is a mutagenic agent originated from different sources including cigarette smoke, exhaust fumes and overcooking. It is also generated endogenously during oxidative stress as a byproduct of lipid peroxidation. *E. coli* AlkB (EcAlkB) and ALKBH3 dioxygenases are DNA repair enzymes that remove alkyl lesions from bases via an oxidative mechanism restoring native DNA structure. They belong to the superfamily of 2-oxoglutarate (αKG) and Fe(II) dependent dioxygenases. AlkB is induced within *E. coli* system of adaptive response to alkylating agents (Ada response). Our *in vivo* data show that HPA shows mutagenic properties and, generated in plasmids, causes (respectively) A→C and A→T transversions. We established the optimal pH, Fe(II) and αKG concentrations for enzymatic reaction. Our data proved that the protonated form of HPA is preferentially repaired by EcAlkB and ALKBH3 *in vitro.* Moreover, the number of reaction cycles carried out by an AlkB molecule remains limited and reached 38 ± 4 enzymatic cycles before its total inactivation. Molecular modeling of the AlkB/HPA and ALKBH3/HPA complexes demonstrated that the R stereoisomer in the equatorial conformation of the HPA hydroxyl group is strongly preferred, while the S one seems to be susceptible to AlkBdirected oxidative hydroxylation only when HPA adopts the syn conformation around the glycosidic bond.

COMPUTATIONAL ANALYSIS OF EPS15 3'UTR IN DIFFERENT SPECIES

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EPS15 gene encodes a conservative protein, participating in EGFR pathway, regulation of cell growth and mitogenic signals, control of cell proliferation, assembly of clathrin-coated pits etc. Post-transcriptional regulation of this gene by microRNAs – 18-30 nt non-coding RNA molecules that mainly negatively regulate gene expression – is poorly studied.

The aim of our study was to analyze 3'UTR of EPS15 and find potential binding sites for microRNA for the following species: *H. sapiens, B. taurus, M. musculus, R. norvegicus, G. gallus* and *D. melanogaster.* To analyze sequence similarity of EPS15 3'UTRs we used blast and clustal omega. To predict target sites for microRNAs in 3'UTR of EPS15 TargetScan and microRNA.org servers were used, while for the pathway analysis for microRNAs we used miRPath server.

Comparison of 3'UTRs revealed that for the majority of analyzed organisms full-length 3'UTR in known, except *R. norvegicus*, in which 3'UTR we did not find any known canonical poly(A) signals 10-40 nt upstream the end of the sequence. It may indicate incomplete 3'UTR sequence available from GenBank. Then, we found that 3'UTRs of *M. musculus, R. norvegicus, G. gallus* and *D. melanogaster* were similar less than 50%. At the same time, the degree of 3'UTR similarity between *M. musculus* and *B. taurus* constitutes 63.26%, between *H. sapiens* and *M. musculus* – 66.51%, between *H. sapiens* and *B. taurus* – 76.74%. Based on these results, we suggested the presence of similar regulatory elements in 3'UTRs of *M. musculus, B. taurus* and *H. sapiens.*

To confirm our suggestion, we analyzed 3'UTRs of *H. sapiens, M. musculus* and *B. taurus* and found target sites for highly conserved microRNAs: miR-196a, miR-23a/b, miR-130a, miR-26a/b, miR-19a/b, miR-145, miR-205. Identified microRNAs were enriched for the processes where EPS15 is involved in: viral process, epidermal growth factor receptor signaling pathway, ion binding, cellular protein modification process, nucleic acid binding transcription factor activity, toll-like receptor TLR1:TLR2 signaling pathway, toll-like receptor TLR6:TLR2 signaling pathway, nervous system development, Fc-epsilon receptor signaling pathway.

Obtained results showed the degree similarity of EPS15 3'UTRs in several vertebrate species and existence of several conservative microRNA target sites. Such conservation can suggest functionality of these sites since they were retained in the process of evolution but this need to be confirmed experimentally.

MUTATIONS IN *EPHA1* GENE ASSOCIATED WITH INTELLECTUAL DISABILITY AND ALZHEIMER'S DISEASE

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Whole exome sequencing (WES) has become a highly efficient genomic tool to study intellectual disability (ID) etiology in clinical diagnostic and novel causative mutations. But, detection of novel genetic factors requires further studies of its association with ID or related pathologies. In recent reports we presented WES results of two affected siblings with ID identified two missense mutations in the coding region of the *EPHA1* gene (c.1475G>A - rs11768549 and novel c.1891G>A). Both affected siblings were compound heterozygotes. *EPHA1* polymorphisms rs11767557 and rs11771145 was also documented in recent genome wide association studies to be strongly associated with other neurodisorder - Alzheimer's disease (AD).

Presented study is aimed to clarify the role of mentioned EPHA1 gene mutations and polymorphisms as genetic factors of ID and late onset AD (LOAD) pathogenesis. Assays for the detection of *EPHA1* SNPs based on PCR-RFLP analysis were developed. The comparative analysis of genotypes distribution was performed in a group of 65 patients with mild ID, 75 LOAD patients and group of 250 healthy volunteers from Ukraine (control group). The significant differences ($P < 0.05$) in allele frequencies of rs11768549, rs11767557 and rs11771145 between ID patients and controls were identified. Statistical analysis showed significant association of rs11768549A (OR=3.96; 95% CI=1.13-13.89); rs11767557T (OR=1.99; 95% CI=1.18-3.37) and rs11771145G (OR=1.55; 95% CI=1.02-2.37) alleles with higher risk of ID. Our results also suggest that EPHA1 rs11767557T allele is associated with higher risk of LOAD (OR=2.09; 95% CI=1.14-3.85).

We propose *EPHA1* gene rs11768549, rs11767557, rs11771145 polymorphisms as a new markers of genetic susceptibility for ID and rs11767557 – for LOAD. C.1891G>A substitution was not detected in any of investigated 250 persons from control group as well as among 65 ID and 75 AD patients. Moreover, 1891G>A substitution was not identified in any population investigated in frame of EXAC, HapMap and 1000 Genomes Projects. Therefore, we suppose that *EPHA1* c.1891G>A (p.Gly631Arg) is mutation with an extremely low frequency that indicates its high pathogenicity. Our results allow us to hypothesize that missense mutations in *EPHA1* gene may be responsible for autosomal recessive ID. Thus, our summarized findings are consistent with the fact that adults with a childhood history of specific learning and ID are in risk to develop dementia, including AD.

MICRORNA- AND RBP-BASED REGULATION OF GENES INVOLVED IN THE REMODELING OF ACTIN CYTOSKELETON

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Remodeling of actin cytoskeleton is an essential step for many cellular processes in normal and pathological conditions such as cell migration, invasion, endo- and exocytosis, cell division. To date, many proteins deeply involved in this process are characterized including adaptor proteins such as ITSN1, serving as a scaffold for the assembly of multiprotein complexes. In addition, ITSN1 plays important roles in clathrin-mediated endocytosis, neurotransmission and is overexpressed in Alzheimer's disease and several cancers. Despite intense studies, there are still many gaps in the understanding of the regulation of actin cytoskeleton remodeling on post-transcriptional level, particularly by microRNAs and RNA-binding proteins (RBPs).

The aim of our work was to predict computationally target sites for microRNAs and RBPs for several genes involved into the remodeling of actin cytoskeleton with further confirmation of microRNA target sites in 3'UTR of ITSN1.

For the search of RBP target sites, we selected 49 transcripts of genes participating in the reorganization of actin cytoskeleton and analyzed by RegRNA 2.0 and Scan For Motifs servers resulted in the identification of Musashi binding elements (MBE) as conserved for 37 transcripts. Our previous results indicated that MBE is a most common regulatory element for FEI nucleation hub at the initial step of clathrin-mediated endocytosis that includes FCHO1/2, EPS15 and ITSN1/2 proteins. Based on the absence of *in silico* or experimentally verified model for Musashi 1 or Musashi 2 (MSI1/2) proteins (that bind MBE), we made primary models for these proteins using SWISS-MODEL and Chimera 1.11 software. MSI1 and MSI2 are 78% homologous proteins, each containing two consecutive RNA-recognition motifs (RRM), via which MSI1/2 bind target mRNA and influence its stability. Previously, we found several microRNA target sites in 3'UTR of the short isoform of ITSN1 (ITSN1-S) including miR-30, miR-19 and miR-181 families playing roles in several cancers. To confirm these predictions we performed luciferase assay with reporter plasmid containing full-length 3'UTR of ITSN1-S and above mentioned microRNAs in MDA-MB231 cells. According to the results, binding sites for miR- 19a-3p and 181a-5p were established to be functional to downregulate ITSN1-S expression while the functionality of the site for 30a-5p is ambiguous.

In conclusion, obtained data show the importance of microRNA and RBP-mediated regulation of actin cytoskeleton remodeling genes that can be important for the study of cancer development.

SPERM-MEDIATED GENE TRANSFER (SMGT) IN DUCK (*ANAS PLATYRHYNCHOS*)

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Over the past decades, sperm-mediated gene transfer (SMGT) has attracted the attention of many researchers. Despite the fact that it has become a well-accepted method, SMGT is still developing new approaches to make this technique even more widespread and reliable. This method is based on the ability of spermatozoa to bind exogenous genes *in vitro* and deliver them to the oocyte during fertilization.

The transgenic duck, apparently, could function as a bioreactor for the production of commercially or pharmaceutically important proteins that are difficult or impossible to produce economically in prokaryotic systems. Gene transfer technology can also be a useful addition to traditional methods of breeding poultry in improving commercially important production characteristics, such as growth and disease resistance.

The present study used CRISPR/Cas9 system to mediate the HDR directed EGFP gene integration into the duck's host genome by the SMGT.

A total of 20 parents (13 males and 7 females) of Shaoxing ducks were used for sperm-mediated gene transfer. In the experiment, we obtained 13 F0 birds (2 males and 11 females). Five females and one male contained the transgenic structure as detected by PCR in DNA which was extracted from blood. Therefore the exogenous DNA insertion efficiency was 46.2% (6/13).

Transgenic transmission to the next generation was verified by crossing 13 (F0) ducks with non-transgenic poultry and obtaining their offspring. One female and two male did not produce any descendants. The offspring (F1) analysis revealed, a total of 8 (F0) female ducks transmitted the transgene to their descendants. Total in F1 we obtained 111 offspring (from the 10 ducks), among which 36 ducks (32.4%) were positive for EGFP gene. Also, one duck with a transgene detected in its blood did not pass on to its descendants, and 3 ducks with no transgene detected in their blood, found it at their descendants.

Thus, the data obtained indicate a high level of transgene transmission to offspring, and the possibility of using this technology to produce a transgenic duck.

HYPOXIC REGULATION OF ENDOPLASMIC RETICULUM STRESS RESPONSIVE GENE EXPRESSIONS IN IRE1 KNOCKDOWN GLIOMA CELLS

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The endoplasmic reticulum stress is an important regulator of glioblastoma multiforme growth. IRE1 signaling is a central and most conservative branch of the unfolded protein response and inhibition of this signaling pathway leads to a suppression of glioma growth. Hypoxia is another important factor to glioma development. A better knowledge of tumor responses to a hypoxic condition is required to elaborate therapeutical strategies of cell sensibilization based on the blockade of survival mechanisms. The aim of this study was investigation of the effect of IRE1 (inositol requiring enzyme 1) knockdown as well as hypoxia on the expression of eukaryotic translation initiation factor 2 alpha kinase 3 (*EIF2AK3*), activating transcription factor 6 (*ATF6*), clusterin (*CLU*), adhesion G protein-coupled receptor E5 (*ADGRE5*), leukemia inhibitory factor (*LIF*), phosphoserine aminotransferase 1 (*PSAT1*) and tetraspanin 13 (*TSPAN13*) genes in U87 glioma cells with hopes of elucidating their involvement in the development and progression of glioblastoma.

In this study we used two sublines of U87 glioma cells. One subline was obtained by selection of stable transfected clones with overexpression of vector (pcDNA3.1), which was used for creation of dominant-negative constructs (dnIRE1). This untreated subline of glioma cells (control glioma cells) was used as control 1. The second subline was obtained by selection of stable transfected clones with overexpression of dnIRE1 and has suppressed both protein kinase and endoribonuclease activities of IRE1. The expression of a subset of 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.

It was shown that the expression levels of *ATF6, CLU, ADGRE5, LIF, PSAT1,* and *TSPAN13* genes are significantly down-regulated in glioma cells with the knockdown of IRE1 signaling enzyme. It was also shown that in glioma cells subjected to hypoxia the expression levels of *PSAT1, TSPAN13,* and *EIF2AK3* genes were up-regulated, whereas the expression of ATF6 gene was down-regulated. At the same time, the expression levels of *LIF, CLU,* and *ADGRE5* genes did not change in response to hypoxic treatment. Furthermore, inhibition of IRE1, a key effector of an unfolded protein response pathway, modified the effect of hypoxia on the expression of most studied genes. This data clearly demonstrated that IRE1 signaling participates in hypoxic regulation of numerous gene expressions, but molecular mechanisms of its role in this regulation warrant further investigation.

The present study demonstrates that IRE1 knockdown down-regulated the expression of most studied genes and modified hypoxic regulation of their expression and that these changes possibly contribute to the suppression of glioma growth in cells without IRE1 signaling enzyme function.

PREDICTING MODEL OF REGULATORY RELATIONSHIPS BETWEEN LONG NON-CODING RNAS ANRIL/XIST AND DISEASE-ASSOCIATED MICRORNAS

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Long noncoding RNAs (lncRNAs) are defined as a class of important heterogeneous ncRNAs with the length more than 200 nucleotides. The recent study suggested that lncRNAs ANRIL and XIST participate in development of several unisex tumors via binding disease-specific microRNAs. ANRIL and XIST can act as decoys, containing sequences that mimic miRNA target sites to titer these miRNAs away from their primary targets thereby act as molecular sponges.

The aim of the research is to predict and identify miR-lncRNA (XIST and ANRIL) interactions in several selected cancers – breast cancer, glioblastoma, neuroblastoma.

We used LncRNADisease, Lnc2Cancer, Diana Tools to analyze present expression data of lncRNAs ANRIL and XIST for selected diseases *in silico.* To find possible involvement of target microRNAs in various diseases we used HMDD v2.0 and Mir2 Disease databases.

Using computational analysis of disease-associated microRNAs (Diana Tools), we predicted potential microRNAs for sponge activity of ANRIL (miR-15, -125, and -7 family) and XIST (miR-30, -15, -7 family). Then, we used HMDD v2.0 and Mir2Disease databases to find possible involvement of these microRNAs in various cancers. MicroRNAs from miR-15 and miR-7 families are predicted to bind XIST and ANRIL lncRNAs that can suggest the potential link between these lncRNA in varios cancers.

In conclusion, we suggested our prediction model of potential regulatory network of long noncoding RNAs ANRIL and XIST, which gives an opportunity to obtain a more comprehensive picture of epigenetic regulation.

CONSTRUCTION, CLONING AND EXPRESSION OF THE FVIII GENE WITHOUT B DOMAIN IN CHO-DHFR CELLS

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Heamophilia type A is a genetic disorder that impairs body's ability to make blood clots, which is a process needed to stop bleeding. Factor VIII, an essential blood coagulation protein, is a key component of the blood coagulation system. Factor VIII is encoded by the FVIII gene. Defects in this gene results in hemophilia A, a well-known recessive X-linked coagulation disorder.
The aim of our work was to obtain cDNA of factor VIII without B domain. The expression of B-domain deleted FVIII in mammalian cell cultures is higher than that of full-length FVIII molecules. The FVIII factor was made of synthetic oligonucleotides. We performed gene assembly from the long synthetic oligonucleotides (35-50 bp) that were ligated. PCR was used to amplify 4374 bp DNA fragment. Using innovative method we put together all of the fragments of the gene and cloned it into one common vector. After digestion the DNA fragment was inserted into pCG/dhfr eukaryotic expression vector. The pCG/dhfr plasmid contained CMV promoter, dhfr gene, MTX resistant variant, with promoter SV40 and the human FVIII factor without domain B sequence. The CHO cells were transfected by lipofection method with PEI/DNA complexes. Positively transfected cells were selected in selective medium (without thymidine and hypoxanthine) using dihydrofolate reductase (dhfr) selection system. The gene copy number in this cell line was increased by a stepwise increase in the methotrexate concentration. To determine whether FVIII was produced by the transfected cells, cell medium was collected and tested by Western blot using site-specific anti-Factor VIII monoclonal antibody.

EXPRESSION OF THE FVIII GENE WITHOUT B DOMAIN IN *E. COLI* BACTERIAL EXPRESSION SYSTEM

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Factor VIII (FVIII), an essential blood coagulation protein, is a key component of the fluid phase blood coagulation system. The role of the FVIII is to increase the catalytic efficiency of factor IXa in the activation of factor X. Variants of these factors frequently lead to severe bleeding disorders.
Mammalian cells can produce catalytically active proteins with all necessary posttranslational modifications. However, breeding such cells is time consuming and very expensive, and the amount of the product obtained is usually low. In contrast to eukaryotic cells, bacterial culture is inexpensive and allows to obtain large quantities of recombinant proteins in short time.
The aim of our work was to obtain recombinant factor VIII in the so far unused prokaryotic system. The FVIII gen was made of the synthetic oligonucleotides. We performed gene assembly from the long synthetic oligonucleotides (35-50 bp) that were ligated. PCR was used to amplify 4374 bp DNA fragment. Using innovative method we put together all of the fragments of the gene and cloned it into one common vector. After digestion, the DNA fragment was inserted into the pDB prokaryotic expression vector. The pDB plasmid contained deoP1P2 promoter, tetracycline resistant variant and the human FVIII factor without domain B sequence. Plasmid pDB/FVIII was transformed to the *E. coli* strain by electroporation. Protein expression was confirmed by MALDI-TOF/TOF mass spectrometry.

CONSTRUCTION, CLONING AND EXPRESSION OF THE ANTI-CD22 MONOCLONAL ANTIBODY IN THE *E. COLI* BACTERIAL EXPRESSION SYSTEM

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Antibodies, otherwise known as immunoglobulins (Igs), belong to one of the most important achievements of modern medicine, which have conditioned its rapid development. Antibody applications cover a wide range of aspects of laboratory diagnostics and therapy of infectious, neoplastic or autoimmune diseases. They are also used in transplantology and anticoagulant therapy.

The smallest fragment of the antibody that retains the affinity and specificity for the parent antibody is scFv (single-chain variable region fragment antibody). ScFv is an unnatural minimal antibody in which the variable regions of the heavy and light chains are linked by a flexible peptide linker. Such minimal antibodies have many advantages in therapy and diagnosis due to the small size and the ease of mass production. In comparison to monoclonal antibodies *in vivo*, scFvs are released more quickly from the blood and penetrate tissues quickly and evenly. Most of the products tested in clinical trials are whole antibodies produced in mammalian cells. Intensive research are being carried out on the production of antibody fragments in bacterial expression systems such as *E. coli*.

The aim of this work was obtaining the single chain of the anti-CD22 monoclonal antibody fragment in *E. coli*. This fragment consists of the heavy chain V_H, the light chain V_L and the short flexible link $(Gly_4Ser)_3$. The obtained protein is constructed as a fusion gene - Ubi::CD22, where the ubiquitin (Ubi) is used as a signal protein. This protein is responsible for protein modification in eukaryotes called ubiquitination, a phenomenon which does not occur in bacteria. In spite of that, it has been shown that proteins fused to ubiquitin undergo greater expression in *E. coli* and are easier to purify and renaturate than nonhybrid foreign proteins. We obtained a satisfactory level of expression of fusion proteins Ubi::CD22 in *E. coli* and a visible amount of the CD22 protein after cleavage of the ubiquitin.

INFLUENCE OF OLIGORIBONUCLEOTIDES ON THERMAL STABILITY OF METHYLTRANSFERASE ECORI

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DNA methylation is a prerequisite for the realization of the functions of cellular "immunity" by regulating the expression of genes during the development of pathological states. RNA-based oligoadenylates drugs that can bind and affect the work of epigenetic regulators and transcriptional proteins through interaction, with regulatory domains can be used as safe analogs. At the moment, a number of inhibitors of DNA-methyltransferases are known, but their mutagenic and toxic effects are a significant disadvantage of these compounds. Natural and synthetic oligoadenylates that can bind and affect the work of epigenetic regulators and transcriptional proteins through interaction with regulatory domains can be used as safe analogs.

In our research, we focused on the investigation of the thermal stability of protein methyltransferase EcoRI in the presence of 2′-5′ oligoadenylate 2′-5′-A_3 and its analog 2′-5′ A_3-epo, total yeast RNA and complex total yeast RNA with alcohol sugar – D-mannitol. The method of fluorescence spectroscopy was used in our study. The fluorescence melting curves were obtained from fluorescence spectra of protein- oligoribonucleotides solutions measured at different temperatures (30 to 75 °C). For estimating the dissociation constant (K_d) next formula was used $K_d = \frac{(1-\theta)(D-(\theta P_0))}{\theta}$.

The thermal denaturation profiles of methylase EcoRI alone and in the presence of 2′-5′-A_3, 2′-5′ A_3-epo, total yeast RNA, and yeast RNA:D-mannitol complex were obtained and analyzed. It was shown that addition of 2′-5′-A_3 and 2′-5′ A_3-epo to methylase EcoRI leads to the shift of protein melting temperature in higher temperature region. For example, the melting point of EcoRI is 38 °C. After adding 2′-5′-A_3 and 2′-5′ A_3-epo to the solution the temperature of protein melting point is shifted to 41 °C. After adding total yeast RNA:D-mannitol and RNA to the solution the temperature of protein melting point is shifted to 62 and 53 °C. The magnitude of the structural changes is given relatively weak (micromolar) dissociation constant for the interaction between these molecules. For example, the dissociation constant between EcoRI and total yeast RNA is K_d = 0.31 ± 0.07 μM and between EcoRI and total yeast RNA with – D-mannitol complex – K_d =0.15 ± 0.02 μM. On the other hand, the dissociation constant between EcoRI and 2′-5′-A3 is K_d = 0.20 ± 0.03 μM and between EcoRI and 2′-5′ A_3-epo – K_d = 0.03 ± 0.006 μM.

So, we assume that protein methylase EcoRI become more stable under the presence of oligoribonucleotides. We can suppose that thermal stabilization of protein indicates the interaction between oligoribonucleotides and methylase EcoRI that leads to changing of its conformation.

GENE EXPRESSION IN HUMAN PLACENTA: ON THE WAY FROM FOLATE-RELATED ONE CARBON SUBSYSTEM TO TRANSCRIPTOME

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Background. The human placenta is the highly specialized organ of pregnancy. It originates from the trophectoderm of the blastocyst and develops into a complex functionally active structure that mediates the tight interactions between the mother and fetus. Growth and function of the placenta are precisely regulated and coordinated; their derangements lead to pregnancy complications e.g. life-threatening preeclampsia (PE). The objective of our study is to build an integrated understanding of gene expression in healthy and PE–affected human placenta by analyzing the expression of genes responsible for the folate-related one carbon metabolism (FOCM) as a subsystem suspected in PE pathogenesis and creating a specialized database of gene expression for the systems analysis of genome wide gene expression.
Methods. Collection of placental samples from I and III trimesters of gestation; cultivation of placental explants; RT-qPCR; WB; methods of bioinformatics.
Results. FOCM and related methionine cycle and transsulfuration reactions comprise a core metabolic system which provides support for the basic needs of dividing cells: rapid ATP generation, *de novo* purine synthesis; the synthesis of S-adenosylmethionine (SAM) to sustain the cellular methylation and glutathione production to maintain an appropriate cellular redox status. The expression of corresponding *GART, ATIC, MTR, CBS, ADO* and *SUOX* genes declined from first trimester to term, while *CDO* expression increased by more than 10 times. *GCLC* and *GCLM* levels changed the least of all. The gene *GART* underwent the alternative polyadenylation; the abundance of the short form prevailed in the I trimester and decreased at term. We have shown that in addition to transported taurine it is synthesized in placenta along *ADO*-dependent pathway. The cultivation of placental explants with 20-40 mM homocystene to imitate clinical hyperhomocystenemia revealed inhibition of *de novo* purine synthesis and proliferation, increase of apoptosis and SAM/SAH disbalance. To move from core FOCM subsystem to the whole transcriptome, we collected cDNA microarray expression data of more than 1000 biological samples of healthy and PE-affected human placenta available in open access and created the specialized database (http://194.44.31.241:24173/). We, stratified the data according to the term of pregnancy, maternal age, diagnosis and type of placental structure and created several case-control groups.
Conclusion. The adverse effect of hyperhomocystenemia in pregnancy is realized in particular by changes in the core FOCM. Creation of the specialized data base of gene expression in human placenta and stratification of data with formation of case-control groups are the basement for new discoveries in PE area.

IRE1 KNOCKDOWN LEADS TO SUPPRESSION OF GLIOBLASTOMA GROWTH

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Tumor growth is tightly associated with the endoplasmic reticulum stress response signalling pathways and hypoxia, which are linked to the neovascularization and cell death processes. The endoplasmic reticulum has an essential position as a signal integrator in the cell and is instrumental in the different phases of tumor progression because the signaling pathways elicited by endoplasmic reticulum stress sensors are connected to metabolic pathways and to other plasma membrane receptor signaling networks. The IRE1 (inositol requiring enzyme 1) is a central mediator of the unfolded protein response and its inhibition leads to suppression of tumor growth through down-regulation of the angiogenesis and proliferation processes. It contributes to changes in the expression profile of many regulatory genes resulting in proliferation, angiogenesis, and apoptosis.

We have shown that inhibition of IRE1 suppresses glioma cell proliferation and tumor growth by affecting the expression of genes encoding the tumor suppressors, oncogenes, and TNF receptors as well as apoptosis related factors, protein kinases, and key transcription factors. While studying the expression of genes encoding proliferation related factors, ubiquitin specific peptidases and some other proteins such as IL13RA2, KRT18, CD24, NAMPT, MEST, ING1, ING2, MYL9, BET1, TRAPPC3, ENDOG, LONP1, TSFM, and MTIF it was shown that IRE1 protein kinase and endoribonuclease have different impact on gene expression specifically activating or suppressing various signaling pathways and that the regulation of gene expression for THFRSF21/DR6, GADD34, DATF1, TP53, PLK1, PLK2, ING1, ING2, ATF3, ENDOG, ENDOG, and TSFM are specifically controlled by IRE1 protein kinase activity.

Furthermore, we have shown that hypoxia, which is obligate interconnected components of malignant tumor growth, affects almost all studied genes and that inhibition of IRE1 signaling network mostly modifies the expression of proliferation related genes contributing to the intensity of glioma cell proliferation. We have identified several perspective genes which expression significantly changed in glioma cells with inhibited both enzymatic activities of IRE1 or its endoribonuclease only and some of these genes can be perspective targets to the design of novel compounds for therapeutic strategies to manipulate levels of endoplasmic reticulum stress in diseases.

METABOLIC REPROGRAMMING OF MITOCHONDRIA IN IRE1 KNOCKDOWN U87 GLIOMA CELLS

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Numerous studies have established the link between tumor progression and endoplasmic reticulum stress. Gliomas use endoplasmic reticulum stress signaling pathways to adapt to stress conditions and rapid proliferation. It has been demonstrated that inhibition of IRE1, a key mediator of the unfolded protein response, leads to significant anti-proliferative effect in glioma growth through down-regulation of proliferation and angiogenesis processes. It is possible that this anti-proliferative effect is also mediated by polyfunctional proteins ACADM (Acyl-CoA Dehydrogenase Medium Chain), ACO2 (Aconitase 2), ARG2 (Arginase 2) and GOT2 (Glutamic-Oxaloacetic Transaminase 2), which are possibly integrated in IRE1-dependent metabolic reprogramming of mitochondria and play some other vital functions. The main goal of our study was to investigate IRE1-dependent mechanisms of the expression of nuclear genes encoding mitochondrial proteins in glioma cells for evaluation of their possible significance in the IRE1-mediated inhibition of glioma growth.

The expression level of ACADM, ACO2, ARG2, and GOT2 mRNAs as well as ACTB mRNA were measured in U87 glioma cells by quantitative polymerase chain reaction. In this work we used U87 glioma cells and their sublines with full (kinase and endoribonuclease) and partial (only endoribonuclease) inhibition of IRE1 signaling enzyme function, transfected by dominant-negative constructs dnIRE1 and dnrIRE1, respectively.

Inhibition of IRE1 signaling enzyme function down-regulates the expression of *ACADM*, *ACO2* and *GOT2* genes and increases the expression level of *ARG2* gene in comparison with the control glioma cells. It was shown that ablation of endoribonuclease function does not change the expression of *ACADM* gene, indicating the involvement of IRE1 protein kinase, but not IRE1 endoribonuclease activity in the IRE1-mediated regulation of this gene expression. At the same time, mechanisms of regulation of *ACO2, ARG2* and *GOT2* gene expressions are more complicated, because the expression level of these genes is significantly increased in glioma cells without endoribonuclease activity and down-regulated in glioma cells with total inhibition of the IRE1 enzyme function.

The results of our work demonstrate the presence of kinase- or endoribonuclease-mediated mechanisms of the IRE1-dependent regulation of the expression of nuclear genes encoded mitochondrial proteins related to metabolic reprogramming of mitochondria and it is shown that the expression of all studied genes is responsible to IRE1-mediated endoplasmic reticulum stress signaling in gene specific manner because IRE1 knockdown significantly affects their expression level.

CHIRALITY DURING PROTEIN BIOSYNTHESIS: THE ROLE OF HYDROXYL GROUPS OF tRNA^{TYR} IN D-TYR AMINOACYLATION AND PROOFREADING REACTIONS AGAINST ITS INVOLVEMENT IN TRANSLATION

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The biological world requires the homochirality of macromolecules. Chirality during protein biosynthesis is essential for cell survival and for the composition of natural proteins with cognate amino acids. The selectivity for L-amino acids during translation is based on the stereochemistry of RNA. Additionally, the catalytic site of the majority of aminoacyl-tRNA synthetases is also stereospecific. Tyrosyl-tRNA-synthetase (TyrRS) is known to esterify both enantiomers of Tyr (L- and D-Tyr). Together with cysteinyl-tRNA-synthetase (CysRS) they are nonspecific to the acylating position of tRNA (2' or 3'-position of hydroxyl groups of A76 moiety), binding L-amino acids to the 2' and 3'-OH of tRNAs. To overcome the lack of TyrRS editing domain, an additional enzyme, D-aminoacyl-tRNA-deacylase (DTD), exists. DTD is the *trans*-editing factor, which possesses as a stereospecific checkpoint during protein biosynthesis. It hydrolyses the ester bond between noncognate D-amino acid and tRNA.

However, the involvement of hydroxyl groups of tRNA^{Tyr} in D-Tyr attachment by TyrRS and further hydrolysis of D-Tyr-tRNA^{Tyr} misacylated complexes by DTD remained unknown. In our research, we focus on the investigating of the primary sites of L- and D-Tyr binding to tRNA^{Tyr} from *Thermus thermophilus* and hydrolysis the substrates with D-Tyr using *in vitro* assays. [^{32}P]-labelled substrates of wild type tRNA^{Tyr} and its 2' and 3'-deoxyA76 derivatives were used in kinetics experiments. The catalytic data for D-Tyr reveal in the 467-fold reduction of aminoacylation activity of 2'd A76 and a 6-fold increase in activity of 3'd A76 tRNA^{Tyr} comparing to the A76 tRNA^{Tyr} with both hydroxyl groups. Therefore, D-Tyr primarily binds to the 2'-OH. Neither 2'd- no 3'd-D-Tyr-tRNA^{Tyr} have been hydrolysed even by 10 μM DTD. Markedly, the transacylation from 2'-OH to 3'-OH is essential for this hydrolysis and the presence of free 2'-OH group. Thus, deacylation assays of misacylated D-Tyr-tRNA^{Tyr} resulted in the 2'-OH-assisted hydrolysis from 3'-OH moiety. Concluding this, the 2'-OH of tRNA^{Tyr} is the initial site of D-Tyr misaminoacylation and it also assists in deacylation during hydrolysis reaction.

THE MOLECULAR CONSTRUCTOR PROGRAM BUILDS STRUCTURAL TEMPLATES OF PROTEINS ACCORDING TO THEIR DETERMINING NUCLEOTIDE SEQUENCES

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The Molecular Constructor (MC) program was created to construct an individual structural template for any protein with an undefined experimental structure and no homology on the basis of information contained in the determining its nucleotide sequence of mRNA. Until now, the idea of coding the information about the spatial structure of proteins in the genome has not been sufficiently substantiated. This is due to the categorical nature of the known postulate that only the amino acid sequences of proteins are encoded in the genome. At the same time from the concept of the primary structure of the protein in the form of the amino acid sequence, the characteristic of the peptide bonds between the amino acid residues involuntarily eludes. This means that they are identical, but it is not so. A hypothesis was proposed that the information of the third nucleotide of codons should be re-coded into the corresponding rotamer of the peptide bond directly by the most three-dimensional structure of isoacceptor tRNAs. The concept of rotamers of the peptide bond as the main element of the protein structural template formed during matrix synthesis at ω = 120° (R-rotamer), -120° (L-rotamer) and 0° (0-rotamer) is introduced.

The conformers of the periodic secondary structure (right or left helix, β-sheet) in the protein are encoded by repeating the corresponding codons at the same nucleotides at their third position, which leads to the decoding of the patterns of rotamers of the peptide bounds: Rn, Ln or 0n. For example: the sequence (GCC/G)n encodes the right spiral of polyalanine (Rn is the prototype of all α- and 3/10-helix), whereas (GCA)n – the left helix (Ln represented in turns), and (GCT)n – β-strand (0n).

Geometrical algorithm of the MS-program includes such components:

- library of atoms and amino acid residues in a Cartesian coordinate system;
- rotamers of peptide bond;
- table of the genetic code of the protein structural pattern;
- conditions for coding conformers of the secondary structure.

For structural template of protein, the MC-program calculates the coordinates of each atom, taking into account the covalent radii of the latter, the lengths and multiplicities of the bonds, and the angles between them. As a result, based on the decoded information of the file with the nucleotide sequence of mRNA (input file .dne), the MC produces an output file .pdb with the coordinates of all the atoms of the realized protein template and visualizes its 3D-structure.

HSP90α EXPRESSION IN THE SMALL INTESTINE AND HSP70 BLOOD SERUM LEVELS IN RATS WITH CARRAGEENAN-INDUCED ENTERITIS

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Introduction. Heat shock proteins (HSPs) are highly conserved molecular chaperones involved in protein folding and refolding. Two representatives of stress-inducible high-molecular-weight chaperones – HSP90 and HSP 70 – cooperate with each other to unfold misfolded and alternatively folded proteins. It has been reported that they are upregulated in inflammation. However, the role of the chaperones mentioned above is not fully elucidated in carrageenan-induced intestinal inflammation.
The aim of the study was to assess HSP90α expression in the small intestine and HSP70 levels in blood serum of rats with chronic carrageenan-induced enteritis.
Materials and Methods. Twenty female WAG rats were used in this study. They were randomly subdivided into two groups with ten animals in each. The rats from Group 1 were orally daily administered 1% κ-carrageenan solution. Carrageenans are sulfated polysaccharides used in food industry as thickeners and emulsifiers. However, they are known to induce inflammation. Carrageenan intake resulted in the development of enteritis confirmed morphologically. Control group consisted of intact healthy animals. Specimens of small intestine were collected to analyze HSP90α expression immunohistochemically using antibodies to HSP90α (Thermo Fisher Scientific, UK). HSP70 high sensitivity ELISA kit produced by Enzo Life Sciences (USA) was used to determine blood serum HSP70 levels. Mann-Whitney U test was used to compare two independent groups of variables.
Results. HSP90α was found to be overexpressed in the small intestine of rats with chronic carrageenan-induced enteritis. A lot of HSP90α-expressing cells covered intestinal villi in the animals from Group 1.
In addition, it was found that HSP70 serum levels were over 2.5-fold higher in the animals with enteritis compared with the control group. It is conceivable that overactivation of HSPs is compensatory for the intense oxidative protein modification due to the development of oxidative stress found by us in carrageenan-induced intestinal inflammation and overproduction of pro-inflammatory cytokines.
Conclusion. Chronic carrageenan-induced enteritis is associated with the HSP90α overexpression in the intestinal tissue against the background of elevated HSP70 concentrations in blood serum. HSP90α and HSP70 may be related to the development of the intestinal inflammation caused by carrageenan.

SITE-DIRECTED MUTAGENESIS OF TRYPTOPHAN RESIDUES IN THE STRUCTURE OF TYROSYL-tRNA SYNTHETASE *BOS TAURUS*

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Tyrosyl-tRNA synthetase (TyrRS, EC 6.1.1.1) is one of the key enzymes of protein biosynthesis in both pro- and eukaryotes. Bovine (*B. taurus*) cytoplasmic TyrRS is one of the best studied mammalian aminoacyl-tRNA synthetases. This enzyme forms a homodimer of two 59.2 kDa subunits, each of 528 amino acid (aa) residues. N- and C-terminal domains of the enzyme subunit are connected by a long disordered 17 aa linker. The NH2-terminal catalytic domain comprises a "minimal" 39 kDa TyrRS and has full catalytic activity *in vitro.* The C-terminal domain formed by aa residues Val363-Ser528 is 166 aa long and reveals the 52.7% identity to the mammalian cytokine endothelial monocyte activating polypeptide II (EMAPII), which activates monocytes and endothelial cells – an effect first discovered at cancerogenesis induced with chemicals.

The purpose of this work was the replacement of tryptophan residues in the structure of mini TyrRS at alanine with the aim to study the structural and functional properties of the enzyme by fluorescence spectroscopy technique.

The genetically engineered *E. coli* XL10Gold strain transformed with plasmid pET30a-39KYRS was used to obtain plasmid DNA. Site-directed mutagenesis of tryptophan codons, replacing them with codons of alanine in the nucleotide sequence of the cDNA of the catalytic module of aminoacyl-tRNA bovine synthetase cloned in the recombinant plasmid pET30a-39KYRS, was performed with the modified QuikChange PCR method with long primers. For sequencing, standard primers for vectors of the pET T7 series were used to confirm the planned mutations.

All the planned substitutions of tryptophan codons for alanine codons in the nucleotide sequence of the target enzyme were obtained using designed primers, Phusion polymerase and QuikChange mutagenesis method. The effectiveness of the mutagenesis was 100%.

The principle of sequential mutagenesis of amino acid residues in the structure of the catalytic module of tyrosyl-tRNA synthetase using the same primers used in the work can be used to obtain multiple single-type mutations in the structure of other proteins.

The authors express gratitude to: Corresponding Member of NAS of Ukraine, head of the Department of Protein Engineering and Bioinformatics, Dr. Sci., Professor Kornelyuk O. I.

OBTAINING THE LIRAGLUTIDE PEPTIDE IN THE UNIVERSAL EXPRESSION SYSTEM IN *ESCHERICHIA COLI* BACTERIAL CELLS

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Incretins are a group of hormones, that in healthy people account for 70% of insulin secretion during a meal. They have a beneficial effect on the body's sugar metabolism by, among others, increasing insulin secretion in the pancreas, decreasing glucagon secretion and increasing insulin sensitivity in the cells. Exenatide and liraglutide achieve their therapeutic effect through the structural similarity to GLP - 1; namely, they bind to the appropriate receptor and mimic the functions of the hormone. Other substances belong to inhibitors of the dipeptidyl peptidase IV enzyme (DPP-IV), which is a physiological GLP-1 inactivator contributing to its degradation. These include exenatide (Byetta), liraglutide (Victoza), sitagliptin (Januvia), saxagliptin (Onglyza) and vildagliptin (Galvus). Incretin drugs can be used with "traditional" antidiabetic medications as a form of combination therapy. However, high costs limit their use in the treatment of the type 2 diabetes.

The liraglutide gene sequence was designed based on the oligonucleotides with codon optimization adapted for expression in a bacterial host. The pT7RSNHULir and the pIBA-Lir expression vectors were obtained. Optimization of bacterial cultures of *E. coli* Bl21(DE3) transformed with the plasmid pT7RSNHULir and bacterial cells of *E. coli* IBA transformed with the plasmid pIBA-Lir was performed. The digestion of the Ub::Liraglutide protein with the UBPD2C protease analogue was achieved. The presence of peptides: ubiquitin and liraglutide was confirmed by MALDI-TOF/TOF mass spectrometry. The next step will be to develop an optimal method in pilot scale for purifying the incretin obtained.

POSTER SESSION VIII. PROTEOMICS

BRADYKININ B2 RECEPTOR AND DOPAMINE D2 RECEPTOR COOPERATIVELY REGULATE LEPTIN- AND INSULIN-DEPENDENT SIGNALING PATHWAYS IN HUMAN NEURON-LIKE CELLS

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Bradykinin receptor type 2 (B2R) and dopamine receptor type 2 (D2R) belong to the wide family of G protein-coupled receptors. Recently, structural interactions between B2R and D2R have been demonstrated, involving modification of typical signalling pathways of these receptors, such as Ca^{2+} release and cAMP production. B2R and D2R are involved in pathologies associated with dysregulation of energy homeostasis. In the brain, leptin and insulin are major signals that regulate energy homoeostasis and body adiposity. This study aimed to evaluate the mediation of B2R and D2R in the regulation of leptin and insulin signalling in neuronal cells.

Differentiated human neuroblastoma cell line (SH-5YSY) endogenously expressed B2R and D2R. The treatment of cells with leptin or insulin induced changes in B2R and D2R expression. In turn, bradykinin, a B2R agonist caused an enhanced expression of leptin and insulin receptors. Contrarily, sumanirole, a selective D2R agonist did not affect the expression of insulin and leptin receptors. A co-stimulation of differentiated cells with receptor agonists altered Ca^{2+} release compared with cells stimulated separately with these agonists. A modified activation of selected transcription factors (STAT3, MAPK and Akt) was also observed. Since two D2R isoforms, short and long forms are constitutively expressed in neuronal cells, additional studies were performed to determine the roles of these isoforms in the observed effects. These experiments were carried out in HEK293 cells transfected with a plasmid that encodes these proteins and demonstrated different signalling in response to sumanirole action, suggesting a more accentuated role of the short isoform.

Our study demonstrated a cooperative action of B2R and D2R agonists in neuronal cells pretreated with leptin or insulin. It seems that sumanirole is able to attenuate the pro-inflammatory action of bradykinin through changes in signaling pathways responsible for leptin and insulin action in neuronal cells.These findings may contribute to a better understanding of the mechanisms that regulate food intake at the neuronal level in relation to inflammatory processes developed during diverse diseases, such as obesity or diabetes.

This work was supported by the grant No 35p/13/2016 funded by Leading National Research Center (KNOW).

EFFECT OF HB-EGF INTERACTION WITH HEPARAN SULFATE PROTEOGLYCANS ON GROWTH FACTOR INTERNALIZATION TO CYTOPLASM

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Heparin-binding EGF-like growth factor (HB-EGF) is a member of epidermal growth factor family. HB-EGF promotes keratinocytes and fibroblasts proliferation and migration, which is critical for skin epithelialization during wound healing. Binding between HB-EGF and first (EGFR) or fourth (HER4) receptor class HER (human EGF receptor) types leads to the activation of the signaling pathways involved in regulation of gene expression, cell migration and proliferation.

The aim of the research was to study the role of the heparin-binding domain in the structure of human HB-EGF and it's influence on the growth factor's biological activity, namely the ability to form a stable complex with receptors and undergo further intracellular trafficking.

In this research we use fluorescently-labeled recombinant derivatives of full-length soluble sHB-EGF and its truncated form - sHB-EGFΔ84–106 - with deletion of the heparin-binding domain. Obtained results showed that the absence of heparin-binding activity of HB-EGF impairs its ability to promote the cells proliferation, but didn't affect the specific bindings with receptors and contributed to the high-affinity interactions. It was found that full-size form of HB EGF is characterized by a lower intensity of translocation to the cytoplasm in the ligand-receptor complex due to interaction with plasma membrane heparan sulfate proteoglycans (HSPGs). Also, we examined the trafficking route of full-size and mutant forms of sHB-EGF by comparing their localization to endosomal pathway markers associated with clathrin-dependent endocytosis. Using the confocal microscopy we investigate the colocalization of these proteins with Rab5a-labeled early endosome, as well as Rab7 and Rab11a markers of late and recycling endosomes, respectively.

Accordingly, we identified some patterns that shed light on the molecular mechanisms of sHB-EGF binding with EGFR and further internalization to cytoplasm and importance of interaction with HSPGs in these processes.

ICD-SENSITIVITY OF IRON (II) CLATHROCHELATES TO GLOBULAR PROTEINS

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Macrocyclic cage complexes iron (II) clathrochelates possess a wide range of bioactivities (inhibition T-7 RNA polymerase; cytotoxicity to cancer HL-60 cells; amyloid fibrilization suppression). It was discovered that clathrochelates bearing rigid phenyl-carboxy groups upon binding to bovine serum albumin/BSA induce strong specific circular dichroism (ICD) signal in visible spectral range (350-600 nm). The key role of the phenyl-carboxy substituents for the supramolecular binding of macrocyclic compound to protein and for the ability to give ICD response was reported. This ICD effect caused an interest in study of such cage complexes as potential chiroptical probes for protein sensing. Here we explored ability of the clathrochelates bearing binding-able carboxy- and hydroxy- groups on "flexible" alkyl linkage to induce ICD response in the presence of various globular proteins: BSA and human serum albumin/HSA, beta-lactoglobulin/BLG, lysozyme, trypsin and insulin.

Clathrochelates with alkyl-carboxy group(s) (**1**, **2**) give CD signals upon binding to albumins or BLG. Alkyl-carboxy substituted clathrochelates are able to discriminate albumins by the CD-signals intensity (3 mdeg/BSA and 25 mdeg/HSA for compound **1**) or by spectra shape (compound **2** gives bands with reversed signs, but close intensities - 8 mdeg/BSA, 10 mdeg/HSA). Also **1** induces the strong signal in presence of BLG (18 mdeg). Alkyl-carboxy terminated clathrochelates with lysozyme/trypsin/insulin had low-intensity CD signal (up to 2 mdeg).

The ICD-bands of the clathrochelates bearing alkyl-hydroxy group (**3**, **4**) in presence of studied proteins were insignificant (0.2-2.3 mdeg), that could point out weak protein-clathrochelate supramolecular binding.

Thus, the ability of clathrochelates with flexible alkyl-carboxy groups to give strong ICD-response upon binding to proteins was first shown. CD-sensitivity of these compounds to arrangement of the protein binding sites (here ability to discriminate BSA and HSA) makes them prospective scaffolds for development protein-sensitive chirooptical probes.

The project is funded by the EU Horizon 2020 research and innovation programme under Marie Skłodowska-Curie grant agreement No 778245.

N-ALKYL FUNCTIONALIZED STYRYL CYANINES FOR FLUORESCENT DETECTION OF NUCLEIC ACIDS

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Nucleic acids (NA) detection by fluorescent dyes is widely used for different biomedical applications from gel electrophoresis to cell organelles visualization. One of the common techniques is based on a fluorescent dye conversion from weakly emissive to strongly emissive state upon NA binding. Styryl-cyanines among the dyes are able to effective NA sensing.

Here we study the influence of the N-alkyl-substituents in benzothiazole styryl cyanine molecule on its properties as probe for detection of NA. For this purpose, dyes fluorescent sensitivity to NA in solution and at post-electrophoresis visualization were characterized together with their photostability.

In the unbound state, the styryl cyanines are weakly fluorescent (6-35 a.u.); their excitation/emission maxima are in the 524-544/591-601 nm ranges. The ability to increase the fluorescent signal ranges in the range 14-78 times for the dyes bearing different N-alkylaryl substituents (compounds **1-5**). N-alkyl substituent could determined preference of dye to NA type: the higher preference for RNA (53 times) compared with dsDNA (14 times) was possessed by the dye with N-alkylphenantroline group (compound **2**). The moderate quantum yields (up to 25 %) for the dyes in complex with dsDNA were obtained. The ability of studied styryl cyanines to visualize DNA fragments (50-1031 b.p.) after gel electrophoresis was shown; higher discernibility of DNA staining (at 2-4 ng/lane DNA concentrations) by the dye with N-alkyldipyridyl group (compound **3**) compared to commonly used ethidium bromide was observed.

The dye with alkyl-carboxy group (compound **6**) slightly increase (up to 2.8 times) its emission intensity in presence of NA (due to effect of negatively charged group). However, the presence of this group strongly increases the intrinsic photostability of the dye upon (470 nm) LED irradiation. At the same time incorporation of alkyl-aryl groups to the dye molecule results in decrease of its intrinsic photostability.

The structure of N-alkyl substituent could strongly affect the fluorescent sensitivity of the stryrylcyanine dye to NA and its preference dsDNA/RNA. Despite the long N-alkyl linkage between the terminal functional group and dye chromophore it is able to change the intrinsic photostability of the dyes.

TRANSLOCATION DOMAIN OF DIPHTHERIA TOXIN (DT) PARTICIPATES IN THE REALIZATION OF ANTITUMOR EFFECT OF NON-TOXIC RECOMBINANT DT DERIVATIVES

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CRM197 protein is a non-toxic diphtheria toxin (DT) point mutant that is used in anticancer therapy. This toxoid was suggested as a potent medication for receptor-targeted triple negative breast cancer therapy (Nam S.O., et al., 2016). However like the native DT, CRM197 has a high immunogenicity, causing side effects in patients. The aim of the present work was to evaluate the cytostatic effect of less immunogenic non-toxic recombinant derivatives of DT on triple negative breast cancer cells *in vitro.* For this purpose recombinant derivatives of DT – CRM197, SbB (subunit B) and Rd (receptor domain) have been produced in *E. coli* cells, purified by Ni-NTA agarose affinity chromatography, and their effect on the growth of individual colonies of human triple-negative breast cancer MDA–MB–231 cells was characterized by such parameters as average colony area, perimeter and circularity index.

According to the obtained results, CRM197 and SbB, whose molecules contain the translocation domain (Td), exhibited the same cytostatic effect against MDA–MB–231 cells, reducing the area and perimeter of individual colonies. Rd protein did not affect the last two parameters, which characterize the colonies size, but changed a form of colonies edge, as evidenced by an increase in circularity index.

Since antiproliferative effect of DT derivatives was observed only in the presence of Td in their molecules, it was supposed that this domain can be involved in realization of cytostatic effect probably due to its pore-forming activity in relation to lipid membranes (Donovan J.J., et al., 1981; Kagan B.L., et al., 1981). To explain proliferation slowdown the hypothesis was proposed that Td can disrupt the endocytosis process in cells by breaking down ionic balance between endosomal lumen and cytoplasm, which may result in a delay of endosome maturation and disorder of intracellular physiological conditions.

It was concluded that Rd and Td, unlike the catalytic domain of CRM197, play an important role in the implementation of cytostatic properties. Based on the obtained results we supposed that SbB, which comprise both Rd and Td, may be the least immunogenic structural DT fragment that can be used as functional analog of CRM197 and antitumor agent.

Financial support. The publication contains the results of studies conducted by Grant of the President of Ukraine for competitive projects (project number F66) from the State Fund for Fundamental Research. The project title: Study of Diphtheria Toxin Recombinant Fragments as Potential Biopharmaceuticals in Cancer Therapy, state registration number: 0116U006914, and by the Young scientists Premium from the Administration of Palladin Institute of Biochemistry of the NAS of Ukraine for the best scientific work, 2016.

ITSN1 IS A POTENTIAL PARTNER OF MICROTUBULE-ASSOCIATED PROTEIN TAU

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Microtubule-associated protein tau promotes polymerization of tubulin, regulates stability of microtubules and determines microtubule spacing. Under pathological conditions, tau aggregates into insoluble filaments, which is accompanied by synaptic dysfunction and neural cell death in a range of neurodegenerative disorders – tauopathies. Despite of its importance, pathogenesis of these disorders is still only poorly understood.

The aim of our study was to find new potential partners of tau, which would expand our understanding of the interactome of cytoskeletal proteins as well as define possible regulatory motifs affecting gene expression and translation of these proteins.

We hypothesized that intersectins (ITSNs) – an evolutionarily conservative family of SH3-domain containing scaffold proteins, involved in multiple processes and pathologies including clathrin-mediated endocytosis, actin cytoskeleton regulation and neurodegeneration – could be among such partners. Tau is known to interact with some SH3-containing proteins such as BIN1. Using ScanSite web server we predicted several interaction sites with ITSN1 SH3 domains. To confirm our suggestion, we overexpressed the recombinant GST-tau in *E. coli* and investigated the possibility of its interaction with endogenous short isoform of ITSN1 (ITSN1-S) in MCF7 cell line using the GST pull-down method. Obtained results showed the direct interaction between recombinant GST-tau and endogenous ITSN1-S. Moreover, we performed computational search of microRNAs predicted to co-regulate both ITSN1-S and tau. Using TargetScan web server, we found miR-34 (previously confirmed for tau by other authors) and miR-181 families as potential regulators of ITSN1-S and tau. According to pathway analysis, these microRNA families involved in Alzheimer's disease, regulation of cytoskeleton and neural transmission – processes common for both tau and ITSN1-S.

Taken together, these data suggest possible interaction and co-regulation of microtubule-associated protein tau and ITSN1-S.

FTO DIOXYGENASE STRUCTURE AND ITS INTERACTORS –- BIOPHYSICAL STUDIES

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AlkB is dioxygenase that repairs alkylated DNA and RNA containing 3-methylcytosine or 1-methyladenine by oxidative demethylation in *Escherichia coli*. The family of mammalian AlkB homologs counts nine members, i.e. ALKBH1–8 and FTO (fat mass- and obesity-associated protein). FTO protein consists of two domains. N-terminal domain responds for enzymatic activity and similar with other ALKBH proteins, while C-terminal domain function is poorly known. *In vitro* FTO protein expressed in *E. coli* is DNA/RNA demethylase with a strong preference for 3-methylthymidine (3-meT) in single-stranded DNA or 3-methyluracil (3-meU), but the role of this protein in whole metabolism is still subject of study. To evaluate a role of FTO, series of biophysics analysis was performed on protein expressed in eukaryotic system. Liquid chromatography coupled to tandem mass spectrometry (LC-MS-MS/MS) exhibits three phosphorylated serines in N-terminal domain of FTO which were absent in the protein expressed in *E. coli*. Usage of microscale thermophoresis technique (MST) shows ability of FTO to dimerization in presence Fe^{2+} and α-ketoglutarate. Moreover, low resolution structure of homodimer was obtained by Small-angle X-ray scattering (SAXS). Bioinformatics analysis shows potential place of interaction between C-terminal domain of FTO and Calmodulin (CaM) – the presence of signaling protein in all types of cell, which substrate specificity may change upon calcium binding. This state was proved by MST. FTO and CaM form strong complex in the presence of Ca^{2+}, Fe^{2+} and α-ketoglutarate (K_d = 7.6 nM). Further analysis with usage of Hydrogen-Deuterium Exchange technique shows significant changes of CaM conformation upon complex formation without serious changes in FTO structure. We postulate that, this interaction may be dependent on calcium metabolic pathways because changes in CaM take places in calcium binding sites.

IDENTIFICATION OF ISOZYMES OF MALATE DEHYDROGENASE ABLE TO BIND THIAMINE

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In protein extracts from synaptosomal membrane preparations, a thiamin-binding protein (TBP) with thiamine triphosphatase (TTPase) activity has previously been detected and partially purified by affinity sorbent (t-AS) and gel-filtration.

In an attempt to identify protein-carrier TTPase activity in the elution fractions with enriched TTPase activity, using MALDI-TOF-mass spectrometry (MS) proteins have been identified with no thiamine (or its homeostasis); among the most representative proteins there are malate dehydrogenase (MDH) and glutamate dehydrogenase (GDH). During the study it is found that the binding specificity of MDH and GDH molecules of thiamine and their affinity for thiamine show several isozymes/isoforms of MDH and, presumably, GDH. Other enzymes from a subclass of dehydrogenases were also detected. Our aim is to identify isozymes/isoforms of MDH that exhibit the ability to bind with thiamine: we used a commercial preparation of malate dehydrogenase from a heart (Sus scrofa, Reanal, Hungary), as the entire procedure of affinity chromatography for protein extract of brain tissue has been completely reproduced. When analyzing the protein elution profile from the t-AS column, several peaks of MDH-ase activity appear. The study uses several methods, including one-dimensional and two-dimensional electrophoresis, indicating the presence of several isoforms of MDH in the preparation. The results of MS studies also show that such proteins bind with thiamine as part of t-AS: putative malate dehydrogenase 1B; malate dehydrogenase, mitochondrial (36 kDa); malate dehydrogenase, mitochondrial isoform X1 (42 kDa), and a number of other proteins, incl. glyceraldehyde-3-phosphate dehydrogenase; 11-cis retinol dehydrogenase; dihydrolipoyl dehydrogenase, mitochondrial; L-lactate dehydrogenase A chain; L-lactate dehydrogenase C chain; mitochondrial 2-oxoglutarate/malate carrier protein.

The data allow us to conclude that thiamine or its biologically active (b.a.) derivatives bind to and affect NAD-dependent dehydrogenases. Preliminary results obtained *in vivo* show that NADP-dependent malate dehydrogenase decarboxylated (malic enzyme) thiamine has no effect; no previous study identified this activity for eluates from t-AS also. Likely, there is a binding site for thiamine or its b.a. derivatives which are located near the NAD-binding site, a result of which the regulatory action of thiamine/b.a. derivatives of thiamine is therefore possible. The mechanism of this kind of regulation requires further study.

DETECTION AND CHARACTERIZATION OF THE INTERACTION BETWEEN RNA-BINDING PROTEIN SAM68 AND SCAFFOLD PROTEIN ITSN1

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An increasing number of proteins are identified as multifunctional or moonlighting. Recently found nuclear-cytoplasmic shuttling of ITSN1 suggests that the protein might regulate maturation of transcripts associated with mitogenic and apoptotic signaling pathways where ITSN1 is involved. Several high-throughput screenings identified potential partners of ITSN1 among RNA-binding proteins (RBP) whereas the relevance of the interactions in a cell is unknown. The aim of the current work was to analyze the interactions between ITSN1 and several RNA-binding proteins.

As some RBPs contain proline-rich regions, the interactions between ITSN1 SH3 domains and these RBPs were analyzed. ITSN1 SH3 domains fused to GST-tag were used to precipitate overexpressed proteins WBP11, SAM68 and LARP6 from HEK293 lysates. It was shown that ITSN1 SH3 domains bind WBP11, SAM68 and LARP6. To confirm the interaction in cells a microtubule bench assay developed in the SABNP Laboratory (Evry University, France) was applied. According to the technique, a protein of interest fused to microtubule-associated domains of Tau is brought onto microtubules in living cells whereas the presence of a protein partner on microtubules reveals the interaction. For this purpose HeLa cells were co-transfected with constructions encoding proteins of interest fused to Tau-RFP and GFP and analyzed using fluorescent microscopy. Among three RBPs (WBP11, LARP6, and SAM68) the interaction was confirmed for ITSN1 and SAM68. The reciprocal analysis using recombinant proteins SAM68-RFP-Tau and GFP-ITSN1 revealed the binding between SAM68 and full-length ITSN1 or truncated forms containing SH3-domains. Proximity ligation assay confirmed that endogenous proteins ITSN1 and Sam68 are localized in close proximity in HeLa cells nuclei. The direct binding of two proteins was checked *in vitro* using purified recombinant proteins ITSN1SH3 and SAM68. As SAM68 is a partner and a substrate for SRC-kinase, using competitive binding assay, it was shown that ITSN1 SH3 domains and SRC SH3-domain do not compete for the binding to SAM68 but interact with different proline-rich regions in SAM68.

As a result, the current data obtained using *in vitro* and *in vivo* techniques strongly suggest that ITSN1 interacts with RNA-binding protein SAM68. It was shown that ITSN1, SRC, and SAM68 form a triple complex suggesting the potential role of the complex in signaling processes in cells cytoplasm and nucleus.

PLATELETS IN COORDINATION OF COAGULATION AND FIBRINOLYSIS

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Introduction. Platelets are crucial for hemostatic response; being key regulators of hemostatic balance they can stimulate two alternative processes: coagulation and fibrinolysis. Platelets have a potential to mediate clot formation through thrombin generation and fibrin polymerization as well as to promote subsequent clot lysis through enhancing plasmin production and its protection from blood inhibitors. In our previous survey on platelet rich plasma (PRP) we have shown that platelets can selectively regulate coagulation and fibrinolysis. Therefore, the aim of the further study was to elucidate the molecular mechanisms of platelet involvement in coordination of coagulation and fibrinolysis processes.
Methods. Coagulation and lysis of freshly obtained PRP were monitored by absorbance measurements at 405 nm using the clot waveform analysis assay for data assessment. Plasminogen binding and thrombin generation was estimated with specific chromogenic substrate assay. Flow cytometry analysis was performed to evaluate changes in structure of platelet membrane after activation with thrombin.
Results and Discussion. Previously we found that the increase in maximum absorbance during platelet-rich plasma clotting was changed by its subsequent rapid decrease due to clot dissolution. To prove the contribution of the plasminogen/plasmin system to spontaneous degradation of PRP-derived clot, PRP was preincubated with 5 and 10 mmol/l 6-aminohexanoate (AHA), a blocker of lysine-binding sites in plasminogen. AHA caused complete inhibition of spontaneous dissolution of the clot. Obviously, plasminogen binding to membrane surface was attenuated.
Resent researches prove the ability of some prothrombin complex (PTC) proteins to bind plasmin(ogen) on platelets. So, PTC can be one of the protein systems responsible for regulation of cooperation between coagulation and fibrinolysis.
By flow cytometry analysis we showed that under thrombin activation, a population of platelets with high level of phosphatidylserine signal was formed, that provide them procoagulant properties due to the binding and activation of prothrombin complex proteins. At the same time, the increasing of plasminogen association with the platelets surface (indicated with the usage of streptokinase) in the presence of PTC was observed.
Conclusions. Therefore, platelets can regulate local hemostatic balance by coordinating coagulation and fibrinolysis processes, making their rates comparable and therefore regulating the size and lifetime of clot, preventing thrombosis. Presumably, prothrombin complex can serve as an executive component of regulatory mechanism by which fibrin clot formation and subsequent lysis can be regulated by platelets.

CHANGES IN MATRIX METALLOPROTEINASE-9 SECRETION, PHAGOCYTES ACTIVATION, MATURATION AND APOPTOSIS, *IN VITRO,* IN RESPONSE TO *MYCOBACTERIUM TUBERCULOSIS COMPLEX* ANTIGENS MPT63 AND MPT83

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Knowledge about mechanisms of pathogenesis and development of tuberculosis (TB) is rapidly growing. Interactions of bacterial pathogenic factors with target molecules lead to particular effects in host. Thus, the investigation of features and functions of a number of tuberculosis bacilli antigens is a prerequisite for prevention and treatment of TB.

The goal of this study was to investigate the influence of *M. tuberculosis* antigens MPT63 and MPT83 on innate immune cells activation markers expression, phagocytosis efficiency and cells signaling pathways changes.

The enzymes of the family of matrix metalloproteinase (MMP) play an important role in many processes at norm and pathology, including granuloma formation, where tuberculosis bacilli could survive and reproduce. Long-term incubation of cells with MPT63 and MPT83 antigens (0.25 and 1.5 μM) has been shown to be accompanied by an increase in the gelatinase activity of enzymes in the culture medium of J774 cells. Moreover, no difference was found between MMP-9 increase of activity in the samples after treatment with 0.25 and 1.5 μM MPT63. However, an increase of gelatinase activity was observed only after incubation with 0.25 μM MPT83 not with 1.5 μM. The level of gelatinous activity of MMP-9 in the medium of intact cells without fetal bovine serum and stimulated with mCherry did not differ.

The secretory antigen of *M. tuberculosis* MPT63 contributed to the deactivation of NF-κB by reducing the levels of p-p65 subunit in J774 cells nuclei, starting from 20 min, which may result in inhibiting of transcription of proinflammatory factors and, as a result, promoting apoptotic effects in macrophages. The apoptotic effects of MPT63 and MPT83 on J774 cells were demonstrated in the experiment with the interaction of annexin V with a plasma membrane of cells after mycobacterial antigens trigger action.

Also, we demonstrate increasing of CD11b (50 and 30%) and F4/80 (19 and 17%) expression on peritoneal macrophages cells surface after treatment with 0.25 μM MPT63 and MPT83, respectively. A significant CD11b expression is associated with increased phagocytosis that was shown by methods of flow cytometry on peritoneal murine macrophages which have higher phagocytosis activity of calcium phosphate nanoparticles and EGFP expressed *E. coli.*

The ability of the recombinant proteins of *M. tuberculosis complex* MPT63 and MPT83 affect the processes of phagocytosis, activation and maturation of macrophages, apoptosis, and enhancing the activity of enzymes that remodulate the extracellular matrix suggests the important pathogenetic role of these antigens in the development of mycobacterial infection.

SEARCH OF NEW PLATELET-BINDING SITES WITHIN αC-REGIONS OF FIBRINOGEN

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Fibrin(ogen) αC-region (Aα220-610) is involved in fibrin polymerization and platelet aggregation interacting with GPIIbIIIa-receptors through RGD-residue (α572-574). The aim of the present work was to investigate the role of conformation and integrity of αC-regions in platelet aggregation.

Three proteases were isolated from cultural solution of *Bacillus thuringiensis* var. israelensis IMV B-7465, the crude venoms of *Agkistrodon halys halys* and *Echis multisquamatis* using several techniques including ion-exchange and size-exclusion chromatography. Aggregation of washed platelets was studied using aggregometer SOLAR-2110.

Identification of hydrolytic products of fibrinogen was performed by SDS-PAGE under reducing conditions with further immunoprobing using the monoclonal antibodies 1-5A (anti-Aα509-610) and II-5C (anti-Aα20-78) and MALDI-TOF analysis on Voyager-DE. We demonstrated that proteases from *A. halys halys* venom, culture media of *B. thuringiensis* and *E. multisquamatis* venom hydrolyzed fibrinogen splitting off Aα414-610, Aα505-610 and Bβ1-42, respectively, and forming desAα414-610, desAα505-610 and desBβ1-42 fibrinogens. It was demonstrated that platelet aggregation rate was not much affected in comparison to control meanings (45%) and was estimated as 25, 32 and 40% in the presence of desAα414-610, desAα505-610 and desBβ1-42 fibrinogen, correspondingly

Using unique proteases we obtained three forms of fibrinogen, differed by conformation and integrity of αC-regions. Two of studied forms of fibrinogen lost their C-terminal ends of different lengths (Aα505-610 or Aα414-610), αC-regions of the third form of fibrinogen-desBβ1-42 remained intact but dissociated from the core of a molecule and from each other. We demonstrated that the removal of Aα414-504 peptide much impaired platelet aggregation. Removing of peptide Aα505-610 only slightly decreased platelet aggregation. However, the rate of platelet aggregation was decreased 1.9 times when peptide Aα414-610 was removed.

Fibrinogen sequences located within peptide Aα414-504 may have platelet-binding sites or be important for stabilizing the structure of the fibrinogen molecule necessary for effective platelet aggregation.

THE ROLE OF FIBRINOGEN BβN-DOMAIN IN THE MIGRATION OF CANCER CELLS

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The BβN-domain is located in the N-terminal parts of both Bβ-chains of fibrinogen molecule. Its Bβ1-42 sequence is known as a multifunctional region interacting with heparin, haemostasis proteins and adhesive receptors of different cells including platelets. This led us to an assumption about possible involvement of this domain in the processes of cancer cell migration. Thus the objective of our study was to obtain the molecule lacking its BβN-domains (fibrinogen desBβ(1-42)$_2$) and to investigate its role in the process of fibrinogen-dependent cancer cell migration.

Fibrinogen desBβ(1-42)$_2$ was derived from human fibrinogen using the highly specific fibrinogenase from *Echis multisquamatis* venom and isolated by gel-filtration on Superdex G-75. The sequence of cleaved peptide was determined by MALDI-TOF analysis, integrity of fibrinogen Aα- and γ-chains was confirmed by SDS-PAGE. H1299 lung cancer cell line monolayers were wounded by scratching and incubated with native fibrinogen or fibrinogen desBβ(1-42)$_2$ in cell culture media for up to 24 h. The wound healing dynamics mediated by cell migration for both fibrinogen forms was analyzed.

It was demonstrated that fibrinogen desBβ(1-42)$_2$ being added to the culture media failed to maintain cell migration of H1299 cell: wound healing with desBβ(1-42)$_2$ was 50% slower then with native fibrinogen when negatively charged cell culture plates were used. Interestingly enough, there was no difference in cell migration on both forms of fibrinogen when cell culture plates with positively charged surface were used. As far as Bβ1-42 sequence is located in negatively charged region of fibrinogen molecule, it could be masked when positively charged plates were used for the adsorption.

Fibrinogen and fibrin BβN-domains contain sites of cell interactions and can be assumed as a target for inhibitors preventing cancer cell growth and metastasis.

ABSTRACTS WITHOUT PRESENTATION

THE NO-SYNTHASE ACTIVITY IN PATIENTS WITH ACUTE DESTRUCTIVE APPENDICITIS

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Objective. The regulation of inflammation and detection of pathognomonic signs of its transition into destructive forms are the urgent problems of biology. This is the base for the medical problem solution of differential diagnosis of phlegmonous form acute destructive appendicitis, which can be treated conservatively, with a gangrenous form requiring immediate surgical intervention. NO is one of the universal biochemical regulators of the immune response. The purpose of the work was to determine the NO-synthase (NOS) activity in patients with acute destructive appendicitis.

Materials and methods. Forty patients with acute phlegmonous appendicitis (APA), 20 patients with acute gangrenous appendicitis (AGA) and 30 healthy volunteers were screened. Determination of of NOS activity parameters was performed in erythrocyte hemolysates by express-method taking into account the activity of its inducible isoform (Sklyarov O.Ya., 2010), stable metabolites of nitric oxide – nitrate (NO_3^-) and nitrite (NO_2^-) serum level using the standard method with Gris reagent (Green L.C., 1982).

Results. It was found that in patients with APA the total NOS activity was 8.80 ± 0.12, in patients with AGA 10.2 ± 0.98, higher than control (6.67 ± 0.23) nmol NADPH/min/mg protein ($P < 0.05$). The activity of indusible NOS (iNOS) in patients with APA (4.68 ± 0.05) was twice as high and in patients with AGA it was 3.9 times higher (8.40 ± 0.23) compared to the control (2.11 ± 0.04) nmol NADPH/min/mg protein ($P < 0.05$). Thus, it was found that an increase in the total activity of NOS due to activation of iNOS is characteristic of destructive forms of acute appendicitis. The content of stable metabolites of nitric oxide in the blood of patients with APA was 1.8 times, with AGA – 2.3 times higher than in control (respectively, 6.84 ± 0.20, 8.90 ± 0.56 vs. 3.80 ± 0.15 mmol/l). The increase occurred due to the growth of nitrite (NO_2^-) and nitrate (NO_3^-) pools with the predominance of nitrites.

Conclusions. It was established that the activation of the NOS activity with the simultaneous predominance of nitrite content was more significant in the case of the acute gangrenous appendicitis in comparison to the phlegmonous form, indicating the presence of nitric oxide in the pathogenesis of gangrenous inflammation. Determination of NOS activity can be used for differential diagnosis of destructive forms of inflammation.

BIOORGANIC MOLECULAR COMPOSITION OF FISH AS A NUTRITION PRODUCT FOR HUMAN

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Fish is a commercial product with the important nutritional value for humans. As a part of themselves bioorganic composition, the fish muscles have proteins and amino acids that are necessary for normal human life. The main biochemical indicators of the fish general state are the contents of proteins, fats and carbohydrates in its tissues. Analysis of these parameters allows estimating the state of fish stocks in natural reservoirs under the conditions of anthropogenic transformation impact on aquatic ecosystems.

The bioorganic composition of muscles was studied in the most representative commercial fish species, as follows: pike-perch (*Sander lucioperca*, L.), european perch (*Perca fluviatis*, L.), freshwater bream (*Abramis brama*, L.), and roach (*Rutilus rutilus*, L.) at the Zaporizke Reservoir (Ukraine). The fish samples were picked up at non-contaminated lower section of the reservoir, and at the Samara Bay polluted with toxic wastes.

Obtained data resulted in reduced protein contents in muscles of the fish from the Zaporizke Reservoir in comparison with the averaged data of other authors. It may indicate weakening of metabolic processes in fish, reduced physiological and functional capabilities, which can lead to decreased immunity, and as a consequence, to increasing incidence and susceptibility to various types of infections. In the Samara Bay, the protein contents were decreased in the muscles of studied fish species, but the significant deviation by 14% was only in bream. The protein content in fish tissues depends on many factors, but is primarily determined by the level of feed base enriched.

The fish from the Samara Bay showed a significant increase of the content of total lipids in the muscles: roach – by 70%, freshwater bream – by 67%, perch – by 31% and pike-perch – by 75%. Probably, increased lipid level in the muscle tissue may indicate a metabolic abnormality due to adverse conditions that have arisen in the area.

In any living organism, including fish, substances that are easily mobilized for the production of energy are carbohydrates, and primarily glucose and a spare carbohydrate – glycogen. The commercial fish species from the Samara Bay showed predominant decreasing the glycogen contents. Thus, in the perch muscles, glycogen level was decreased by 20%, in pike-perch muscles – by 29%.

Therefore, it can be concluded that the anthropogenic pollution of the Samara Bay influences the metabolic processes of the ichthyofauna, and especially the carbohydrate metabolism as decreased glycogen content. Consequently, the carbohydrate metabolism is most sensitive to changes in the environment.

EFFECT OF CHONDROITIN SULFATE ON OXIDATIVE BALANCE IN BLOOD SERUM OF RATS WITH ACUTE HIND PAW INFLAMMATION

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Musculoskeletal disorders and diseases are the leading cause of disability in the world and account for more than one-half of all chronic conditions in people over 50 years of age in developed countries. The pathogenesis of most diseases of the joints is associated with inflammation development. It is known that inflammation processes, coupled with reactive oxygen species generation, also play an important role in musculoskeletal disease pathogenesis.

The purpose of this study was to determine the parameters of lipid and protein oxidation in rat serum during acute hind paw inflammation and with administration of chondroitin sulfate.

The experiments were carried out using white non-strain male rats with initial weight around 180-240 g. The first series modeled acute limb inflammation by single sub-planar administration of 0.1 ml of 1% carrageenan solution to the posterior right paw (equal volume of saline was used as the control). Previously, the animals received a therapeutic intramuscular dose of 3 mg/kg of the chondroprotector Drastop (the active ingredient – chondroitin sulfate) daily during 28 days. The control group of rats received equivalent doses of physiological solution. The blood serum from rats was collected 3 hours after the carrageenan injection.

It was shown that levels of lipid peroxidation products in blood serum of rats during acute hind paw inflammation were increased: diene conjugates – by 59%, TBA-active products – by 48%, Shiff bases – by 34% in comparison with the control group. We have also found changes of protein oxidative modification product level in rats with hind paw inflammation. Neutral aldehyde product level increased by 56%, neutral ketone products – by 62%, alkaline aldehyde products – by 49% if compared with the control group. Also the level of hydrogen peroxide was higher by 26% in rats with pathology. The administration of the chondroprotector was associated with normalization of researched parameters in blood serum of rats with acute hind paw inflammation (the carrageenan injection in the paw).

The results of our experiments show that acute local inflammation in hind paw of rats induces enhanced production of reactive oxygen species, which leads to the activation of lipid and protein peroxidation processes. The injection of the chondroprotector elicits the decrease of most of the researched values to the control levels, which confirms anti-inflammative properties of Drastop.

RESEARCH MOLECULAR MECHANISM OF CORRECTIVE ACTION OF NANOTUBULAR HALLOUZITE IN METABOLIC DISTURBANCES INDUCED BY CYSTIC FIBROSIS IN MICE (GENE delF508)

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The aim of the study was to study the effect of the corrective action of the nanotubular halloysite on the metabolic alkolysis of epithelial cells of the respiratory tract and blood cells in mice with the reproduced mutation of the delF508 gene. The study was carried out on laboratory mice of the experimental and control groups in the amount of 50 individuals in each with the delF508 mutation and the metabolic status - non-respiratory alkalosis. The diagnostic test was in the plasma of mice, the level of Ca^{2+} was 2.6 mmol/l, Cl^{-} - 86 mmol/l, Na^{2+} - 111 mmol/l, pH 7.45, hypokalemia, respiratory insufficiency. The animals of the experimental group with alkalosis received 0.5 ml of 2% nanocomposite halloysite per day for 5 days parenterally with a probe, which is a biocidal sorbent. After 5 days of application of halloysite nanocrystals, the animals improved. Using the ABL-4 analyzer, the parameters of the acid-base state of venous blood were evaluated. Blood sampling was made from the tip of the tail. In the blood samples, the pH, pO_2 (mmHg), pCO_2 (mmHg), actual bicarbonate (HCO_3, mM/l), total CO_2 (TCO_2, mM/l), actual excess bases (ABE, mM/l) and standard blood bicarbonate (SBC, mM/l). A decrease in the pH of the blood plasma to 7.35, an increase in the concentration of glycosaminoglycans, a potassium level increased and was 4 mmol/l, replenishment of electrolytes and H^{+} ions was observed. Statistical processing of the results was carried out according to Student's *t*-criterion using statistical software package "Statisica for Windows 4.0". Using the diffusion NMR spectroscopy (DOSY) method, the molecular process of ion transport and the sorption ability of the influence of nanoclustalloy galloisizite on membranes of epithelial cells in the control and experimental groups of animals were studied. We obtained measurements of the coefficient of self-diffusion of halloysite molecules through the cell membrane of epithelial cells. It was found that halloysite nanocrystals are preferentially sorbed from the near-cellular medium by CO_3 ions. During ion exchange, part of HCO_3 dissociates upon entry into the membrane, followed by the displacement of protons from it. It was found that the decrease in the pH of the blood is due to hydrogen ions of halloysite, which are formed upon the dissociation of some of the HCO_3 ions. Halloysite has a cation-exchange capacity (1.0-1.5 mg-eq/ml), anion exchange capacity (0.2-0.3 mg-eq/ml) and biocidal properties. Nanotubular halloysite consists of silicon-oxygen structures with included magnesium and iron atoms, which, by their structure and properties, ensure the capture of pCO_2. The conducted studies of the acid-base state made it possible to determine the existence of metabolic alkalosis in the blood of the mice of the experimental and control groups. The use of halloysite nanocrystals smooths the disturbances and promotes an increase in the reserve capacity of the organism (the power of the buffer systems). The effect of its influence was manifested in the most complete compensation of the shifts in the acid-alkaline system of the blood, the restoration of metabolic processes and ion exchange.

SHAOXING AND SHAN PARTRIDGE DUCK POLYMORPHISM BY MICROSATELLITE LOCI

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Microsatellite markers are now widely used for the detection and description of micropopulation processes occurring in the populations of domestic animals for the effects of various factors of breeding pressure.

That is why the purpose of our study was the microsatellite analysis of two populations of Shaoxing and Shanma breeds with 19 locuses was conducted.

The selection of birds for the study were carried out on a duck farms in Zhejiang Generation Biological Science and Technology Co., Ltd. and Zhuji Guowei Poultry Development Co, Ltd., and at the laboratory of the Jjejiang Academy of Sciences Institute.

We studied 339 ducks (240 ducks of population I (Shaoxing breed) and 99 ducks of population II (Shanma breed)).

In total of 19 investigated locus in the Shaoxing breed population, two locuses were monomorphic (SMO10 and APL 83). In the Shan Partridge Duck population, only one locus was monomorphic (SMO10).

The number of different alleles (Na) for each polymorphic locus ranged from 2 (APL81, SMO12) to 20 (APL2) in population I and from 2 (APL82, APL81, APL83, SMO12, SMO13) to 17 (APL2) in population II. On the average, one locus had 7,632 alleles in population I and 6,000 of alleles in the population II.

The effective number of alleles (Ne) was 2,747 in Shanma and 2,805 in Shaoxing population, respectively.

The number of alleles and the expected heterozygosity (Hexp) values can provide important information for the discrimination of individuals and breeds. The index of expected heterozygosity ranged from 0.077 to 0.849 in population and from 0.010 to 0.824 in population II.

For each population was found private locus, in population I 12 loci and in population II 9 loci.

The results show high level of polymorphism of the studied populations of ducks. The obtained results can be used in the creation of new lines of ducks. Also these results will provide useful information for genetic diversity studies in ducks and for the development of duck traceability systems in the market.

THE EFFECT OF MELATONIN ON BIOCHEMICAL PARAMETERS IN THE RATS BLOOD PLASMA UNDER CONDITION OF COMBINED ADMINISTRATION OF ETHANOL AND CAFFEINE

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Alcoholism is one of the most serious medical and social problems in many countries. In a modern life ethanol consumption is often combined with the excessive consumption of caffeine.

The aim of the study was to determine changes of carbohydrate, lipid and protein metabolic parameters in the blood plasma of rats under condition of subacute ethanol intoxication combined with the administration of caffeine, and the possibility of their correction by melatonin.

Experiments were carried out on white sexually mature male rats of 180-230 g body weight, which were kept under the standard vivarium conditions. Subacute alcohol intoxication was induced by intragastric administration of 40% ethanol in a dose of 7 ml/kg of body weight daily during 7 days. Caffeine was administered contemporaneously with ethanol intragastrically in a dose of 30 mg/kg of body weight.

It was established that ethanol intoxication in combination with caffeine administration during 7 days resulted in a decrease in concentrations of glucose (by 33%), total protein (by 16%), urea (by 46%) in the blood plasma of rats, and an increase in the concentration of creatinine (by 47%) and total cholesterol (by 54%) compared with control group of animals.

The administration of melatonin in a dose of 5 mg/kg of body weight daily at 20^{00} during 7 days on the background of the combined injections of ethanol and caffeine was accompanied by the normalization of glucose and total protein in the blood plasma of rats. A decrease in urea concentration in the blood plasma under melatonin administration was less significant than in untreated with melatonin animals, but still remained below the control group by 14%. The administration of melatonin also prevented the increase in cholesterol and creatinine levels in the blood plasma of rats treated with both ethanol and caffeine.

The positive influence of melatonin on carbohydrate, protein, and lipid metabolism is likely due to its hepatoprotective effect related with antioxidant properties. By stabilizing cellular hepatocyte membranes, preventing oxidative modification of proteins, melatonin is able to avert hepatocyte cytolysis, normalize metabolic processes and mitigate the toxic influence of ethanol and caffeine on the liver.

SHAN PARTRIDGE AND SHAOXING DUCKS GERMLINE CHIMERAS' REPRODUCTIVE ABILITY

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Production of transgenic birds has a wide application in modern biotechnology. There are various methods of birds transgenesis and production of germline chimeras is one of the effective and often used method.

Low survival rate and weak reproductive ability of the produced chimeras are main disadvantages of this method. In order to produce germline chimeras, the embryos of the Shan partridge duck were used as recipients, and blastodermal embryos of Shaoxing fetuses were used as donors. Recipients were sterilized using ultraviolet light irradiation.

Application of this method produced ten germinate chimeras (6 males and 4 females) which were grown to puberty. Survival rate of recipient embryos following injection of donor blastomeres was 11.02% (13/118). The resulting chimeras were paired with the ducks of the Shaoxing breed.

Analysis of the phenotype of the obtained animals indicates the presence of signs of chimerism of different parts of the body according to the color of the plumage, as well as the coloring of claws.

Analysis of the phenotype of descendants of chimeras shows the transmission of signs of color of tail and claw color which are almost completely corresponded to the phenotype of donors. The eggs (n = 3493) were collected and incubated. In total, there were 890 descendants from chimeras, with 768 eggs that were inseminated by spleen (males) chimeras and 122 eggs from chimera females.

The fertility rate was 66.3% (2314/3493), hatchability was 55.0% (1920/3493), respectively. Analysis of genotypes at 23 microsatellite loci indicates that the level of chimerism of germ cells of germline chimeras was 73.01% (561/768) from male chimeras and 65.6% (80/122) from females. The descendants of animal chimeras 72.0% (641/890) carried microsatellite loci of donors.

The indicators of sperm production of male chimeras D63208, A62587, D63372, A61417, A61633, 0914 were within the range of 0.1 to 0.7 ml. A comparison of the sperm production of male chimeras with animal donors and recipients was characterized by a high concentration and speed of motility, which confirms the good quality of the sperm produced by germinate chimeras.

Thus, the obtained data indicates the positive outlook on usage of the Shan partridge and Shaoxing duck model for creating transgenic ducks.

FEATURES OF PROTEIN METABOLISM DURING INFLAMMATION UNDER TERMS OF TRANSDERMAL INJECTION OF GINGER EXTRACT

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In recent years, interest in plant medicines, which have a mild physiological effect, minimal toxicity, non-addictive, absence of immunosuppression, the possibility of prolonged use, has increased. One of the medicinal plants used in traditional medicine for many years in the treatment of various diseases is the ginger root, which contains a number of physiologically active substances.

The aim of the research was to study the biochemical changes in blood samples during the carrageenan-induced inflammation applying treatment using the ointment based on ginger extract.

The study was conducted on 20 males of white rats (2 groups of 10 animals each: 1st group – control (without treatment), 2nd group – application of 0.025% ointment with ginger extract 24 hours after phlogogen inoculation). Inflammation was caused by sub-planar injection of 0.1 ml of 1% carrageenan to the plantar fasciitis of the hind limb of rats. Indicators of protein metabolism (total protein and protein fraction ratio) were performed prior to the study and on days 1, 3, 6 and 8 of the experiment. All animal studies complied with the rules of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes.

According to the results of the research, it was found that phlogogen injection in all groups of animals within 1 day resulted in almost 2-fold hypoproteinaemia in comparison with initial numbers and contributed to a change in the albumin-globulin ratio (hypoalbuminaemia) on average by 35% while the level of some globulins of the so-called "proteins of acute phase" increased 2 times compared with normal physiological parameters.

According to literary sources, hypoproteinaemia in the conditions of carrageenan inflammation is associated with the release of proteins from the vascular channel into the inflammatory foci and exudate accumulation, thereby hypoalbuminaemia caused by the increase of the interleukin-1 level and the activation of the immunological process leading to the increased immunoglobulin production. Application of an ointment based on ginger extract stimulated a gradual, significant increase in total protein, and on the 8th day of the experiment, the return of protein content and albumin-globulin ratio to background values was observed. Whereas the partial restoration of protein metabolism was observed only on the 14th day of the study in the control group.

BENEFITS OF FENUGREEK BASED BIONANOCOMPOSITE TREATMENT ON PLASMA BRANCHED CHAIN AMINO ACIDS PROFILE IN OBESE RATS

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Amino acid profiles of valine, leucine and isoleucine from rat serum were investigated. Total amount of valine in Control (C) group was 12.5 and 20.3 μg/ml in group with experimental obesity (HCD group), caused high calorie diet intake. Valine level increased 1.6 times. Bionanocomposite (BNC) treatment decreased serum level of valine in groups C_BNC to 12.1 and 14.6 mkg/ml in HCD_BNC that means 1.4 times lower to HCD group, respectively.

Similar results were detected in the levels of other BCAAs such as leucine and isoleucine. The levels of leucine increased 1.7 times in HCD group compared to Control, from 13.5 to 22.4 μg/ml. Bionanocomposite reduced serum level of leucine in 1.5 times, from 22.4 to 15 μg/ml.

The level of isoleucine in HCD group was 1.8 times higher compared to Control group, 15 to 8.5 μg/ml. At the same time we determined 1.6 times lower amount of this amino acid in HCD_BNC group comparing with HCD, from 15 to 9.4 μg/ml.

This BCAAs increase insulin secretion by Langerhans pancreatic cells and activate mTOR signalling pathways. Branched-chain amino acids and particularly leucine are suggested to regulate muscle protein synthesis by activation of the mTOR pathway and thereby to stimulate protein synthesis on the translational level. Amino acid content is important index in diagnostics of diseases, which positively correlated with the clinical, biochemical and hormonal parameters in the subjects with obesity and/or insulin resistance.

We have shown that high-calorie diet intake is able to increase the levels of BCAAs in rat serum. BNC treatment reduced serum levels of these amino acids, so we can say about positive effect of this treatment. Studies in this field can increase knowledge about the mechanisms of obesity prevention and metabolic syndrome related traits using herbal supplements such as the bionanocomposite.

MILK PHOSPHOLIPIDS IN THE CORRECTION OF FUNCTIONAL STATE OF THE LIVER UNDER THE TETRACYCLINE-INDUCED HEPATITIS

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Tetracycline is one of medical preparations with direct cytotoxic action on the liver. Thus it is widely used in pharmaceutical studies of therapeutic effectiveness of hepatoprotective preparations. The aim of the present work was to determine liver functional state and reparative properties of milk phospholipids under tetracycline induced hepatitis in rats. To achieve this, Wistar rats were administered 250 mg/kg of 4% tetracycline hydrochloride suspension once a day intragastrically. As the corrective therapy, 1% solution of BAS "FLP-MD" was administered in liposomal form based on milk phospholipids. Under modeled steatohepatitis, significant destructive changes were observed in the cell membranes of hepatocytes in experimental rats. It was confirmed by higher activity of transaminases (activity of AST increased 4 times, that of ALT 1.7 times) and the AST/ALT ratio was increased 2.4 times. The synthesis of clotting factors in livers of sick animals was inhibited. The content of fibrinogen in blood decreased by 44.2%, factor II (prothrombin) by 27.8%, Xa-factor by 27.9%, and protein C by 40.6%. The animals also had hypochromic anemia which develops under pronounced azotemia and bilirubinemia. The calcium-phosphor metabolism and hyperkalemia were observed. These changes can negatively affect trans-membrane transport processes. Milk phospholipids administration in the form of liposomal BAS "FLP-MD" to sick animals have a pronounced membranotropic and reparative effect on injured hepatocytes, restore metabolism, and prevent possible complications (anemia, coagulopathy, nephropathy, and osteopathy, parenchymatous jaundice and intoxication). According to the obtained results, the liposomal BAS "FLP-MD" based on milk phospholipids may be used in prophylactics and pharmaceutical correction of steatohepatitis of medical origin.

LARVAL TEMPERATURE EFFECTS ON THE DEVELOPMENTAL TIME AND LIFESPAN OF *DROSOPHILA MELANOGASTER*

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According to the developmental theory of aging, there is an assumed causal relationship between period of pre-adult development and the individual lifespan. Longevity of an organism may be 'developmentally programmed' by nutrition and other environmental factors during development. *Drosophila melanogaster* is widely used as a model object for prediction study of the developmental theory of aging. In the present study we examined the body weight, lifespan and expression levels of longevity-associated genes, such as *Hsp70, InR, Sir, dTOR* and *dFOXO*, in adult fruit flies, which were exposed to different temperatures.

A wild-type Oregon-R strain of D. melanogaster was used in the study. Flies were kept at different temperatures (20.0; 22.5; 25.0; 27.5 and 30.0 °C) during the larval stages of development. Analysis of gene expression was performed with RT-qPCR using SYBRgreen dye. The statistical significance of difference between biomarker parameters was determined by Kruskal-Wallis test and the one-way ANOVA followed by Tukey HSD post-hoc tests to account for multiple comparison.

The imagoes development period increased 1.7 times when the developmental temperature decreased from 27.5 to 20.0 °C. The maximum weight of imago was found in flies that were kept at 22.5 °C. When the environmental temperature increased from 22.5 to 30.0 °C, the weight of flies decreased (male – by 42%, female – by 36%). The highest mean and maximum lifespans of the flies were observed at 22.5 °C and was significantly decreased at other developmental temperatures compared to control (25.0°C), $P < 0.05$. In male flies, the developmental temperature significantly affected *InR* expression level ($P = 0.04$), while in females changes in genes expression were observed for *Hsp70* ($P = 0.003$), *InR* ($P = 0.003$), *Sir* ($P = 0.003$), *dTOR* ($P = 0.006$) and *dFOXO* ($P = 0.007$).

The obtained data provides additional evidence of a significant effect of developmental temperature on the lifespan of flies and highlights its influence on the expression levels of longevity-associated genes.

PRESYNAPTIC DYSFUNCTION UNDER EXPERIMENTAL VITAMIN D_3 DEFICIENCY: IMPAIRED EXOCYTOTIC NEUROTRANSMITTER RELEASE AND INTRACELLULAR Ca^{2+} HOMEOSTASIS

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Vitamin D_3 being as an important neurosteroid regulates the expression and functioning of a range of brain-specific proteins required for a delicate balance between neurotransmitters uptake and released. Prolonged vitamin D_3 deficiency (VDD) leads to reduced expression of a number of genes involved in neurotransmission, neuroplasticity and neuroprotection. According to a range of experimental research vitamin D_3 deficiency is implicated in delayed neurological impairments (multiple sclerosis, schizophrenia, mental retardation).

Detailed analysis of neurosecretion at the cell level has not been provided earlier for vitamin-D-deficiency (VDD). Earlier we revealed then VDD in rats is associated with elevated level of cholesterol in synaptic vesicles and plasma membrane. Our aim was to evaluate the changes in excitatory neurotransmission and stimulated release of major inhibitory neurotransmitter gamma-aminobutyric acid (GABA) under VDD and after treatment with vitamin D3 (VDD+D3).

Unstimulated (tonic) release of glutamate from nerve terminals was increased in VDD rats. Extracellular glutamate concentration was also higher (3.10 ± 0.27 vs 5.00 ± 0.23 nmol/mg of protein) under VDD (n = 10, $P \leq 0.05$) and partially restored to control value after treatment with vitamin D_3 (4.40 ± 0.47 nmol/mg of protein). Unstimulated [^{3}H]GABA release from VDD synaptosomes was increased in comparison with both control and VDD+D_3 whereas the stimulated GABA release was attenuated by 24%. Generation of reactive oxygen species (ROS) in presynapse was intensified and free intracellular [Ca^{2+}] in presynaptic nerve terminals tended to be higher (57.3 ± 56.1 nM vs 78.2 ± 5.3 nM, n = 8, $P \leq 0.05$) under VDD. Intensified ROS production and elevated free cytosolic [Ca^{2+}] under VDD may be the reason for high tonic release of both neurotransmitters. On the other hand, high impact of cholesterol on kinetic of membrane fusion (that we demonstrate previously) may underlie the decline in stimulated secretion. Data suggested that only GABA transport was restored with subsequent vitamin D_3 therapy.

Our data demonstrated that during postnatal development both excitatory and inhibitory neurotransmission are impaired under VVD. Potentially excitotoxic concentrations of glutamate in synaptic cleft and decreased stimulated GABA release under VDD suggests the predisposition for neuronal damage.

TRACKING THE ENDO- AND EXOCYTOSIS IN PRESYNAPSE AND QUANTITATIVE ANALYSIS OF NEURON-SPECIFIC PROTEINS

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Neurotransmitter release, a key aspect of interneuron signalling, is governed by the life cycle of synaptic vesicles that deliver neurotransmitters to the synaptic cleft. Neurotransmitters are actively accumulated or synthesized in presynaptic boutons, stored in synaptic vesicles (SVs) and released during exocytosis. Separate steps of exocytosis, i.e. SV docking, activation of the fusion machinery (priming) and finally Ca^{2+}-dependent membrane fusion can be studied in living neurons and in model systems.

The methods proposed in this study allow tracking endocytosis with carbon nanodots (CNDs), estimation of the functional state of isolated synaptic vesicles, their fusion competence in pre-existing clusters and the depolarization-induced secretion of CNDs. We applied the fluorescent CNDs as novel fluorescent markers of exo-/endocytosis and compared this approach with other techniques for characterization of SV turnover (FM1-43 destaining, pH-sensitive dye dynamics, flow cytometry etc.).

Synaptic plasma membranes were visualized by the fluorescent membranotropic probe R18. Based on spectrofluorimetric measurements we estimated the excitation and emission spectra of R18 and CNDs and used both probes in confocal imaging of isolated brain nerve terminals (synaptosomes).

As a wide range of transmembrane and soluble neuronal proteins are implicated in the exocytotic neurotransmitter release the fluorescence-activated cell sorting (FACS) can be applied for relatively fast and complex analysis of cell marker expression in different animal models.

After isolation of primary cells from the brain the populations of astrocytes, neurons, oligodendrocytes and microglia can be separately studied based on labeling with cell-specific markers.

Using the staining with anti-Tubulin beta III one can focus on the population of neurons, apply antibodies against the surface or intracellular protein of interest and quantitatively analyse the expression of SNARE-proteins, neurotransmitter receptors, transporters, adaptor proteins in neurons. In contrast to immunohistochemistry, being a quantitative technique, FACS allows detecting much smaller differences (as significant) in surface and intracellular protein expression between groups.

NON-INVASIVE DIAGNOSTICS OF UNFAVOURABLE COURSE OF COMMUNITY-ACQUIRED PNEUMONIA IN CHILDREN

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Objective: to determine a diagnostic role of markers and risk factors promoting unfavourable development of community-acquired pneumonia on the basis of biochemical analysis of pulmonary expiratory content.

Materials and Methods. Twenty six children with verified community-acquired pneumonia were examined on the basis of Pulmonological Department of the Regional Pediatric Clinical Hospital in Chernivtsi (Ukraine) by means of simple random sampling applying "case-control" method. They were treated in-patiently no longer than 12 days (I group), and 36 children were treated in-patiently for more than 2 weeks (II group). An average age of the representatives from I group was 8.60 ± 0.84 years including 53.8% of boys. An average age of patients from II group was 10.10 ± 0.79 years ($P > 0.05$), including 55.6% boys ($P > 0.05$). The groups did not differ considerably by the main clinical characteristics.

Results. The clinical groups were not found to differ much by their severity and form of pneumonia, although the patients from group II more often developed complication in the form of exudative pleurisy (8.3%, $P < 0.05$) while pulmonary complications were not available in group I. The children who required longer in-patient treatment due to unfavourable course of pneumonic process were found to have the signs of intensified protein oxidative modification in the expired air condensate. Thus, the content of aldehyde- and keto-derivatives of 2,4-dinitrophenylhydrasones of the main character in group I was in an average 30.0 ± 3.64 E 430 mmol/g of protein against 76.10 ± 8.64 E 430 mmol/g of protein, and of the neutral character – 3.80 ± 0.21 and 8.40 ± 0.84 E 370 mmol/g of protein respectively ($P < 0.05$). At the same time, the content of these products of protein oxidative modification can be used as a marker of an unfavourable course of pulmonary parenchyma inflammatory process and the risk of a long in-patient treatment. For example, the content of aldehyde- and keto-derivatives of 2,4-dinitrophenylhydrasones of the main character prevailing 55 E430 mmol/g of protein as a test concerning unfavourable long course of pneumonic process was characterized by specificity 90.9%, predicted value of a positive result 98.8%, post-testing probability of the result 90.7%. And odds ratio in this case was 8.0 (95%) with a relative risk of event 2.0.

An average content of nitrogen monoxide metabolites in the expired air condensate in group I was 38.30 ± 9.04 and 48.70 ± 6.88 μmol/l in group II ($P > 0.05$). And the content of nitrogen monoxide metabolites more than 38 μmol/l was indicative of the necessity for a long in-patient treatment of pneumonia: odds ratio - 6.9, relative risk of event 2.6; post-testing probability 65.6%, and sensitivity of the test 84.6%.

Conclusions. Therefore, in case of community-acquired pneumonia in children requiring in-patient treatment the content of products of protein oxidative modification and nitrogen monoxide metabolites in the pulmonary expired air increases that can be used as non-invasive diagnostics and detection of risk concerning unfavourbale course of infectious-inflammatory process in the alveolar tissue.

EFFECT OF THE ETHYL ESTER OF 4{(2-ETOXY-2-OXOETHYLIDEN-4-OXO-1-(4-DIFLUORMETOXYPHENYLTHIAZOLIDINE-2-ILIDEN]-HYDRAZONO}-1-METHYLPYRAZOLE-3-CARBONIC ACID ON THE CONTENT OF GLYCOGEN AND ACTIVITY OF GLYCOSE-6-PHOSPHATASE IN THE TISSUES OF RATS SUFFERING FROM THE DEXAMETHASONIC DIABETES

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There are about 250 mln people suffering from diabetes in the world. Some sensitizers such as thiazolidindiones can be involved in treatment of this sickness.

Aim. Investigation of effect of the ester of 4{(2-etoxy-2-oxoethyliden-4-oxo-1-(4-difluormetoxyphenylthiazolidine-2-iliden]hydrazono}-1-methylpyrazole-3-carbonic acid (RC) on the content of glycogen (GN) and activity of glycoso-6-phosphatse (G-6-PH-ase) in the tissues of rats suffering from the dexamethasonic diabetes (DD).

Experimental Methods. This research has been performed in compliance with Bioethical Expertise of Preclinical and Other Scientific Researches Conducted on Animals (Kyiv, 2006). An array of the male 18 month old non-linear white rats was divided into the following subgroups: 1) control (intact); 2) rats suffering from DD for the daily subcutaneous injections of 0.125 mg/kg of dexamethasone; 3) rats suffering from DD with additional oral intake of 0.021 mmole/kg of RC. All blood samples were taken from the tail vein before decapitation on the 14^{th} day of experiment and the level of the basal glycemia (BG) was determined by using OneTouchUltra (LifeScan, USA). A content of GN in the tissues was determined by Morris' method, while Swanson's method was employed to determine an activity of G-6-PH-ase. A software complex Statistica 10 by StatSoft Inc. was used to perform statistical analysis of the obtained data. Given these data, the use of Mann-Whitney test was considered sufficient for valid conclusions. Differences were considered to be statistically significant at $P \leq 0.05$.

Results. The level of BG in the DD group of rats was found to be twice higher than that in the control group; G-6-PH-ase activity in the liver and kidney tissues were by 53 and 44% correspondingly higher than those in the control group. The level of GN in the skeleton muscles of the rats with DD was by 48% lower while in their liver tissues it was by 38% lower than those for the control group. The level of BG for the rats taking RC on background of DD was only 25% above the control group level while a content of GN in the tissues and activity of G-6-PH-ase in kidney were approximately same for the experimental and control groups; activity of G-6-PH-ase in the liver tissues was by 15% higher than that in the control group.

Conclusion. It can be seen that RC facilitates decreasing of BG, promotes restoration of the content of GN and the activity of G-6-PH-ase in the tissues of rats suffering from DD.

SOME SPECIAL FEATURES OF PRO-/ANTIOXIDANT STATE OF THE RATS KIDNEY UNDER MERCURY CHLORIDE INTOXICATION

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Rats poisoning with mercury dichloride solution leads to destruction of the cell membrane and causes activation of free radical oxidation process of macromolecules. It activates antioxidant defense system of the animal organism, which is responsible for decontamination of active form of oxygen. Processes of antioxidant protection play an important role in pathogenesis of different diseases, because emergence of imbalance between activation of macromolecules' free radical oxidation and failure of antioxidant protection system can speed up the development of different pathological processes that are the basis of the renal diseases.

The processes of molecules peroxide oxidation in rats kidneys in case of sulema (mercurius corrosivus) nephropathy with salt loading were investigated on white male rats. The content changes of thiobarbiturate acid products and oxi-modified proteins in rats' kidneys in case of salt loading on sulema nephropathy background were found out. It was registered that salt loading of 3 or 0.75% led to content increase of TBA-RP in comparison with control in different kidneys layers. The effect of 0.1% sulema (mercurius corrosivus) solution in dose of 5 mg/kg of the body weight of animals caused increased indexes of free radical oxidation products, both lipids and proteins, under both types of salt loading relatively to control.

For instance, we found that 0.75% salt loading caused the increase of TBA-RP content indexes in comparison with the control by 63% in the renal cortex, 2 times in the renal medulla, and 2.5 times in the renal papilla in comparison with control.

We studied the changes of glutathione-S-transferase and catalase enzymes activity from the antioxidant protection system. We found the decrease of catalase activity in the renal medulla and papilla in case of salt loading after influence of mercury dichloride. However, animals' intoxication with sublimate caused glutathione-S-transferase activity increase in the renal cortex and medulla in comparison with control regardless of salt loading concentration. In conditions of sublimate intoxication and 0.75% salt loading the same index decreased for renal papilla in comparison with control values and did not change at 3% salt loading.

Therefore, rats' intoxication with mercury dichloride solution leads to the destruction of the cell membrane and causes activation of macromolecules' free radical oxidation process. In turn, this stimulates the antioxidant system of the animal's organism, which takes part in neutralization of oxygen active forms.

MODELING THE INFLUENCE OF ESTROGENS *IN VITRO* ON THE LEVEL OF REDUCED GLUTATHIONE IN ORGANS OF RATS OF DIFFERENT AGES

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Glutathione is one of the most powerful cellular non-azimic antioxidants that determines oxidative-reduction alarms; it is vital for the detoxification of xenobiotics, and it regulates cell proliferation, apoptosis, immune function and fibrogenesis. The effect of estrogen compounds may cause a decrease in the level of glutathione against the background of the increase in the content of active forms of oxygen as well as it can be a protector by increasing the concentration of thiol groups, which may be explained by the type of xenoestrogens and their doses.

Model experiments were carried out on the female rats of the Vistar line, which were given the food treated with the "Cinestrol" drug – a derivative of stilben, which according to the chemical structure, is different from steroid estrogen hormones (female sex hormones), but the biological and therapeutic properties close to them – in the amount of 2 mcg per 1 kg of mass. Food was exposed to exoestrogen during 45 days. At the beginning of the experiment, the age of the experimental animals was 3 months – in the prepubertal period (group II, $n = 6$) and 6 months – mature (group IV, $n = 6$). Control groups consisted of intact animals of the corresponding age (group I, $n = 6$ and III, $n = 6$). The study was conducted in accordance with the requirements of Directive 2010/63/EC on the protection of animals used for scientific purposes. The serum of blood, brain, liver and kidney of rats has been investigated. The state of antioxidant defense was determined by the level of reduced glutathione (Owens, Belcher, 1965). Statistical data processing was carried out using the Statistica 6.0 application package (StatSoft, USA). The difference between the comparable values was considered probable at $P < 0.05$.

The results of our research have shown the increase in the level of reduced glutathione in the liver of younger females by 8.7%, in older individuals – by 9.4%. In the brains of rats, in the pubertant period, the increase in content was 8.8 and 7.5% in adult males. In the kidney tissue of experimental rats, there was a tendency to increase the level of reduced glutathione. For blood serum of rats from group II, there was an increase in the content of reduced glutathione by 7.7% from group IV – by 8.5%. The increase in the content of reduced glutathione was found to be due to the increase of free SH groups in the reaction medium.

The obtained data indicate an antioxidant mechanism of estrogen protection that does not depend on receptor binding: estrogen effects at the cellular level are associated with intracellular signaling pathways and antioxidant enzymes.

RANKL-RANK-NF-κB SIGNALING PATHWAY OF THE BONE TISSUE IN TYPE 1 DIABETES AND AFTER VITAMIN D_3 ADMINISTRATION

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Type 1 diabetes (T1D) is a complex endocrine disorder, its development being accompanied by a number of side effects. While some of them are well-studied (nephro-, neuro-, hepatopathies), the mechanisms of diabetes-related osteoporosis are still poorly understood. T1D-induced secondary osteoporosis can be linked to disturbances of osteoblast-osteoclast balance and impaired functioning of the receptor activator of nuclear factor κB ligand (RANKL)-receptor activator of nuclear factor κB (RANK)-nuclear factor κB (NF-κB)-nuclear factor of activated T-cells c1 (NFATc1) pathway in bone tissue. Vitamin D_3 (cholecalciferol), which is an active modulator of bone resorption/formation, is considered as potential agent in the treatment of diabetes-induced osteoporosis. The study was aimed at characterizing diabetes-induced changes in signaling through RANKL-RANK-NF-κB-NFATc1 in the bone tissue in experimental T1D and to assess the efficacy of vitamin D_3 supplementation. Diabetes was induced by a single intraperitoneal injection of streptozotocine (STZ) in a dose of 55 mg/kg of rat b.w. After 2 weeks, diabetic animals were divided into two groups treated with or without D_3 (600 IU/kg b.w., *per os*) for 30 days. We showed a significant increase in the level of the RANK protein (1.34-fold, $P \leq 0.05$) in the bone tissue of diabetic animals compared vs. control. The increase in the RANK was accompanied by elevated levels of downstream transcription factors. We observed an elevation in protein synthesis of both total and phosphorylated (at Ser 311) NF-κB (1.4- and 2.3-fold, respectively, $P \leq 0.05$) in bone tissue of rats with T1D. There was also a significant 2.0-fold increase in the level of NFATc1 protein in diabetes vs. control. These data indicate a shift in the osteoblast-osteoclast balance towards osteoclastogenesis. In contrast, a reduction in the synthesis of osteocalcin (1.36-fold, $P \leq 0.05$), which is the marker of osteoblast-dependent bone formation, has been demonstrated, that suggests a disrupted functioning of osteoblasts leading to a decrease in osteosynthesis. No significant changes in the protein level of the RANKL were detected in diabetes. Abnormal osteoblast-osteoclast balance was accompanied by a significant decrease in the vitamin D_3 bioavailability in T1D animals, as was evidenced from 40% drop in the content of blood serum 25OHD as compared with control. Vitamin D_3 treatment led to the normalization of investigated parameters of diabetic rats. Thus, normalization of vitamin D status of the diabetic animals helps to restore osteoblast/osteoclast balance in bone tissue and prevent secondary osteoporosis development.

EFFECT OF NANOCERIA ON THE PROTEIN SYNTHESIS FUNCTION IN SALIVARY GLANDS UNDER MONOSODIUM GLUTAMATE-INDUCED OBESITY

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Obesity is associated with hyposalivation, development of xerostomia, increased viscosity of saliva. However, pathogenetic mechanisms of changes in salivary glands under the experimental obesity has not yet been completely elucidated and the ability to correct these changes with nanoceria.

The aim of the study was to investigate the influence of nanoceria on the activity of α-amylase and ornithine decarboxylase (ODC) in the tissues of salivary glands in monosodium glutamate (MSG)-induced obese rats.

The study was carried out on 48 rats of both genders. The animals were divided into four groups: I – intact control (4-month rats), group II – newborn rats subcutaneously in the volume of 4 mg/g MSG administered at 2, 4, 6, 8, 10 day of life, group III – intragastric administration of nanocrystalline cerium dioxide at a dose of 1 mg/kg volume of 2.9 ml/kg against the background of glutamate-induced obesity, the fourth group of animals treated with a solution of sodium citrate intragastrically in the volume of 2.9 ml/kg (solvent of nanocrystalline cerium). Introduction of nanocrystalline cerium dioxide solution starting from 4 weeks after birth (after weaning from the mother) and continued intermittently two-week course in 2 weeks.

The changes in a body weight were analyzed in rats of all groups during 4 months. Body mass index was calculated. Four-month-old animals were decapitated, removed and weighed visceral fat was measured. We determined α-amylase activity and ornithine decarboxylase activity in the homogenate of salivary glands.

We observed the development of visceral obesity in 4-month MSG rats ($P < 0.001$). We established the decrease of visceral obesity under intragastric administration of nanoceria against the background of MSG-induced obesity ($P < 0.05$). It was found a significant 1.06 times decreasing of the activity of α-amylase ($P < 0.05$) and significant 1.47 times decreasing of ODC activity ($P < 0.05$) in MSG-induced obese rats compared with the control group. Under the administration of nanoceria α-amylase activity was significantly increased by 1.09 times ($P < 0.05$) and ODC activity was significantly 1.45 times increased ($P < 0.05$) in salivary glands compared to the group of animals without correction.

Thus, nanocrystalline cerium dioxide administration improves the protein synthesis function in salivary glands under the MSG-induced obesity in rats.

TRANSGENE INTERFERON α2b DOWNREGULATED HUMAN MGMT PROTEIN *IN VITRO*

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Human repair protein MGMT defended cells from minor but very mutagenic and apoptotic DNA damage by O6-methylguanine. It restores DNA in rapid and irreversible suicidal reaction of transfer methyl adduct directly from O6-position of guanine to its own cysteine. But in cancer cells MGMT expression could lead to worse response to alkylating chemotherapy and even to resistance to this cure. So, MGMT status is important to choose the right therapeutic strategy that could include also immunotherapy by interferons. but we do not have much data about its influence on MGMT expression.

To investigate interferone the influence on MGMT expression we treated two cell lines, HEp-2 (Human epithelial type 2 cells, considered to originated from a human laryngeal carcinoma, HeLa contaminanted) and E8 (derived in our laboratory from embrio) by transgene interferon α2β (trINF) in concentration: 2000, 200, 20 and 2 IU/ml for HEp-2 and 200, 20 and 2 MO/mL for E8. Also we use mock control by treating cells with trINF diluent and intact control where cells were in the same conditions as in the experiment. Cells were collected and lysed, protein concentration measured using NanoDrop 2000c, for sds-acrylamide electrophoresis we loaded 100 ug of crude protein to each line. Separated protein were transferred on pvdf membrane in SemiDry blotter system. Western Blot performed using monoclonal anti-MGMT antibody (cat #NB100-168, MT 23.2, Novus Biologicals,), as loading control we used densitometry on pvdf membrane stained with Coomassie 350 G.

We observe MGMT downregulation on protein level by trINF in all range of analyzed concentrations in both cancer-originated HEp-2 and non-cancer E8 cells. In HEp-2 trINF lead to statistical significant downregulation in conc 2000, 2 IU/ml ($P < 0.0005$) and 20 IU/ml ($P < 0.05$). In the case of E8 cells we could speculate that trINF downregulated MGMT protein but statistically significant level of this downregulation we observed only in concentration of 200 IU/ml ($P < 0.05$). In other concentration trINF tended to decrease MGMT level but without statistical significance.

A NOVEL PLATELET AGONIST FROM SKIN SECRETIONS OF *BUFO BUFO*

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Skin secretions from many species of anurans (frogs and toads) are a rich source of peptides with broad-spectrum activities that may be developed into agents with therapeutic potential, particularly for topical applications. As far as our previous studies have shown that the components of crude skin secretions of *Bufo bufo* induce platelet aggregation, the aim of this study was the identification of platelet agonist from skin secretions of this toad.

Adult specimens of *B. bufo* (Kyiv region, Ukraine) were used in this research. Crude secretions were obtained by washing the skin with distilled water after mechanical stimulation of glands, than centrifuged to remove debris and lyophilized (Telstar LyoQuest). The 50 mg of lyophilized skin secretions was dissolved in 1 ml of 0.05 M Tris-HCl and centrifuged at 10 000 g for 5 min. The supernatant was diluted three times with buffer for application and applied to ion exchange column of DEAE cellulose. Chromatographic assays were performed using a system for liquid chromatography (BioRad, USA). The bound proteins were eluted by increasing salt gradient applying the buffer that contained 0.05 M Tris-HCl and 1 M NaCl (flow rate – 1 ml/min). Fractions were collected in 2.5 ml volume and the potential to induce platelet aggregation was tested for each of them. Platelet aggregation was measured in rabbit platelet-rich plasma ($2x10^5$ cells/μl) by aggregometer AT-02 (Medtech, Russia). Only one active fraction was indentified. It was then lyophilized, dissolved in 2 ml of upH2O and applied to the G200 Sephadex gel filtration column (flow rate – 1 ml/min). Samples were collected in 5 ml volume and the potential to induce platelet aggregation was tested. One fraction showed the ability to induce platelet aggregation in the dose-dependent manner. The sample of this fraction was lyophilically dried and subjected to SDS-PAGE (18% (w/v) separating gel). The presence of three protein zones ranging from 65 to 97 kDa was shown.

The two-stage chromatographic separation on the DEAE cellulose ion exchange column and Sephadex G200 allowed collecting a protein fraction, which are considered being active agonists of platelet aggregation. The elaboration of its mechanism of action are required in further investigations.

IN VITRO CULTIVATION AND REGENERATION EFFECT ON *CRAMBE ASPERA* BIOCHEMICAL PROPERTIES AND GENETIC STABILITY

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Crambe aspera M. Bieb. is a fodder crop, food and oil culture. *In vitro* techniques application result in a relatively high propagation coefficient even for the species with problematical *in situ* and *ex situ* reproduction. These methods provide a long-term plant species conservation with a possibility of thorough study, though *in vitro* cultivation may cause somaclonal variation and changes in plants biochemical properties.

For estimation of the impact on *in vitro* cultivation, plants regenerated from lateral buds from *in vitro* and *in vivo* cultures were used for antioxidant activity (AOA), polyfructan and total soluble protein content analysis. AOA was determined using DPPH (2.2-diphenyl-1-picrylhydrazyl) radical scavenging method. Total soluble protein content was measured applying Bradford method. Polyfructan content determination was based on ketosugars ability to color in the acidic environment with resorcinol. Moreover, for elimination the risk of somaclonal variations emergence polymerase chain reaction was used for genetic stability investigation of *C. aspera* plants with SSR (*gHGL218091, gHGL110933, gH032602*, Na10-F08) and ISSR (UBC827, UBC864, UBC890, A17898, B17899, IS-05) DNA marker systems.

There was no significant difference in plants protein content due to *in vitro* cultivation. AOA was higher in not aseptic *C. aspera* plants than in aseptic ones (88.07% for *in vivo* grown plants and 69.53% for *in vitro* grown, relatively to ascorbic acid AOA). Polyfructan content was also lower for *in vitro* grown plants samples (1.82 ± 1.04 mg/g) comparing to *in vivo* grown plants (15.38 ± 1.92 mg/g of fresh weight).

In vitro cultivation due to different factors (long-term cultivation, type of explants, indirect organogenesis, medium composition, stress etc.) could cause polyploidy, mutations and somaclonal variations. *C. aspera* genotypes from *in vitro* and *in vivo* conditions analyzed by means of preselected SSR and ISSR markers showed that genome didn't undergo considerable changes after *in vitro* establishment. Therefore, we can assume that the difference in the biochemical properties appeared as a response to specific environmental conditions and was not caused by genetic alterations.

SELECTIVITY STUDIES FOR ZEOLITE-BASED CONDUCTOMETRIC SENSOR FOR AMMONIUM DETERMINATION UNDER VARIED PARAMETERS OF WORKING BUFFER SOLUTION

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As a large group of naturally occurring aluminosilicates, zeolites have been recently shown to be prospective receptor probes for sensing of a variety of analytes. Being formed of the ordered crystalline three-dimensional network consisting of cage-like cavities and channels, zeolites can provide molecular sieve function and thus enable targeting with suitable selectivity. The analytically important properties of zeolites are derived from their intrinsic ion exchange capacity that makes it possible to use these affordable and chemically stable materials as host structures for development of advantageous analytical tools for a wide range of applications.

Natural zeolite clinoptilolite was used in this work as a recognition element of a conductometric sensor for ammonium detection. Selectivity of clinoptilolite toward NH_4^+ has been reported earlier and attributed to substitution of cations of a smaller ionic radius (Na^+) with cations of a larger ionic radius (NH_4^+) that is of higher thermodynamic profitability. In the sensor design, zeolite was immobilized on the surface of gold interdigitated electrodes by adhesion and separated from the environment with perfluorinated polymer Nafion®. The sensor performance was evaluated in phosphate buffer solution, CHES buffer, HEPES buffer and Tris-HCl buffer to study a dependence of the sensor selectivity and sensitivity on the presence of Na^+ in the environment. The sensor signals (within 0-25 mM ammonium concentration range), compared in differential mode of conductometric measurements and using electrochemical impedance spectroscopy, revealed significant effect of sodium in phosphate buffer solution on the sensor sensitivity to NH_4^+. Investigation of the ion exchange properties of clinoptilolite toward amine-containing compounds is planned for the next stages of the work to study the feasibility of zeolite co-immobilization with biologically active compounds to develop a series of biosensors for detection of other important analytes with high sensitivity and selectivity.

Developed here zeolite-based conductometric sensor is easy to fabricate, has high operational and storage stability and other satisfactory analytical parameters that makes it promising to use it for further application in real sample analysis.

A POSSIBLE MOLECULAR MECHANISM OF ANTIVIRAL ACTIVITY OF THE PLANT PREPARATION "ALTABOR"

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Natural products of plant origin are widely used in the traditional herbal medicine and considered as the sources for various pharmaceutical applications. Different classes of phenolic compounds such as ellagitannins have shown significant biological effects in animal and human studies like antioxidant, anti-inflammatory and anticancer actions. These properties of ellagitannins are associated with multi-target action. Several studies have been initiated to assess possible antiviral activities of ellagitannins. Altabor is the plant drug that was obtained by "Borschagivsky Chemical-Pharmaceutical Plant". The active substance of the product is dry extract of infructescences of grey and black alder. It consists of a mixture of oligomeric ellagitannins. According to an experimental study of the Altabor, the polyphenolic composition of the extract has antiviral properties against several viruses of influenza, vesicular stomatitis and herpes simplex. The mechanism of antiviral action of Altabor is the interferon synthesis induction, inhibition of influenza virus neuraminidase and herpes virus-specific thymidine kinase. The aim of our work was to investigate the Altabor substances DNA-binding ability and to study its direct effect on DNA and DNA-dependent RNA syntheses *in vitro.* The next task was to identify the mode of interaction of the main components of Altabor substances with enzymes by using computational approaches.

The investigated extract effect on transcription and replication processes in vitro was studied by DNA-dependent RNA polymerase system of bacteriophage T7 model and PCR.

It was shown that the ellagitannins complex of Altabor has no direct interaction with DNA, but can effectively inhibit both DNA and RNA syntheses in model systems. In spite of the fact that the extract contains a long list of constituents, the significant role for drug efficiency is related to 4 compounds – ellagitannins glutinoin, pedunculagin, praecoxin and davuriciin. Computation studies showed that each of ellagitannins efficiently occupies the active site of enzymes on a stage of promotor binding and transcription or replication initiation.

In conclusion, complex investigation of dry extract of grey and black alder fruits demonstrated the effective inhibition of DNA and RNA syntheses *in vitro.* Analyses of our results showed that the biological effect of ellagitannins can be explained by their synergistic action on several virus-specific DNA and RNA metabolic processes in the infected cells.

ACTIVITIES OF ALANINE AND ASPARTATE AMINOTRASFERASES IN THE BLOOD SERUM OF STERLET OF DIFFERENT AGE

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Ukrainian fishing industry possesses the important place in animal husbandry production. Because of intensification and growing scientific content in fishing industry fish-breeders should have not only practical skills but also deep fundamental knowledge in fish biology. The understanding of biochemical processes is necessary for the development of practical guidelines in the fishing industry. The age-depending dynamics of enzymes activity in sterlet organism is poorly studied that makes such studies perspective and important. Evaluation of the activity of different enzymes in fishes of different age is also interesting for studying the adaptation processes in sterlet organism. In this regard, the purpose of the work was to study the activity of some enzymes of the class of transferases in the blood of sterlet of different ages.

The object of the study was sterlet 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, that were kindly provided by the fish farm “Osetr” in Ukrainka village of Obukhivskii district of Kyiv region. All samples were collected at the beginning of May that allowed us to avoid the influence of the seasonal and oxygenation factors on enzyme activity.

Activities of aspartate aminotransferase (AST, EC 2.6.1.1) and alanine aminotransferase (ALT, EC 2.6.1.2) were determined by Reitman's and Frenkel's method using kits purchased from “Felicit”.

We found out the age-dependent changes in ALT and AST activity. ALT activity of 3-year-old and mature fish was higher than in the blood serum of 2-year-old fishes by 1.5 and 50.2%, respectively. The highest AST activity was shown for mature fish; it was higher by 42.6% compared with indices for 2-year-old fish. It should also be noted that the activity of ALT in the blood of sterlet is increased to a greater extent than the activity of AST. Obviously, this is due to the fact that AST is contained in the mitochondria of hepatocytes and in order to penetrate into the blood, it is necessary first to penetrate through the membrane of mitochondria, and then – through the cell membrane.

With age, in the blood serum of sterlet, an increase in the activity of enzymes of the class of transferases (alanine and aspartate aminotransferases) was observed, which, along with the characteristic changes in the individual development of fish, could be caused by both ecological and nutritional factors.

FEATURES OF BRONCHIAL ASTHMA PROGRESS IN CHILDREN UNDER DIFFERENT ACTIVITY OF N-ACETYLTRANSFERERASE

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Recent studies have considerably expanded the views on the mechanisms of bronchial asthma (BA) development. Given that the gene of N-acetyltransferase (NAT2) determines the polymorphism of the enzymes responsible for the biotransformation of xenobiotics, nowadays much attention is being paid to its study in connection with the increasing influence of the environmental factors on the formation of childhood asthma. NAT arylamine is a unique family of enzymes that are involved in biotransformation and detoxification of xenobiotics and play an important role in the pathogenesis of atopy and BA, since they regulate the metabolism of some endogenous substrates (serotonin, leukotriene E4, etc.) involved in the development of bronchospasm and airways' inflammation.

The aim of the study was to increase the effectiveness of asthma management in school-age children, taking into account the individual activity of NAT.

There has been conducted the examination of 118 children with BA. In all children there has been identified (by the method of V.M. Prebstyng and V.I. Gavrilova) such genetic marker as a type of acetylation, which are characterizing the particularities of the II phase of the xenobiotics' biotransformation system. Two clinical groups were formed: I group consisted of 68 children with slow acetylating type (SAT), and the second (II) group included 50 patients with fast acetylating type (FAT). These survey results were analyzed by the methods of clinical epidemiology, considering the odd ratio (OR) and relative (RR) risks of an implementation of event with estimation of their 95% confidence level (95% CI).

The results of the survey have shown that in the I group an intermittent, and persistent (mild, moderate and severe) course of asthma was noted, respectively, in 2.9 ± 1.1%, 4.5 ± 1.3%, 44.1 ± 6.0% and 48.5 ± 6.8% of patients. Among the representatives of the II group, the corresponding patients' distribution by disease severity was as follows: 14.0 ± 3.8% ($P < 0.05$), 4.0 ± 1.2%, 50.0 ± 7.0% and 32.0 ± 6.6% (in all cases $P > 0.05$). Thus, in patients with SAT there has been registered increased risk of the development of severe BA (RR = 1.5 [95% CI: 0.9-3.0], OR = 2.0 [95% CI: 1.1-4.2]) compared with children of II group. There has been shown that the presence of a SAT confers the possibility with a sufficient sensitivity (82.0%) and a negative predictive value (95,0%) of the result to predict the possibility of a severe asthma attack. At the same time, the predicting risk of occurring of severe asthma attack in patients with a SAT in comparison with asthma children with FAT has been characterized by: RR = 3.4 [95% CI:2.4-4.8], OR = 3.9 [95% CI: 0.9-7.5].Thereafter, the presence of SAT in asthmatic children increases the chances of developing a severe asthma attack 3.9 times.

MACROELEMENT COMPOSITION OF RAT MYOCARDIAL TISSUES AND STRUCTURAL-FUNCTIONAL CHANGES OF HEART UNDER CONDITIONS OF ARTIFICIAL HYPOBYOSIS

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The study of the biochemical characteristics of homeostasis of an animal organism in conditions of hypobiosis, in particular, the macroelement composition of heart tissues, is important because they are obviously related to the structural and functional characteristics of this organ.

The studies were carried out on white, non-breeding male rats weighing 180-200 g, which were kept under standard vivarium conditions and divided into two groups: 1st – control (intact animals), 2nd – experimental (the state of hypobiosis). Each group had 7 rats. The content of macroelements of the heart tissues of the rats was determined using atomic emission spectrometry with inductively coupled plasma on the atomic emission spectrometer Iris Intrepid II XSP (Thermo Scientific, USA). Structural-functional changes of the heart of rats were observed by magnetic resonance imaging (on a scan of Achieva 1.5 T, Philips Medical Systems Nederland B.V.) and contrast computed tomography (Brilliance CT 64-sectional configuration, Philips Medical Systems, Cleveland, Inc., USA).

In the tissues of the heart of rats, under conditions of artificial hypobiosis, changes in the content of macroelements were observed: an increase in Potassium, Sodium, and Ferrum and a decrease in Calcium. The increase in Potassium and the decrease in Calcium, probably, explain the decrease in cardiac activity, which was confirmed by studies of structural and functional characteristics of this organ. The actions of hypobiotic factors revealed a significant narrowing of vessels of the heart of rats, as well as a 3-fold decrease in the velocity of the vascular bed compared with the control. There was a significant decrease in the total volume of the heart of animals of the 2nd group and its chambers, as well as a decrease (6 times) in the amplitude of cardiac contraction and contractile capacity of the heart muscle.

The revealed changes in the macroelement composition of the tissues of the heart of rats in the state of artificial hypobiosis and structural and functional characteristics of this organ can be indicative of the fact that under the conditions of hypoxia, hypercapnia, and hypothermia, the introduction of animals into a hypo-biological state may be one of the future promising methods of anesthesia and analgesia in the veterinary and medical practice.

ASSESSMENT OF THE IMPACT OF SELECTED HISTONE DEACETYLASE INHIBITORS ON THE EPITHELIAL MESENCHYMAL TRANSITION (EMT) IN BREAST CANCER CELL LINES

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Background: Histone deacetylase inhibitors (HDIs) are a group of targeted anticancer drugs which exhibit pro-apoptotic, cell-cycle arrest and anti-metastatic activity in many types of cancer cells, including breast carcinoma. Currently, four HDIs (vorinostat (SAHA), belinostat (PXD-101), romidepsin (FK-228), and panobinostat (LBH-589)) have been approved for T-cell lymphoma and multiple myeloma treatment. Although HDIs have been shown to effectively induce the suppression of migration and proliferation of breast cancer cells, the anti-metastatic mechanism of HDIs still remains poorly understood. Nevertheless, since both anti-invasive and pro-invasive properties have been described, there is some disputation about the effect of HDIs on invasion of breast cancer cells. EMT is a multifarious series of cellular and molecular alternations by which epithelial cells reduce their epithelial character and acquire a mesenchymal phenotype. During this process cells lose their cell-cell adhesion and polarity and transform to fibroblastoid cells with migratory and invasive abilities. EMT resulting in increased metastatic and invasive properties of neoplastic cells and drug resistance. However, the molecular mechanisms underlying this transition are poorly understood.
Methods: Expression of E and N cadherin at the mRNA and protein levels was evaluated using the qPCR technique, western blotting and immunocytochemistry using confocal microscopy, respectively. Assessment of inhibition of migration after HDIs treatment was made by the xCELLigence RTCA impedance monitoring.
Results: Our study revealed that two histone deacetylase inhibitors - valproic acid (VPA) and vorinostat (SAHA) can significantly increase the mRNA and protein expression of E cadherin (epithelial marker) in T47D, MCF-7 and MDA-MB-231 breast cancer cell lines, suggesting that VPA and SAHA can inhibit the EMT process in these cells. Interestingly, in MDA-MB-468 cells, we observed that the expression of N cadherin was upregulated after VPA and SAHA treatment. Moreover, VPA and SAHA regimen resulted in morphological changes and inhibition of migration in all analyzed breast cancer cells in a time-dependent manner.
Conclusions: VPA and SAHA could serve as promising agents in the personalized therapy of breast cancer. Identification of molecular targets may provide a novel potential opportunity to improve therapeutic strategies of breast carcinoma.

ATTENUATION OF LYSYL OXIDASE HYPERACTIVITY IN RATS' BLEOMYCIN-INDUCED LUNG FIBROSIS

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The diminishing of Lysyl oxidase (LOX) hyperactivity by semicarbazide containing preparations was investigated in Bleomycin-induced lung fibrosis rat's model.

The primary function of Lysyl oxidase is to oxidize amine substrates to reactive aldehydes, which results in the crosslinking of collagen and elastin. LOX expression is markedly elevated at lung fibrosis, liver cirrhosis, atherosclerosis, scleroderma, and desmoplastic tumors featured by prominent symptom of fibrosis. LOX blocking is a perspective therapeutic way to cure such disorders.

Bleomycin-induced lung fibrosis rat's model was used for the stimulation of LOX and other amine oxidases (semicarbazidesensitive amine oxidase (SSAO), diamine oxidase (DAO), polyamine oxidase (PAO)) activity in the lung tissue. Two preparations: sodium semicarbazyde (CEM), a well-known irreversible Cu- and quinone-containing amine oxidase inhibitor, and creatine monohydrate modified with hypochlorite (CrM) were applied to decrease this hyperactivity. There were 4 groups of Wistar rats: Group 1 control; Group 2 positive bleomycin (BLM) control; Group 3 BLM+ CEM, and Group 4 BLM+ CrM. All rats but the intact group were intratracheally injected once with BLM at a dose of 5 mg/kg in 0.4 ml of water. Group 3 and Group 4 received CEM in a dose of 0.005% or CrM in a dose of 2%, respectively, with drinking water during 2 months of the preparatory period and 2 weeks after BLM instillation. All rats were sacrificed at the 14^{th} day after BLM-stimulation. Histological studies have confirmed the formation of lung fibrosis, and amine oxidases activity was determined in lung tissues. Obtained data have showed the activity (μmol H_2O_2/min/mg of proteins) of LOX – (8.1 ± 0.6); SSAO – (5.4 ± 0.9); DAO – (3.5 ± 0.5); PAO – (6.6 ± 0.9) in the intact group. The development of lung fibrosis resulted in the increasing of these enzymes activity in the group 2 (BLM): LOX – 2.2 times; SSAO – 3 times; DAO – 1.4 times; PAO – 1.9 times. The application of two preparations has normalized these parameters. In the group 3 (BLM+CEM) and group 4 (BLM+CrM) the activity of these enzymes was reduced as compared to the group 2 (BLM): LOX – 2.3 and 1.5 times; SSAO – 3.2 and 2.8 times; DAO – 1.9 and 1.2 times, respectively. PAO, flavine-containing enzyme, was insensitive to preparations. Obtained results have proved the CEM and CrM efficiency in suppression of LOX and other Cu- and quinone-containing amine oxidases hyperactivity.

CRISPR-CAS9 AS PROMISING TECHNOLOGY TO REVERT CHROMOSOME TRANSLOCATION IN K562

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Chromosome rearrangements can serve as sourse for genetic variability, however, in mammalian organisms they often cause different pathological abnormalities. Such chromosome alterations produce loss of heterozygosity or fusion of genes leading to their deactivation or overexpression that influence different signaling pathways. Start of chromosomal rearrangements is initiated by two or more double-strand breaks (DSB) in the chromosomes of a cell. Unrepaired DSBs could be harmful and deadly lesion to a cell, so different pathways for efficient fixing of such DNA damage has been evolved. There are two main DSB repair mechanisms: homologous recombination (HR) and non-homologous end-joining (NHEJ). HR require a homologous template as a donor sequence for repair and is restricted to certain cell cycle stages. Whereas NHEJ directly ligates broken ends whether a homology template is present or not and is much more error prone than HR.

In our investigation of a hybrid oncogene BCR-ABL generated by the chromosomal rearrangement in chronic myeloid leukemia (CML), we are testing the possibility to revert chromosomal translocation t(9;22) (q34.1;q11.2) to the original state. CRISPR-Cas9 technology can be efficiently applied for induction of DNA DSBs at proper positions. As a model system we used K562 cell line derived from cells of a CML patient which contains oncogenic p210-type of BCR-ABL gene. We analyzed available NGS data for K562 and determined sequences of the breakpoints in Philadelphia chromosome and chromosome 9. The breakpoint sequences between bcr and abl parts on both chromosomes were selected as targets to design gRNAs for the specific action of Cas9. Using two different vectors that contain sequences encoding Cas9, gRNA and reporter genes, EGFP and mCherry, we plan to evaluate cell transfection efficiency and perform cell selection. Repair of the DSB may either occur by NHEJ or by HR, but it is known that NHEJ is more active in human cells. We suppose that our approach of targeted genome editing with CRISPR-Cas9 technology may become an attractive alternative to existing therapeutic strategies based on kinase inhibitors. However CRISPR-Cas9 has some limitations in terms of accurancy and specifity that should be considered and yet to be overcome.

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K

L

M

N

O

P

R

S

T

U

V

W

Y

Z