Institute of Molecular Biology and Genetics
NAS of Ukraine

ALL-UKRAINIAN CONFERENCE ON MOLECULAR AND CELL BIOLOGY WITH INTERNATIONAL PARTICIPATION

dedicated to the heroic struggle
of the Ukrainian people against russian invadors

June 15-17, 2022

CONFERENCE PROCEEDINGS

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24 th February 2022 Russia invaded Ukraine and this terrible war with destruction of civil infrastructure, including cultural, educational, and scientific objects interrupted the scientific work in our country. A lot of scientists were displaced within Ukraine or abroad. Our foreign colleagues immediately demonstrated great support and created a lot of opportunities for Ukrainian scientists in their countries. Despite this, most scientists stayed in Ukraine, some of them even in temporary occupied territories. Therefore, Young Scientist Council and in the Scientific Council of the Institute of Molecular Biology and Genetics NAS of Ukraine created the idea of All-Ukrainian conference with international participation with the aims to encourage Ukrainian scientists, to give the opportunity to colleagues from abroad to demonstrate their staunch support to Ukraine and to keep scientific process ongoing even on the background of the war.

The All-Ukrainian Conference on Molecular and Cell Biology with international participation was held as an online event on Zoom platform, from 15 th to 17 th of June 2022.

KEYNOTE SPEAKERS

Pernilla Wittung-Stafshede
Chalmers University of Technology, Gothenburg, Sweden

Cecilia Lanny Winata
International Institute of Molecular and Cell Biology, Warsaw, Poland

Petr Svoboda
Institute of Molecular Genetics ASCR, Prague, Czech Republic

Michał Komorowski
Institute of Fundamental Technological Research, Polish Academy of Sciences, Warsaw, Poland

Volodymyr Berest
V.N.Karazin Kharkiv National University, Kharkiv, Ukraine

Andrii Domanskyi
University of Helsinki, Finland,
Orion Pharma, Turku, Finland

Anton Nekrutenko
Penn State University, PA, USA

Andreas Ladurner
Ludwig-Maximilians-Universität Munich, Germany,
Eisbach Bio GmbH
Planegg/Martinsried, Germany

Jan Barciszewski
Adam Mickiewicz University,
Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland

Mikko Airavaara
University of Helsinki, Helsinki, Finland

And **Vitaly Kordium (*Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine) with* special *lecture***

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PROGRAM

of

All-Ukrainian Conference on Molecular and Cell Biology

with international participation,

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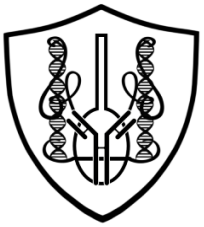

Wednesday, June 15		
09:00	**OPENING REMARKS**	
KEYNOTE LECTURES		
9:20 - 10:00	**Pernilla Wittung-Stafshede** ***Chalmers University of Technology, Gothenburg, Sweden***	***Protein Misfolding and Aggregation – from mechanism to disease with focus on a-synuclein in Parkinson's disease***
Coffee break		
10:10-10:50	**Cecilia Lanny Winata** ***International Institute of Molecular and Cell Biology, Warsaw, Poland***	***Constructing the gene regulatory network underlying heart development using genomics***
10:50-11:30	**Petr Svoboda** ***Institute of Molecular Genetics ASCR Prague, Czech Republic***	***Small RNA pathways in mammals***
Coffee break		
Microbiology and biotechnology		
11:45	**Daria Yarynka** **Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine**	***Highly selective chip-based fluorescent sensor systems for in-field analysis of mycotoxins***
12:00	**Iryna Bida** **D.K.Zabolotny Institute of Microbiology and Virology, NAS of Ukraine, Kyiv, Ukraine**	***The reduction of sulfate of gypsum by sulfidogenic microbiome for the precipitation of toxic metals***
12:15	**Natalia Borzova** **D.K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine, Kyiv, Ukraine**	***Cross-linked glycosidases aggregates for oligosaccharides and flavonoids hydrolysis***

12:30	**Larysa Macewicz** **Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine**	***Biotechnological dermal coatings for the regeneration of traumatic skin demages***
12:45	**Svitlana Dekina** **A.V.Bogatsky's Physico-Chemical Institute, NAS of Ukraine, Kyiv, Ukraine**	***A Novel Enzymatic Wound Burn Dressing Gel of Carbopol***
DINNER		
14:15	**Kseniia Berketa** **Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine**	***Development and optimization of the novel conductometric biosensor for determination of arginine in aqueous solutions***
14:30	**Yuliya Sklyarenko** **Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine**	***Investigation of biocompatibility and osteogenic potential of biomaterials based on hydroxyapatites in vitro***
14:45	**Kateryna Rudnieva** **Kyiv Regional Clinical Hospital, Kyiv, Ukraine**	***Molecular epidemiology of Carbapenemase-producing hospital-acquired Klebsiella pneumoniae in Ukraine***
15:00	**Ganna Kulichkova** **Institute of Food Biotechnology and Genomics, NAS of Ukraine, Kyiv, Ukraine**	***Biogas production in laboratory conditions.***
15:15	**Irena Hlushchuk** **University of Helsinki, Faculty of Pharmacy, Helsinki, Finland**	***Domain-Independent Inhibition of CBP/p300 Attenuates α-Synuclein Aggregation***
15:30	**Olena Moshynets** **Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine**	***Azithromycin reveals biofilm–inhibitory activity and potentiates non-bactericidal colistin methanesulfonate against Klebsiella pneumonia***
15:45	**Taras Baranovskyi** **Kyiv Regional Clinical Hospital, Kyiv, Ukraine**	***Azithromycin may potentiate bactericidal agents during M/PDR Klebsiella pneumoniae-associated noso-comial infections: case series***
Coffee break		

Genetics and epigenetics		
16:15	**Yelyzaveta Budakva** **Institute of Pig Breeding and Agricultural of Production, National**	***Determination of contamination of DNA samples using***

	Academy of Agrarian Sciences of Ukraine, Poltava, Ukraine	***mitochondrial DNA markers***
16:30	**Taras Oleksyk** **Oakland University (MI, USA) / Uzhhorod National University (Uzhhorod, Ukraine)**	***Genome Diversity In Ukraine: Introduction to unique variation, structure and admixture in whole genome sequences of Ukrainians***
16:45	**Anastasiia Satyr** **Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznan, Poland**	***Nanopore sequencing as an efficient method to study structural variation in Arabidopsis thaliana population***
Wine and poster session (part 1)		
17:00-18:00	**Mariia Zhytnikova** (Kharkiv, Ukraine) **Room1** ***The Protein-Nucleic Acid Structural Database with Information on Accessible Surface Area (ProtNA-ASA): updated version***	
	Vitalii Derkachov (Ivano-Frankivsk, Ukraine) **Room2** ***Ethanol attenuates toxic effects of arginine excess in Drosophila melanogaster***	
	Olga Brieieva (Kyiv, Ukraine) **Room3** ***DNA double strand breaks in acute myeloid leukemia blasts of different maturity***	
	Yelyzaveta Kulahina (Kyiv, Ukraine)**Room4** ***Study on ubiquitination of deletion mutants of MRPS18-2 in mammalian cells***	
	Valeriia, Shcherbina (Kyiv, Ukraine) **Room5** ***Expression pattern of MRPS18 family of genes in embryonal brain tumors***	
	Veronika Bakhmat (Kyiv, Ukraine) **Room6** ***Study of analytical characteristics of biosensor based on urease for silver (I) determination***	

Thursday, June 16		
KEYNOTE LECTURES		
9:00-9:40	**Michał Komorowski** ***Institute of Fundamental Technological Research Polish Academy of Sciences Warsaw, Poland***	***Making sense of signaling complexity***
9:40-10:20	**Andrii Domanskyi** ***University of Helsinki, senior researcher at the Orion Corporation Orion Pharma, Turku, Finland***	***Targeting pathological protein aggregation in neurodegeneration: dead end or a new hope?***
Coffee break		
10:35-11:15	**Volodymyr Berest** ***Department of Molecular and Medical Biophysics, V.N.Karazin Kharkiv National University, Kharkiv, Ukraine***	***Molecular interactions of the antimicrobial peptide with nano-sized delivery vehicles broaden its therapeutic efficiency***
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Molecular biology and bioorganic chemistry		
11:45	**Anastasiia Nefodova** **Educational and Scientific Centre «Institute of Biology and Medicine», Taras Shevchenko National University of Kyiv, Kyiv, Ukraine**	***Hematological markers of low-grade systemic inflammation in rats with different models of Alzheimer's disease.***
12:00	**Maksym Sobolevskyi** **Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine**	***Optimization of application of SPR biosensors for detection of oligonucleotide sequences of the Philadelphia chromosome***
12:15	**Volodymyr Prokopiuk** **Research Institute of Experimental and Clinical Medicine, Kharkiv National Medical University; Institute for Problems of Cryobiology and Cryomedicine, NAS of Ukraine, Kharkiv, Ukraine**	***GdVO4:Eu3+ nanoparticles affect proliferation of fibroblast culture in vitro***
12:30	**Anton Tkachenko** **Research Institute of Experimental and Clinical Medicine, Kharkiv National Medical University, Kharkiv, Ukraine**	***Erythrocytes don't internalize $LaVO_4:Eu^{3+}$ nanoparticles***
12:45	**Volodymyr Lushchak** **Vasyl Stefanyk Precarpathian National University,** **Ivano-Frankivsk, Ukraine**	***Posttraumatic stress disorder: biochemical aspects***

DINNER		
14:00	**Daryna Mruga** **Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine**	***Research of ALT-sensitive amperometric biosensor characteristics***
14:15	**Bohdana-Myroslava Briantseva** **Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine**	***Non-nucleoside MGMT inhibitors modulates anti-tumor effect of alkylating drug***
14:30	**Daria Biliai** **Yuri Fedkovych Chernivtsi National University, Institute of Biology, Chemistry and Bioresources, Chertnivtsi, Ukraine**	***5S rDNA intergenic spacer of Aconitum species: molecular organization and application for barcoding***
14:45	**Dmytro Gerasymchuk** **Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine**	***Endocytic proteins ITSN1/2 form a complex with Tau***
15:00	**Ausra Domanska** **University of Helsinki, Helsinki, Finland**	***Characterization of inhibitor-binding pocket in enteroviruses and it's role in genome release***
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Molecular oncology		
15:30	**Nadiia Lypova** **School of Medicine University of Louisville, Louisville, KY, USA**	***PFKFB3 as a target of compensatory cell signaling in response to EGFR inhibition in non-small cell lung carcinoma***
15:45	**Andrea Rasola** **University of Padova, Italy**	***The molecular chaperone TRAP1 in cancer: a master metabolic switch of tumor cells***
16:00	**Sergii Konovalenko** **RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine**	***The results of a study of the combined effects of doxorubicin and laser irradiation on the survival of MCF7 and MCF7-DOX culture cells***
16:15	**Anastasiia Hubiernatorova** **Institute of molecular biology and genetics, NAS of Ukraine, Kyiv, Ukraine**	***Tristetraprolin in cancer: treat or trick?***
Wine and poster session (part 2)		
16:30-18:00	**Olga Sideleva** (Hawaii, USA) **Room7** ***Leptin induced changes in gene expression, barrier integrity and cell morphology in human airway epithelial cells***	
	Ianina Pokholenko (Kyiv,Ukraine) **Room8** ***A biomimetic 3D model to reproduce wound-associated biofilm-related infectious process in vitro***	

	Mariia Usenko (Kyiv, Ukraine) **Room9** ***SPA–β-Lactamase protein as a secondary immunoreagent***
	Polina Pikus (Kyiv, Ukraine) **Room10** ***The therapeutic effect of mesenchymal stem cells derived from the human umbilical cord, using different methods of delivery to an animal model of liver cirrhosis***
	Olha Demkiv (Lviv, Ukraine) **Room11** ***Prussian blue nanocomposite coupled with carbon matrix as artificial peroxidase in glucose biosensor***
	Nataliia Shcherbak (Kyiv, Ukraine) **Room12** ***Genetically modified fodder crops expressing the Colicin M gene***
	Nataliia Liubas (Lviv, Ukraine) **Room13** ***The effect of thiosulfonates esters on the glutathione link of antioxidant protection in rats.***
	Pashynska V. A (Kharkiv, Ukraine) **Room14** ***Drug`s transmembrane transport facilitation by penetration enhancing agents: model study of intermolecularinteractions of dimethyl sulfoxide with the drug`s and membrane phospholipids molecules***

Friday, June 17		
KEYNOTE LECTURES		
9:00-9:40	**Anton Nekrutenko** **Penn State University** **PA, USA**	***GalaxyProject.org: An open global system for the analysis of biological information***
Coffee break		
Molecular physiology and biophysics		
9:50	**Mariia Ursatyi** **Yuriy Fedkovych Chernivtsi National University, Educational and scientific institute of Biology, Chemistry and Bioresources, Chernivtsi, Ukraine**	***Impact of dietary protein deficiency on the state of the glutathione system in the liver of rats of reproductive age under toxic injury with acetaminophen***
10:05	**Olga Tarnopolska** **Bogomoletz Institute of Physiology, NAS of Ukraine, Kyiv, Ukraine**	***The influence of trivalent metal ions on LCC-channels of the nuclear membrane of the cerebellar Purkinje neurons.***
10:20	**Viktor Martyniuk** **Educational and Scientific Centre «Institute of Biology and Medicine», Taras Shevchenko National University of Kyiv, Kyiv, Ukraine**	***Influence of electromagnetic radiation of millimeter range on the optical properties of the hemoglobin***
10:35	**Sonia Nevelchuk** **Bogomoletz Institute of Physiology, NAS of Ukraine, Kyiv, Ukraine**	***Interplay between local cell morphology and kinetics of hippocalcin calcium-dependent insertion***
Coffee break		
KEYNOTE LECTURES		
11:00-11:40	**Andreas Ladurner** ***Biomedical Center Munich, Department of Physiological Chemistry Ludwig-Maximilians-Universität, Munich, Germany; CSO and Co-Founder Eisbach Bio GmbH Planegg/Martinsried, Germany***	***PARPs, DNA damage and cancer treatment***
Molecular physiology and biophysics		
11:40	**Oleksandra Fedchenko** **Bogomoletz Institute of Physiology,**	***Calcium-dependent hippocalcin distribution***

	NAS of Ukraine, Kyiv Academic University, Kyiv, Ukraine	*between different subcellular compartments*
11:55	**Yuriy Danylovych** O.V.Palladin Institute of Biochemistry, NAS of Ukraine, Kyiv, Ukraine	*Properties of mitochondrial no-synthase activity in smooth muscle*
Coffee break		
Cell biology		
12:25	**Tetiana Bukreieva** Institute of Molecular Biology and Genetics, NAS of Ukraine, Kyiv, Ukraine	*T cell response is associated with cytokine and miRNA levels in patients with COVID-19*
12:40	**Serhii Beschasnyi** Kherson State University, Kherson, Ukraine	*Carbon monoxide and their donor (CORM-2) affect on skin wound healing through action on AQP3-channels*
12:55	**Yuriy Kolupaev** Yuriev Plant Production Institute, National Academy of Agrarian Sciences of Ukraine; State Biotechnological University, Kharkiv, Ukraine	*Participation of a signal molecule H2S in induction of wheat seedlings heat tolerance*
DINNER		
14:00 **PANEL DISCUSSION** **Viruses, evolution and the struggle for survival** **Human progress in diagnosis, therapy and prevention.**		
KEYNOTE LECTURES		
15:40-16:20	**Jan Barciszewski** *NanoBioMedical Centre at Adam Mickiewicz University,* *Institute of Bioorganic Chemistry, Polish Academy of Sciences,* *Poznan, Poland*	*RNA in the syringe*
16:20-17:00	**Mikko Airavaara** *Faculty of Pharmacy, University of Helsinki,* *Helsinki, Finland*	*Reporter pharmacology – new tools to develop drugs and quantify therapeutic efficacy*
17:00 **Vitaly Kordium,** *Institute of Molecular Biology and Genetics, Kyiv, Ukraine* ***SPECIAL LECTURE***		
18:00	**CONFERENCE CLOSING REMARKS**	

OPTIMIZATION OF APPLICATION OF SPR BIOSENSORS FOR DETECTION OF OLIGONUCLEOTIDE SEQUENCES OF THE PHILADELPHIA CHROMOSOME

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[2]*Department of Physics and Technological Bases of Sensory Materials, V.Ye.Lashkaryov Institute of Semiconductor Physics, NAS of Ukraine, Kyiv, Ukraine*

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Aim. The aim of this research was to evaluate the immobilization conditions of probe sequences and the effect of the sequence of target oligonucleotide on the efficiency of subsequent hybridization of immobilized oligonucleotides with target oligonucleotides.

Methods. Urea, KH_2PO_4, 6-mercapto-1-hexanol, SSC and citrate buffer were obtained from "Fluka" (Switzerland); all reagents were of analytical grade. All solutions were made with deionized MilliQ water. Single-stranded oligonucleotide probes, functionalized at the 5′-end with a thiol group connected by a hexamethylene linker (HS-$(CH_2)_6$-ssDNA), and oligonucleotide targets were obtained from Metabion International AG (Germany).To investigate the processes of oligonucleotide immobilization and hybridization, we used the two-channel SPR spectrometer "Plasmon SPR6" that was developed at the V.Ye.Lashkaryov Institute of Semiconductor Physics of the NAS of Ukraine. The buffers used during the immobilization of thiolated probes on the gold surface were 0.5 M KH_2PO_4 buffer and 0.02, 0.1 and 0.25 M citrate buffer. For hybridization of target oligonucleotides with immobilized probes, the target sequence solution of various concentrations in the 2×SSC buffer solution is injected into the measuring flow cell and exposed for 10 min.

Results. To study selectivity of the developed biosensors, the following target DNA sequences were used: P1, which consists of 12 nucleotides of the BCR gene and 12 nucleotides of the ABL1 gene of the site of their junction and Bcrex14, which was derived from the normal gene BCR. We have chosen a sequence, which is complementary to P1, as a target oligonucleotide mod-Ph for immobilization on the sensor surface. The effect of point mutations in target oligonucleotides on their hybridization with immobilized probes was studied on the model of probe sequences PNt, P2t and P3t as well as their corresponding complementary target sequences, derived from the rpoB gene of *M. tuberculosis*. The results of the experiments indicate that all developed biosensors were able to differentiate between target oligonucleotides. The highest values of selectivity of SPR biosensor were achieved by immobilising thiolated probes in the citrate buffer solution, whereas the biosensors with the highest sensitivity were obtained by using 0.5 M KH_2PO_4 solution as the environment of immobilization. The limit of detection of P1 target oligonucleotide by the studied SPR biosensors was determined at 50 nM.

Conclusions. Among the SPR biosensors, developed during our research, the biosensors, modified with the probe oligonucleotides using citrate buffer solution, were determined as the most promising in selective detection of oligonucleotide sequences of the Ph chromosome. The results of hybridization experiments suggest that the immobilized probe sequences for a clinical DNA biosensor should be chosen taking into account the most probable point mutations of the target.

MOLECULAR EPIDEMIOLOGY OF CARBAPENEMASE-PRODUCING HOSPITAL-ACQUIRED KLEBSIELLA PNEUMONIAE IN UKRAINE

Rudnieva K.L.[1], Baranovskyi T.P.[2], Potochilova V.V.[1], Iungin O.S.[3], Pokholenko Ia.O.[4], Spiers A.J.[5], Moshynets O.V.[4]

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Aim. Carbapenems are the latest line of defense for the treatment of the most common bacterial infections and Carbapenemase-producing opportunists are often associated with ineffective treatment and infectious outbreaks. However, little is known about the molecular characteristics of the carbapenemases produced by hospital-acquired *Klebsiella pneumoniae* in Ukraine. The project 'Development of combined therapy for severe *Klebsiella pneumoniae*-associated nosocomial infections to overcome the antibiotic resistance' (2020.02/0246) was supported by the National Research Foundation of Ukraine (0120U104850).

Methods. 65 MDR and 6 nonMDR *K. pneumoniae* strains isolated from Ukrainian patients at the Kyiv Regional Clinical Hospital in 2020 were analysed. Identification of microorganisms and determination of sensitivity was performed using a VITEK 2 compact 15 microbiological analyser. AmpliSens MDR MBLFL (AmpliSens, Russia) and AmpliCens/OXA-48-FL (AmpliSense, Russia) were used for molecular characterization.

Results. The most widespread carbapenemase was blaNDM (New Delhi Metallo-β-Lactamase) belonging to metallo-β-lactamase class B. blaNDM detected in 97% of the MDR strains and also in 5 of the 6 non-MDR strains randomly included in the survey, suggesting a critically high blaNDM distribution amongst these hospital isolates. blaVIM was much less common, detected only in 2 MDR strains, and not found in non-MDR isolates. Two other carbapenemase genes, class A carbapenemase blaKPC (*Klebsiella pneumoniae* carbapenemase) and plasmid transmissible class D carbapenemase blaOXA48 were widely represented in the MDR collection. In particular, blaKPC was detected in 55 strains and blaOXA48 in 29 strains. Both carbapenemases were also detected in nonMDR isolates, and blaOXA48 was present in 3 of the 6 nonMDR isolates and blaOXA48 in one nonMDR isolate.

Conclusions. This is the first report of carbapenemase-producing *Enterobacteriaceae* in Ukraine in which four carbapenemases from three classes were detected. The most common carbapenemases in both MDR and non-MDR isolates were blaNDM , blaKPC and blaOXA48 (detected in 97%, 85% and 45%, respectively, of the *K. pneumoniae* collection), and the most rare was blaVIM (3%).

GENETICALLY MODIFIED FODDER CROPS EXPRESSING THE COLICIN M GENE

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Background. The widespread use of antibiotics has led to the emergence and development of multidrug-resistant bacterial strains. In animal husbandry antibiotics are very often used prophylactically to prevent the spread of pathogenic bacterial strains. Colicin M is a non-antibiotic antimicrobial protein produced by some *E. coli* strains and has already been shown as an effective agent against pathogenic one.

Aim. The goal of our work was to obtain genetically modified (GM) fodder crops expressing antibacterial recombinant protein – colicin M and to test the antimicrobial activity of these plants against laboratory *E. coli* strains.

Methods. Transgenic plants of alfalfa (*Medicago sativa*, also called lucerne) and carrot (*Daucus carota*) were obtained via *Agrobacterium*-mediated transformation with vector contains colicin M coding gene (cma) under the control of 35S promotor and selective bar gene under control of nos promotor. We used PCR analysis with primers specific for target genes to confirm the presence of the cma and bar genes in the genome of obtained plants. Antimicrobial activity of colicin M-containing transgenic plants extracts against laboratory *E. coli* strains: DH10B and XL1Blue was determined by soft agar overlay assay.

Results. Transgenic plants of alfalfa and carrot were selected for their ability to grow on a nutrient medium containing 5 mg/L of phosphinothricin. The transgenic nature of obtained plants was confirmed using the PCR method. RT-PCR (reverse transcription PCR) also was performed on previously selected transgenic plants to detect colicin M gene transcriptions. Transgenic lines of alfalfa and carrots which showed significant antibacterial activity against two *E. coli* strains using the soft agar overlay assay were selected.

Conclusions. We have demonstrated antibacterial properties of transgenic alfalfa and carrots plants expressing the colicin M gene, which were tested on *E. coli* DH10B and XL1-Blue strains. Results available in the literature and the results of our study have already shown that recombinant plant-derived colicin M will also be effective against pathogenic strains, therefore transgenic colicin M-producing fodder crops may be promising future candidates for agricultural use.

THE EFFECT OF PENTAFLUOROETHOXYBENZOIC ACID DERIVATIVES ON MUSHROOM TYROSINASE

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Background. A promising path to combat skin disorders – melasma, lentigo, age spots and trauma-induced hyperpigmentation – is development of new tyrosinase inhibitors. Due to the side effects only a few from numerous known tyrosinase inhibitors are used in clinical therapeutic applications and in cosmetic industry. It is necessary to search for highly active, low-cost, easy-to-prepare tyrosinase inhibitors with minimal adverse effects.

Aim. The aim of this work is to search for new effective tyrosinase inhibitors using an enzyme isolated from *Agaricus bisporus*.

Methods. Tyrosinase from *Agaricus bisporus* was isolated: 1 kg of mushrooms was homogenized with 2 dm^3 of an aqueous solution containing 1% of ascorbic and 0.2% of benzoic acid. The enzyme was precipitated by ammonium sulfate to 80%, was dissolved in distilled water and dialysis at 0°C. In tyrosinase preparation the content of protein by the Lowry-Hartree method, activity by Ltyrosine (monophenolase activity) and L-DOPA (diphenolase activity) was determined. Pentafluoroethoxybenzoic acid derivatives were tested for their enzymatic inhibitory activities against tyrosinase by Ltyrosine and L-DOPA. The concentration leading to 50% activity loss (IC_{50}) was calculated by interpolation of the dose-response curves. Benzoic and salicylic acids was employed as a positive standard.

Results. Tyrosinase was isolated from *Agaricus bisporus* with the yield of protein 0.7 mg/g of mushrooms, monophenolase and diphenolase activity 800 and 4500 Unit/(mg protein/min), respectively. Six pentafluoroethoxybenzoic acid derivatives were tested for their enzymatic inhibitory activities against tyrosinase. It was shown that 3-, 4-pentafluoroethoxybenzoic acid and 2-hydroxy-4-pentafluoroethoxybenzoic acid are effective inhibitors of tyrosinase monophenolase (IC_{50} 0.078-1.69 mM) and diphenolase activity (IC_{50} 0.21-5.51 mM). The most active compound, 3pentafluoroethoxybenzoic acid, was the 6.2 folds stronger inhibitor of monophenolase and 2.4 folds of diphenolase activity that benzoic acid which was used as a positive control. The standard enzyme inhibitors (benzoic and salicylic acids) showed almost the same tyrosinase inhibitory activity by L-tyrosine and L-DOPA. In case of pentafluorethoxybenzoic acid derivatives IC_{50} of monophenolase activity are 2.2-3.2 fold less than those of diphenolase. This may indicate a different mechanism of binding of pentafluoroethoxybenzoic acid derivatives and standard inhibitors to tyrosinase.

Conclusions. We considered pentafluoroethoxybenzoic acid derivatives as promising tyrosinase inhibitors that may be applicable for the treatment of diseases associated with hyperpigmentation.

THE INFLUENCE OF TRIVALENT METAL IONS ON LCC-CHANNELS OF THE NUCLEAR MEMBRANE OF THE CEREBELLAR PURKINJE NEURONS

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Aim. The Large Conductance Cation channels (or LCC-channels) are highly expressed in the nuclear membrane of the cerebellar Purkinje neurons [1] and cardiomyocytes [2]. Currently, it is known that these ion channels are permeable to K^+ and Na^+ ions, whereas they are impermeable to Ca^{2+} ions. Some non-specific blockers, such as tubocurarine, bromides of rocuronium, and pipecuronium, of the abovementioned channels have also been identified [3, 4], but the structure and physiological role of these channels remain unknown. The usage of different metal ions as a tool to study the structural features of LCC-channels will allow us to estimate the pore diameter of this channel. Therefore, this work **aim**ed to investigate the effect of La^{3+} and Fe^{3+} ions on LCC-channels functioning.

Methods. The study was performed on nuclei of the cerebellar Purkinje neurons of Wistar rats aged 3-4 weeks. Ion currents' registration through single channels was completed using the patch-clamp technique in a voltage-clamp mode and "excised patch" or "nucleus attached" configurations. The effect of metal ions was investigated by adding their salts to the solution, which was administered to the experimental chamber with nuclei by flow application. Statistical and mathematical processing of research results was performed using Clampfit 10.4 and Origin 9.1 software. To be more specific, the average ion current passing through the channel, the probability of the channel being in the open state (Po), and the occurrence of channel flickering were estimated.

Results. La^{3+} at a concentration of 10 mM causes a significant decrease in the current amplitude was observed (by 45% at the applied potential of -40 mV) ($P \leq 0.01$, $n = 5$). It was also noticeable that La^{3+} causes LCC-channels flickering, which depends on the concentration of the substance and the potential applied. Administration of Fe^{3+} to the medium is accompanied by a slight flickering effect, whereas the average current amplitude through the channels does not change in the presence of the ions. Under the influence of both ion types (La^{3+} and Fe^{3+}), the channel probability of being in an open state (Po) declined gradually in a dose-dependent manner ($P \leq 0{,}05$-$0{,}001$, $n = 3$-7).

Conclusions. Therefore, La^{3+} and Fe^{3+} ions affect biophysical features of the LCC-channels with different effectiveness. The obtained results will be of key importance for assessing structural and functional characteristics of spontaneously active ion channels of the nuclear membrane and revealing their physiological significance.

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PROPERTIES OF MITOCHONDRIAL NO-SYNTHASE ACTIVITY IN SMOOTH MUSCLE

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Aim. Nitric oxide is a universal signaling and regulatory molecule in a cell. 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. Mitochondrial localization of NO-synthase (mtNOS) is today reliably shown in some tissues of mammals. However, the possibility of nitric oxide formation and its molecular physiological properties in smooth muscle mitochondria remain to be clarified. The problem of biosynthesis of NO in myometrium mitochondria is not considered. The aim of this study was to investigate the NO synthesis in mitochondria, the influence of potassium ions on NO production and the dependence of this synthesis on cAMP/PKA pathway.

Methods. Mitochondria were isolated from rat uterine smooth muscle cells using differential centrifugation. NO biosynthesis in isolated mitochondria was studed using NO-specific fluorescent probe DAF-FM-DA by method of flow cytometry. The measurements were performed using COULTER EPICS XL^{TM} (Beckman Coulter, USA) cytometer with an argon laser (λ_{ex}=488 nm, λ_{fl}=515 nm (Fl1 channel).

Results. High activity of mtNOS requires the presence of substrates of respiration, L-arginine, Ca^{2+}, NADPH and tetrahydrobiopterin. The biosynthesis of nitric oxide by mitochondria depends on its energized level: it is stimulated by the addition of respiration substrates (5 mM succinate and pyruvate), suppressed with specific inhibitors of the electron transport chain (5 μM rotenone and 1 μg/ml antimycin A) and protonophore 10 μM CCCP. The activity of mtNOS was significantly inhibited with potassium ions concentration decreased (125–0 mM) and in the presence of potassium channel inhibitors (1 mM tetraethylammonium, 4-aminopyridine, 20 μM glibenclamide, 20 nM charybdotoxin). mtNOS was significantly enhanced by addition of soluble adenylyl cyclase (sAC) activators 10 μM forskolin and 30 mM $NaHCO_3$, as well as by sAC substrate – ATP (0.5–5 mM) and phosphodiesterases inhibitor 1 mM caffeine. It has been shown, that PKA inhibitor – 10 nM PKI, as well as sAC inhibitor 25 μM KH7 lead to a decreased NO synthesis.

Conclusions. The results of the experiment confirm the presence of NO synthesis in mitochondria of smooth muscle cells. The NO synthesis in mitochondria is dependent on the level of inner mitochondrial membrane energization. The potassium permeability of the inner mitochondrial membrane plays important role in the regulation of mtNOS activity. Our data support the idea of cAMP/PKA signaling cascade involvement in NO synthesis in mitochondria.

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THE EFFECT OF LOW CONCENTRATIONS OF PHARMACEUTICALS ON THE MOLECULAR RESPONSES OF STRESS IN DANIO RERIO

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Background. In recent decades pharmaceuticals and personal care products have been determined as the hazardous pollutants because their increasing background concentrations in water bodies and potentials to adversely affect aquatic life. For example, being conveyed in water bodies with runoff and municipal effluents, ibuprofen was found in water bodies within the concentration range 0.648.7 μg L^{-1}. It is expected that such therapeutic concentrations may affect key molecular and cellular events in non-target organisms, but the appropriate information is limited.

To address these knowledge gaps, the **aim** of our research was to study the effect of the common pharmaceuticals in the environmentally relevant concentrations on oxidative stress, electron transport chain, and cytotoxicity in fish.

Methods. The study of the effect of common pharmaceuticals was performed on zebrafish *Danio rerio* as the model. Three groups were settled, one control and two experimental. In the water in which the animals of the experimental groups were kept, the studied pharmaceuticals were added: 1) gemfibrozil (GF) at the concentration of 1 μg L^{-1}, and 2) ibuprofen (IBU) at the concentration of 25 μg L^{-1} which corresponded to the average level of these substances in wastewater.

Results. Gemfibrozil caused a decrease in glutathione and glutathione transferase and an increase in catalase but had no effect on lipid peroxidation and protein carbonylation in zebrafish liver. Ibuprofen affects antioxidant defense system and caused oxidative damage to proteins in zebrafish liver, but also increased vitellogenin-like protein in blood. Lactate dehydrogenase in blood was also found to be higher in the both studied groups. Ibuprofen affects zebrafish health status more profound than gemfibrozil.

Conclusions. Our results showed that pharmaceuticals even in low environmentally realistic concentrations induced profound changes in the stress-responsive systems of zebrafish. This study's results reveal a rather negative effect of both ibuprofen and gemfibrozil on antioxidants, however, only ibuprofen stimulated protein carbonylation. Furthermore, ibuprofen is claimed to be the endocrine disruptor and cytotoxic agent to zebrafish. Ibuprofen and mostly gemfibrozil had the positive effects on lysosomal biogenesis. All mentioned issues raise concerns about the adverse effects of pharmaceutical effluents on life processes of aquatic organisms.

MOLECULAR TARGETS OF ENDOGENOUS PLANT PEPTIDES IN REGULATING PLANT DEFENSE SIGNALING

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Aim. Small secreted peptides act as important endogenous signals playing a role in activation and/or amplification of plant resistance mechanisms in order to counteract invading pathogens. In our study we aimed to expand the knowledge concerning the biological role of SCOOP family peptides and characterize them as inducers of plant defenses.

Methods. In our study we have assessed plant growth performance *in vitro* in media containing synthetic peptides; plant stress resistance level was evaluated based on inoculation tests with *Pseudomonas syringae* pathogen. Potential molecular targets of endopeptides were identified using molecular docking performed with the help of HADDOCK software.

Results. Co-incubation with 1 μM of SCOOP5, 7, 12 or 13 synthetic peptides leads to rapid ROS (reactive oxygen species) burst in *Arabidopsis* leaves. In suspension-cultured cells of *Arabidopsis* SCOOP peptides triggered a production of phosphatidic acid (PA) acting as a second messenger. These two responses can be linked since ROS burst is downstream of PA-induced RbohD activation. Based on the fact that treatment with SCOOP peptides induces a clear defense-like responses in *Arabidopsis*, it was of interest to analyze if stress resistance of SCOOP-treated plants is affected. A biotest with *Pseudomonas syringae* model pathogens showed that a pre-treatment of *Arabidopsis* plants with SCOOP peptides affected the number of CFU accomodated in their leaves. Pre-treatment with 1 μM SCOOP5 and SCOOP13 leads to less CFU number in plant leaves. Yet, for SCOOP7- and SCOOP12-treated plants an increase in CFU number was detected. BAK1 was previously shown as a functional part of SCOOP12 signaling network. It is not known if this is due to direct interactions between the two. A modeled interaction between the SCOOP12 and BAK1 using HADDOCK showed to implicate a kinase domain of BAK1.

Conclusions. Our results corroborate SCOOP5, SCOOP7 and SCOOP13 being a biologically active peptides that act in plant defenses. These endopeptides induce rapid ROS and PA accumulation in plant tissues and affect stress performance of plants. The functional connection of SCOOP peptides to BAK1 as well to other regulatory nods is to be confirmed in further studies.

STRUCTURE OF POLYVINYL ALCOHOL CRYOGELS BY OPTICAL MICROSCOPY

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Background. Polyvinyl alcohol (PVA) due to its unique properties is widely used in many fields, especially in medicine and pharmaceuticals. Physical cross-linking by cyclic freeze-thawing forms a cryogel with special mechanical properties (Crolla J.P., et al., 2021) that can be adjusted to exactly match the properties of soft tissues, making it an attractive candidate for cleaning and immobilization proteins for use in bioseparation and biomedicine. The structure and properties of polyvinyl alcohol cryogels (PVACG) depend on many factors, including both the characteristics of the polymer itself and the conditions of low temperature gelation (Wan W. et al., 2014). For example, molecular weight has a significant effect on the formation of PVACG (Bakeeva I.V. et al., 2019), so with increasing molecular weight the number and size of crystal regions increase due to increasing the length of the polymer chain (Bakeeva I.V. et al., 2019).

Aim. The purpose of this work was to study the structure of PVACG of 2 molecular weights by optical microscopy.

Methods. We used two marks of PVAs: 17-99 and 30-99. Cryogels was obtained by the 1 cycle method of freezing (Chaturvedi A., 2016): 10% PVA solution was poured into Petri dishes (diameter 35 mm) of 2 ml and placed in a freezer (18°C). After 48 hours, transfer the samples to a refrigerator at +4°C for 48 hours. Porous structure of the cryogels was analyzed by confocal microscope Axio Obzerver Z1 (Carl Zeiss, Germany). Zeiss ZEN black software was used for image acquisition.

Results. The nature of structural changes of PVACG is well traced on photomicrographs of thin sections of the corresponding samples. In general, the main elements of the surface microstructure of the PVACG samples, formed from both PVA 17-99 and PVA 30-99, include alternating “threads”. Differences are mainly observed in the size of these structures and orientation. Thus, on the longitudinal sections of PVACG 30-99 these structures are fairly evenly distributed, oriented in one direction and longer. In the case of PVACG 17-99 threads are shorter and chaotically directed. Such changes in the morphology of these cryogels can be explained by the different viscosities of the initial PVA solutions, which are further confirmed by microphotographs with higher magnification.

Conclusions. As a result of this work the obtained cryogels with different structure will be used for further research to develop optimal carriers to preserve the structure and functional activity of proteins.

MOLECULAR ORGANIZATION OF 5S RDNA IN *CUPIDO ARGIADES* (*LYCAENIDAE*)

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Aim. In the last decade, butterflies from the family *Lycaenidae* have been a popular model group in studies of hybridization, speciation, chromosome evolution, ecological specialization, population genomics, and conservation genetics, which can be explained by the highest rate of molecular evolution among butterflies. However, the taxonomy of this family is still insufficiently clarified, which may be due to the high hybridization and also sympatric or cryptic speciation in the group. Because of the high molecular variability, the 5S rDNA non-transcribed intergenic spacer (IGS) region represents a convenient source of molecular markers that can be used to distinguish closely related plant and animal species. In our study, we present results of the cloning and sequencing of the 5S rDNA region of the *Cupido argiades* butterfly.

Methods. Total DNA was extracted from the butterfly body using CTAB. Amplification of 5S rDNA repeated units was performed using the polymerase chain reaction (PCR) with primers RV0803 and RV0804, which are complementary to the conservative region encoding 5S rRNA. Purified PCR products were cloned into pJET1.2 vector (CloneJET PCR Cloning Kit). Colony screening for recombinant plasmids was performed by ampicillin resistance. The recombinant plasmids containing the 5S rDNA inserts were sequenced and analyzed using appropriate software packages (DNASTAR, SnapGene Viewer, Chromas, Geneious).

Results. Five sequences of 5S rDNA IGS were obtained. Analysis of the sequences showed that three clones contain short IGS variant inserts ranging in length from 71 to 78 bp, while two other clones harbor the long IGS variant of 228 bp. We have detected similar short IGS variants in the genomes of other members of the fam. *Lycaenidae*, *Aricia agetis* (GCA_905147365.1) and *Cyaniris semiagrus* (GCA_905187585.1) available in GenBank. Thus, at least two variants of 5S rDNA IGS, which differ significantly in structure and length, appear to be present in the lycaenid genomes.

Conclusions. Analysis of the 5S rDNA IGS sequences of *Cupido argiades* showed that they belong to two distinct classes. Accordingly, two or more different variants of 5S rDNA IGS may can exist in the genomes of butterflies of the family *Lycaenidae*.

APPLICATION OF THE PRINCIPLE OF CLUSTER ORGANIZATION OF A CELL POPULATION IN AGENT-BASED MODELING OF HUMAN SKIN FIBROBLAST GROWTH *IN VITRO*

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Aim. Development and validation of a two-dimensional agent-based computer model of the growth of human skin fibroblasts in culture using the principle of cluster organization of the cell population.

Methods. The Pharo object-oriented programming language was used to create a dynamic agent-based model. The behavior of single agents/cells at each simulation step was determined by a set of simple rules governing the processes of adhesion, migration, proliferation, extracellular matrix production, as well as aging and cell death. To determine the position of the cells and reduce the computational load, the virtual plane was divided into sectors. To determine the parameters of the above processes, the increase of the number of agents during the simulation was compared with the data on the growth of cultured human skin fibroblasts on gelatin, glass, and untreated polystyrene, obtained by us in a real experiment and published earlier. The data of real experiment were approximated by a logistic function. After multiple realizations of the model, the basic parameters were selected at which the sum of absolute deviations between the number of virtual agents and the cells in a real experiment was minimal.

Results. The process of self-organization of cell population should include both the interaction of cells with their nearest microenvironment and be realized at the level of the whole population. In this model the creation of an ordered movement and mutual arrangement of virtual agents is realized through the formation of a set of connected groups of cells – clusters. The emergence of clusters is a random process, and is determined by the presence of several (3-4) agents in close proximity at the initial stage of the simulation (1000-1500 agents). Thus, the previously described by us phenomenon of the formation of lattice “germs” in a growing population of cultivated fibroblasts is modeled. In the course of migration and proliferation, the clusters increase in size and occupy an ever larger area, producing an extracellular matrix. Such a cluster type of movement and growth of a virtual cell population makes it possible to obtain a good approximation to the real growth of human skin fibroblasts on a gelatin substrate. At the same time, a necessary condition is the introduction of the processes of aging and cell death into the model.

Conclusion. The developed model of human fibroblast growth using the mechanism of cluster formation will be useful for predicting the dynamics of the cell population and for in-depth analysis of the role of migration, proliferation, cell aging and matrix production in the behavior of cells growing *in vitro*.

IN VITRO CYTOCOMPATIBILITY EVALUATION OF COLLAGEN DERIVATIVES ISOLATED FROM THE WASTES OF LEATHER PROCESSING

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Aim. Collagen and its derivatives are one of the most frequently used biomaterials in modern tissue engineering applications. Despite its high abundance in the body of different species, collagen use is still limited because of the relatively high cost and shortage of raw materials. The utilization of wastes from the leather processing industry to obtain collagen derivatives can provide a relatively cheap source for the pharmaceutical industry. However, the extensive chemical treatment during the processing of the initial leather material can lead to decreased biocompatibility of obtained collagen derivatives. Therefore, we aimed at studying the effects of collagen derivatives obtained from the wastes of leather processing on the viability and attachment of mammalian cells *in vitro*.

Methods. Limed and delimed pelt were chosen as collagen-containing wastes from the cattle tannery "Chinbar" (Kyiv, Ukraine) for the study. The basic method of collagen acid hydrolysis with acetic acid was used. Dialysis against deionized water and cold sterilization by filtration were applied to samples. The influence of obtained collagen derivatives on the metabolic activity of HEK293 was tested using an MTT assay. To test the ability of developed preparations to promote the attachment and spreading of the cells, the polystyrene Petri dishes were coated with the solution of 1 mg/mL of each sample in 0.05 M acetic acid overnight at +4°C. 2´10^5 HEK293 in DMEM medium were seeded per one 35 mm dish. The cells were incubated for 1 hour at 37°C, 5%CO2. Afterwards, unattached cells were removed, the surface was washed twice with PBS, and the cells attached were stained after Pappenheim. To characterize cell attachment and spreading the morphological spreading parameter (nucleus/cytoplasm area ration) was used.

Results. The data obtained indicate that the preparation of collagen derivatives from delimed pelt exhibited a slightly toxic effect on the HEK293 cells. The total activity of cellular dehydrogenases in the MTT test was inhibited by 31.38±6.3% after 24 hours of exposure at a dose of 0.135 mg/mL. Both collagen derivatives from limed and delimed pelt did not affect the metabolic activity of HEK293 cells at concentrations below 0.1 mg/mL. It was demonstrated that collagen derivatives obtained from limed and delimed pelt were able to promote the attachment of HEK293 cells *in vitro* in serum-free conditions. The cell attached to the polystyrene surface coated with the derivatives had a lower nucleus/cytoplasm area ratio due to enhanced cell spreading as compared to uncoated surface.

Conclusion. It was demonstrated that collagen derivatives obtained from limed and delimed pelt were able to promote the attachment and spreading of HEK293 cells *in vitro* in serum-free conditions.

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INFLAMMATION AND THROMBOSIS IN PATIENTS WITH URGENT COVID-ASSOCIATED CARDIOVASCULAR SURGICAL PATHOLOGY IN THE ACUTE AND POST-COVID PERIODS

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Aim. Study the immunophysiological and hemostasiological preconditions for the formation of complications in patients with COVID19-associated cardiovascular surgical pathology in the acute and post-COVID periods.

Methods. We used photometry, gravimetric assay, chronometry, ELISA, immunoturbidimetry and latex agglutination test.

Results. In patients with COVID-associated urgent cardiovascular surgical pathology in both acute and post-COVID periods, all signs of inflammation are detected, namely: in the acute period SARS-CoV-2 increase in C-reactive protein 16 times in 100% in vascular pathology, 10 times in cardiac pathology, in the post-COVID period increase in 50% – 10 times in vascular pathology, 12 times in cardiac pathology; and prolonged activation of the complement system, which is manifested by increased levels of C3 and C4 components. The hemostasis system in patients with COVID-associated urgent cardiovascular surgical pathology in both acute and post-COVID periods was characterized by divergent changes in the coagulation (thrombosis activity was 4.6 times more pronounced in vascular pathology in the acute period, in cardiac pathology 2 times, and in the post-COVID period in vascular pathology 2.3 times, in cardiac pathology in 1.8 times); anticoagulant (the concentration of antithrombin III) and fibrinolytic units (plasminogen concentration) were reduced in all groups. The concentration of D-dimers is significantly increased in all types of pathology in both acute and post-COVID periods, most pronounced in the group of cardiac pathology in the acute viral period. Increased concentration of D-dimer indicates the activation of fibrinolysis, which was preceded by increased coagulation cascade with excessive formation of insoluble fibrin. An increased concentration of the C3 component indicates complement activation in an alternative way and serves as a prerequisite for enhancing the process of fibrinolysis, namely, a possible increase in the concentration of plasminogen activator. A decrease in the concentration of plasminogen with an increased level of tissue plasminogen activator and a reduced content of plasminogen activator inhibitors cause chronic simultaneous activation of the coagulation and fibrinolysis systems against the background of a decrease in the production of physiological anticoagulants.

Conclusion. Thus, the inflammatory response to SARS-CoV-2 infection leads to significant activation of coagulation – the process of thromboinflammation – with signs of systemic endothelial damage and subsequent loss of normal anticoagulant properties, which requires increased attention to laboratory examination of hemostasis in patients with COVID-associated cardiovascular surgical pathology and using of individualized anticoagulants.

EFFECTS OF MARGARINE ON ACTIVITY OF ANTIOXIDANT ENZYMES IN THE MOUSE LIVER

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Aim. Fats are one of the important nutrients that the body needs to maintain normal functioning. However, the amount and type of fat consumed can have different effects on human health. Margarine is a fatty product that is based on vegetable fats; after their processing, trans-unsaturated fatty acids are formed. Margarine is also a high-calorie product, which in excess can lead to metabolic disorders and obesity. Obesity is characterized by the development of oxidative stress due to the excessive formation of free radicals that damage biomolecules. To detoxify free radicals and related reactive compounds, a number of protective enzymes are activated in the cell, such as glutathione-S-transferase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase. The **aim** of this study was to investigate the intensity of lipid oxidation and the activity of antioxidant enzymes in the liver of mice fed with margarine-containing food.

Methods. Experiments were performed on C57Bl/6j mice. One-month-old animals were divided into two groups. The first group (control) consumed basic food with a fat content of 6.3%. The second group (experimental) was fed additionally with 70% fat margarine. In the experimental group, mice had a choice (to eat basic food or margarine). All mice had unrestricted access to water and food. Each group contained 12 females and 12 males in separate cages (3 mice per cage). The animals were kept on experimental diets for four months. After sacrificing, liver tissues were collected and used for biochemical analysis.

Results. The liver of female mice fed margarine had 67% higher levels of lipid peroxides compared with the control group. At the same time, in males, this indicator was 55% lower than in the control group. In margarine-fed females, the activities of glutathione-S-transferase and glutathione peroxidase were 30% and 119% higher, respectively, and. glucose-6-phosphate dehydrogenase activity was 34% lower than in the control group. In males, the activity of these enzymes remained unchanged with margarine consumption.

Conclusions. Thus, margarine-contain food had sex-dependent effects on parameters of antioxidant status in males and females. A decrease in lipid peroxides with unchanged antioxidant enzyme activity in the liver of males suggests a decrease in the intensity of oxidative stress in this organ. At the same time, the margarine diet induces mild oxidative stress in the female liver.

GENOME DIVERSITY IN UKRAINE: INTRODUCTION TO UNIQUE VARIATION, STRUCTURE AND ADMIXTURE IN WHOLE GENOME SEQUENCES OF UKRAINIANS

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Aim. Ukraine is positioned in the crossroad of the early migration of modern humans and the westward expansion of the Indo-Europeans, and represents an aftermath of centuries of migration, admixture, demographic and selective processes. Since the publication of the first human genome in 2003, and the initial surveys of worldwide variation, efforts have been directed to expand exploration of the human diversity. Our goal was to fill in one of these pieces of the puzzle on the global map of human genomic diversity, namely Ukraine.

Methods. Healthy volunteers from most of the regions of Ukraine were contacted through advertisements and invited for personal interviews at outpatient offices. In our project we sequenced and annotated the entire genomes of 350 individuals for genetic variants, including medically related and functional alleles. In addition, using this data, we conducted population analysis using genomic diversity among ethnic Ukrainians and comparing it to the diversity in other European populations for which whole genome sequences were available in public databases.

Results. This project identified tens of millions of genome variants in Ukrainians, of which approximately half a million were novel genomic SNPs missing from other surveys of genomic diversity. While most of the common variation is shared with other European populations, we found many novel structural variants, indels, copy number variations, single-nucleotide polymorphisms, and microsatellites. Our results indicate that genetic diversity of the Ukrainian population is uniquely shaped by the evolutionary and demographic forces, differs from its neighbors, and this uniqueness cannot be ignored in the future genetic and biomedical studies.

Conclusions. Given the unique history of Ukrainian population, our data contributes a wealth of new information about historically shaped genome diversity, including different risk and/or protective alleles that do not exist nor associate with disease, elsewhere in the world. To our knowledge, this study provides the largest to-date survey of genetic variation in Ukraine, creating a public reference resource aiming to provide data for medical research in a large understudied population. Using bioinformatic analysis, we can ask questions about evolutionary history, ancestry, admixture, and to identify genetic components contributing to genetic diversity and structure of the modern population in the context of European history. The database we created added valuable information to the world's scientific community and laid a cornerstone into the international cooperation in the area of genomics and bioinformatics in Ukraine.

BIOLOGICAL DANGER OF ANTHRAX DURING THE PERIOD OF THE UKRAINIAN-RUSSIAN WAR

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Background. In view of the occupation of the Ukrainian territories by Russia, close attention is paid to means and methods of prevention of infectious diseases common to humans and animals, as it is a biological threat. The most dangerous disease among them is anthrax. Anthrax affects all mammals and humans. On the territory of Ukraine there are more than 11,000 anthrax burials, spores of the pathogen *B. anthracis* are stored in the soil for over a hundred years. The threat of disease is constant. Occupied areas with anthrax burial grounds pose a threat of spreading the pathogen. In the combat zone, the destruction of canned sites and the spreading of contaminated soil is possible. This can result in infection of the territory with anthrax spores for many years, making it dangerous for humans and animals. There is a danger due to the uncertainty of such territories.

Aim. Today, vaccination of humans and animals against anthrax is the only preventive method of confrontation the biological threat.

Methods. Statistical data on anthrax outbreaks and burials in the regions of Ukraine were taken from the "Catalogue of stationary anthrax-infested points on the territory of the Ukrainian SSR 1920-1978 and 1978-2002." (edited by V. Y. Shabliya), obtained from the State Committee of Veterinary Medicine of Ukraine, as well as data from the State Research Institute for Laboratory Diagnostics and Veterinary-Sanitary Examination on outbreaks of anthrax in the territory of Ukraine in 2000-2019. Information on the list of anthrax vaccines used in our country is taken from the site of registration of drugs of the State Scientific Control Institute of Biotechnology and Strains of Microorganisms.

Results. The main way of anthrax prevention among humans is the elimination of the disease among susceptible animals through vaccination. The following drugs are used on the territory of Ukraine: live spore vaccine against anthrax of animals from strain “SB” in the form of a suspension, provided by Sumy Biological Factory State Enterprise Ukraine; live vaccine against anthrax of animals from strain K-79Z in the form of a liquid; vaccine against anthrax of animals from the strain “Sterne 34F 2” in the form of a suspension, provided by Kherson State Enterprise - Biological Factory Ukraine. Thus, there are only live spore vaccines in Ukraine. Antibiotics, antibiotics in combination with an inanimate vaccine or gamma globulin (immunoglobulin) are used to treat the disease in humans. Antibiotics are effective if they are used early in the disease, otherwise the body produces enough toxins to cause the death of the host, even if antibiotic treatment has destroyed all *B. anthracis*. Apply amoxicillin, doxycycline or ciprofloxacin for 4 weeks. Persons who have received emergency prophylaxis are observed for 8 days. Emergency prevention is carried out regardless of whether a person has been vaccinated against anthrax. In the United States and Canada, live, concentrated, or a combination of anthrax vaccines is used to prevent anthrax among people in adverse areas. Vaccination is allowed to persons aged 14 to 60 years. Immunity is created up to 1 year, so revaccination should be carried out annually.

Conclusions. Control of biological threats at the national level is extremely important. In connection with warfare, it is necessary to provide for timely vaccination of animals, which prevents the spread of the disease. Fortifications, funnels from explosions, can lead to landslides and the destruction of anthrax burial grounds. Ignoring these factors threatens the national security of the country, including a possible outbreak of anthrax.

INFLUENCE OF ELECTROMAGNETIC RADIATION OF MILLIMETER RANGE ON THE OPTICAL PROPERTIES OF THE HEMOGLOBIN

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Background. The biological activity of millimeter electromagnetic radiation (MEMR) has long been known that is the basis for its use in medicine. But the primary biophysical mechanisms of this factor at the molecular level remain poorly understood and debated.

So, the features of the influence of microwave radiation on the optical properties of water solutions of hemoglobin with the **aim** to characterize the certain structural state of hemoglobin were investigated in this research.

Methods. The object of the study was the optical properties of aqueous solutions of human hemoglobin in the concentration range of 7 and 15 μM and in the temperature range of 10–40°C. The optical spectra were recorded by using a BiospecMini (Shimadzu) spectrophotometer in the wavelength range of 190–1100 nm. In order to increase the accuracy of the analysis of spectral data, the spectra were normalized relative to the baseline which was taken as a line that ran parallel to the horizontal axis due to the minimum value of the optical density in the absorption spectrum. The values of the absorption maxima and the optical density were used as the main parameters of the absorption spectra. The B-spline function was used to more accurately determine these parameters. Microwave exposure of hemoglobin solutions was performed at a wavelength of 7,1 mm with power that was calculated to be approximately 6 mW. This power of electromagnetic radiation corresponds to the conditional limit range between thermal and non-thermal action of this factor.

Results. The dependence of the optical properties of hemoglobin in the spectral range of heme absorption on temperature was analyzed and the effects of microwave radiation on the optical properties of hemoglobin solutions were considered in the frame of hypochromic effects induced by temperature. It was revealed that the parameters of the absorption spectrum of an aqueous solution of hemoglobin depend on temperature. The optical density in the Sore band and the spectral bands of the oxygenated form of hemoglobin decrease upon temperature increase.

Conclusions. The observed temperature hypochromic effect is associated with increased protein aggregation. The effect of MEMR leads to a small but statistically significant hypochromism in the spectra of hemoglobin absorption that testifies to an increase in protein aggregation under the action of this physical factor that was caused by changes in the hydration of protein molecules. This effect depends on the protein concentration and is not detected at low levels.

ISOLATION OF MACROMYCETE *FOMITOPSIS BETULINA* STRAINS – POTENTIAL PRODUCER OF BIOLOGICALLY ACTIVE SUBSTANCES

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Aim. The polypore fungus *Fomitopsis betulina (Bull.)* B.K.Cui, M.L.Han&Y.C.Dai (also known as birch polypore) had been used in folk medicine for years. The fungus was known by its anti-inflammatory, antimicrobial, antiparasitic and wound-healing properties. Its biological activity is related to polyporenic acids and other substances synthesized by the fungus. That's why *F. betulina* is used as an object of research in different countries by mycologists, biotechnologists, pharmacologists. Studies with pure cultures not only enhance understanding of any natural ecosystem or in the environment, but also are useful in the determination of molecular purposes and screening for novel activity compounds. The aim of our work is to isolate new strains of *F. betulina* in order to investigate their biological activity.

Methods. The fruiting bodies of fungi were collected in two places: the Holosiivskyi National Nature Park, Ukraine and in the city of Sumy in September 2021. The introduction of fungi into the culture was performed according to conventional methods (Bilai et al., 1982; Buchalo et al., 2009). Fragments from internal parts of fruiting bodies were inoculated on Petri dishes with glucose-peptone-yeast agar medium (GPYA), and incubated at 26°C. Cultures were checked regularly for contaminations. Light microscopy was used to evaluate the microscopic morphology. After growth in culture, fungi are identified based on visual characteristics such as colony morphology and color. Stock cultures of introduced fungi were maintained on beer-wort agar slants at 4°C.

Results. Studied fungus from freshly harvested fruiting bodies is quite easily introduced into the culture. Tested GPYA medium was suitable for the introduction and growth for all isolates. Eleven fungal strains of *F. betulina* were isolated from the carpophores grew on *Betula pubescens* Ehrh. only: 1 strain from Sumy and 10 strains from two localities of Holosiivskyi National Nature Park. Vegetative mycelia microstructures as significant key point for verification of isolated cultures were evaluated. All of the isolated fungi have clamp connections – the definitive structures of Basidiomycetes. Verification and identification of isolated pure cultures was also confirmed by primordia formation on the mycelial colony after 3 weeks of growth.

Conclusions. The new strains of *F. betulina* we obtained can be applied in future investigations of their biological and therapeutic activities. It should be noted that new cultures have some advantages over old strains from fungal collections. For instance, new fresh strains may have more biological activity and growth rate. Also, they didn't accumulated mutations, that old cultures gathered during their storage. So, our new 11 strains of *F. betulina* have prospects for further research as biotechnological objects.

EFFECTS OF NACL CONCENTRATION IN THE CULTIVATION MEDIUM ON THE PRESERVATION OF MICROALGAE WITH DIFFERENT DEGREES OF HALOTOLERANCE DURING HYPOTHERMIC STORAGE

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Background. Low temperature storage is widely used to preserve various types of microalgae. But there is not enough information about the long-term effect of low temperatures on the stability of the cells of these biological objects and their functioning.

Aim. Therefore, the aim of the work was to study the effect of hypothermic storage on the viability and motility of microalgae cells of halotolerant *Dunaliella salina* and freshwater *Chlorococcum dissectum* depending on the storage time.

Methods. Microalgae were cultivated at a temperature of 25°C without aeration. Biomass accumulation was carried out in culture flasks (TPP, Switzerland) with a volume of 40 ml under round-the-clock illumination with 3 kLux white fluorescent light on nutrient media containing different amounts of sodium chloride: Ramaraj with 1.5 M and 4 M NaCl content (*D. salina*) and BG-11 with 0 M, 0.06 M, and 0.2 M NaCl content (*Ch. dissectum*). Cold exposure of cultures was carried out for 30 days at a temperature of 4°C without lighting. Motility, concentration, and viability of *D. salina* cells were assessed using a light microscope and by inoculated onto appropriate culture media. Viability assessment of *Ch. dissectum* was carried out by inoculated on agar nutrient media and counting the number of colony forming units (CFU).

Results. Our results showed that the exposure of samples at 4°C for up to 30 days had individual specific species differences. Storage of *D. salina* at low temperatures did not affect the cell concentration during the study period. On day 30, a significant increase in the concentration of *D. salina* cells on the medium content 1.5 M NaCl was observed compared to the control, which indicates an increase in biomass. At the same time, hypothermic storage at 4°C reduced cell motility. A significant decrease by 20% was observed in the culture content 4 M NaCl already on the 10th day and in the culture of 1.5 M NaCl by 40% on the 30th day of exposure. Study of cell viability of microalgae *Ch. dissectum* during hypothermic storage showed that during 30 days of storage at a temperature of 4°C, the number of CFU units increased in all samples, regardless of the NaCl content, compared with the corresponding samples that were cultured under normothermia. At the same time, a more significant increase in biomass was observed in the medium with 0.06 M NaCl. This suggests that the cell culture of microalgae *Ch. dissectum* can be stored at 4°C for 30 days without loss of viability.

Conclusions. Thus, modification of the culture medium by increasing the content of sodium chloride to 4 M for *D. salina* and 0.06 M for *Ch. dissectum* can increase the resistance of cells to low temperatures, and increase the duration of their hypothermic storage.

THE ROLE OF NITRIC OXIDE AND HYDROGEN PEROXIDE IN THE IMPLEMENTATION OF THE STRESS-PROTECTIVE EFFECT OF CADAVERINE ON WHEAT SEEDLINGS UNDER CONDITIONS OF HYPERTHERMIA

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Background. The most common plant polyamines are putrescine, spermidine, and spermine. Cadaverine is the least studied plant polyamine. The influence of exogenous cadaverine on plant resistance to stress factors has little been investigated so far. In the apoplast and peroxisomes, catabolism of polyamines, mediated by diamine and polyamine oxidases, yields hydrogen peroxide, which is an important signaling molecule. Nitric oxide (NO) is another candidate for a role of a signal messenger in the implementation of polyamine action. The stimulation of NO generation, caused by exogenous polyamines, is observed in different plant species, but the mechanism of the effect remains uncertain. The participation of nitric oxide in the accomplishment of physiological (stress-protective) effects of cadaverine has still insufficiently explored.

Aim. The objective of this study was to analyze the effect of cadaverine on the formation of NO in the roots of wheat seedlings. We also attempted to unravel the relations between NO and ROS, as the signal messengers in heat resistance enhancement mechanism caused by this diamine.

Methods. Etiolated seedlings of soft winter wheat cv. Doskonala were used in the work. Three-day-old seedlings were treated with cadaverine at concentrations 1 mM by adding it to the root incubation medium. The roots of the seedlings were also treated for 26 h with one of the inhibitors: the scavenger of NO (0.1 mM 2phenyl-4,4,5,5tetramethylimidazoline-1oxyl-3oxide, PTIO), the inhibitor of diamine oxidase (1 mM aminoguanidine), the scavenger of hydrogen peroxide (0.15 mM dimethilthiourea, DMTU). After the treatments, the seedlings were exposed to 10min damage heating at 45°C in water thermostat. After the heating, all seedlings were transferred to distilled water to grow at 2022°C. In 3 days after the heating, the relative number of the survived seedlings was scored.

Results. The treatment of intact seedlings' roots with cadaverine obviously elevated the content of NO in the tissues as soon as after a 0.5 h contact with this diamine. After 12 h, the effect attained a maximum. The content of hydrogen peroxide demonstrated a similar dynamics. In response to cadaverine, the hydrogen peroxide level began increasing in 0.5 h from the start of the treatment and reached the maximum in 1.52 h followed by some decrease after 24 h. The aminoguanidine (inhibitor of the oxidative pathway of nitric oxide synthesis) prevented the increase cadaverine-induced accumulation of NO. The scavenger of hydrogen peroxide DMTU also attenuated the effect of cadaverine on the NO content. Treatment of the seedlings with DMTU and antagonists of NO diminished the stress-protective effect afforded by exogenous cadaverine.

Conclusions. The conclusion was drawn on the role of NO and its functional relations with ROS in the processes of the cadaverine-induced adaptation of the wheat seedlings to high temperatures.

THE DETERMINATION OF THE ACTIVATION ENERGY OF THE NA, K-ATPASE OF OOCYTES AND EMBRYOS OF THE LOACH

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Aim. The Na-K-ATPase is one of main enzyme that ensures cellular ion homeostasis and membrane transport, also contributes in specialized tissue functions. For example, in the reproductive system this protein is essential for embryo development through its role in the blastocoel formation and cell division of the embryos. The purpose of the study was to investigate the activity of the Na-K-ATPase of loach oocytes and embryos (as test system) during embryogenesis and to determine the activation energy (Ea) of the enzymatic process.

Methods. The ovulation in the females loach (*Misgurnus fossilis L.*) were stimulated by the intramuscular injection of female chorionic gonadotropin (500 units). The eggs were obtained through the 36 h after stimulation, with next fertilizition in Petri dishes by a suspension of the sperms that got according to Neifach method (t=23°C). In this study, the enzymatic activity of the sodium pump were measured directly in the fraction of the plasmatic membrane of oocytes and embryos loach extracts by monitoring the conversion of ATP to ADP in the presence and absence of ouabain. The amount of product Pi were tested by a modified Fiske-Subbarou method. The membrane protein concentration was measured using the Lowry assay. The Ea were determined in the Arrhenius coordinates (lgV from 1/T; with a temperature difference of the 10°C). Differences were considered significant at $P \leq 0.05$.

Results. The oubain-sensitive ATPase activity of the embryos with temperature changes in the incubation medium showed gradually increasing at the studied stages of development compared with ATPase activity of the oocytes (by an average of 23.4±1.6%). The activity of the Na, K–ATPase of the loach embryos reached the maximum value at the stage of the 8 division blastomers (270 min). At the 10 division blastomers no significant changes in the activity of Na, K-ATPase were observed in comparison with the previous stage of the development. The during the first hour after the fertilization, the lipid environment of ATPase was changed, as evidenced by the value of Ea (Ea=20.8 kJ/mol) in the process of hydrolysis of ATP. The gradual decreasing of the absolute values of Ea to the 8 stage of division blastomers (Ea=10.6 kJ/mol) were determined.

Conclusions. This increasing of the enzymatical activity during the early development of the embryos cold-blooded is associated with the processes of embryogenesis and cell membrane of the reorganizations, that is require significant energy expenditure. For the Na, K–ATPase the nonlinear dependence of enzymatic activity on temperature in Arrhenius coordinates were revealed.

CHARACTERISTICS AND BASIC PROPERTIES OF ACTINOBACTERIA FROM MYTILUS GALLOPROVINCIALIS OF ODESSA GULF OF THE BLACK SEA

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Background. Today, both academic and commercial interest in marine actinobacteria is growing. Because they live in a unique environment that promotes the synthesis of new biologically active metabolites with various types of biological activity.

The **aim** of the work was the isolation, primary identification and study of morphological, cultural, physiological, biochemical properties of actinobacteria from the Black Sea mussels (*Mytilus galloprovincialis*).

Methods. Isolation was performed on oat agar with sea salt, Gause 1 and Gause 2. The cultural, physiological and biochemical properties was studied according to generally accepted methods. Identification of strains by fatty acid composition was performed by gas chromatography using an automatic system for the identification of microorganisms MIDI Sherlock. Antibiotic sensitivity and antagonistic activity were evaluated by disc-diffusion method.

Results. During the study, 14 isolates of marine actinobacteria were obtained. By comparing the fatty acid spectra using the MIDI Sherlock library (ACTIN 3.80), all 14 strains of actinobacteria from mussel samples were identified with different similarity indices as belonging to *Streptomyces* genus. The ability of strains *Streptomyces sp.* Myt 2, Myt 6, Myt 7b to produce melanoid pigment was detected on ISP6 and ISP7 media. Isolated strains of actinobacteria are able to attract various sources of carbon into their metabolism. *Streptomyces sp.* Myt 1 and Myt 10 strains do not use fructose and arabinose, and have very moderate growth on media with xylose and sorbitol. Strains *Streptomyces sp.* Myt 12a and Myt 12b were characterized by the lowest ability to involve various carbohydrates in their metabolism. The study of physiological and biochemical properties of actinobacteria strains isolated from mussels found that strains *Streptomyces sp.* Myt 1, Myt 4, Myt 8, Myt 10 do not form hydrogen sulfide. Only strains of *Streptomyces sp.* Myt 1, Myt 4 and Myt 8 from all studied strains of actinobacteria isolated from mussels reduce nitrates to nitrites, but no strain restores to molecular nitrogen. Milk is able to coagulate 42.8% of the studied strains of actinobacteria and only 28.5% peptonize it. All strains of actinobacteria showed complete resistance to penicillin, oleandomycin, nystatin and ampicillin. Partial sensitivity to erythromycin was shown by strains *Streptomyces sp.* Myt 5, Myt 8, Myt 12, and to rifampicin, strains *Streptomyces sp.* Myt 12a, Myt 12b, Myt 10, Myt 8. The greatest antagonistic activity against 12 indicator test-strains were found in following strains of actinobacteria: *Streptomyces sp.* Myt 2, Myt 3a, Myt 3b, Myt 5, Myt 7b, Myt 7ch, Myt 8, Myt 12a and Myt 12b.

Conclusions. According to the literature data, marine actinobacteria is a promising source of bioactive molecules. Therefore, isolation of actinobacteria from marine environments and study of their metabolite profiles is promising for the subsequent search for new antimicrobial compounds.

EFFECT OF THIAZOLE DERIVATIVE COMPLEXED WITH POLYMERIC CARRIER ON CELLULAR ULTRASTRUCTURE OF MURINE LYMPHOMA CELLS *IN VITRO*

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Aim. The newly synthesized thiazole derivative N(5Benzyl-1,3-thiazole-2yl)-3,5-dimethyl-1-benzofuran-2-carboxamide (BF1) in complex with polymeric carriers based on polyethylene glycol (PEG) has been shown significant cytotoxic effect toward the number of tumors cells. We have found that this toxicity is related to activation of apoptosis, DNA damages, arrest of cell cycle on G2/M stage and to activation of the antioxidant defense system. However, the structure of tumor cells under this circumstances was not studied yet. The aim of this study was to investigate the action of the thiazole derivative BF1 conjugated with PEG-based polymeric carrier on cellular structure of NK/Ly lymphoma cells.

Methods. The study was performed on white wild-type male mice with grafted NK/Ly lymphoma. Abdominal drainage of ascites was performed on the day 10 after cell inoculation. BF1, polymeric carrier Th3 and combination of BF1+Th3 (Th4 and Th14) at a final concentration of 10 μM were added to the lymphoma cells and incubated for 15 min. To improve solubility and permeability of studied compounds two different types of the preparation were used. The images of lymphoma cells was obtained using electronic microscopy and analyzed by computer program ImageJ.

Results. The quantitative analysis of electronic photographs of control untreated lymphoma cells has shown that the nucleus takes up a large part (~40%) of the cell area. Each cell contains a number of mitochondria with different sizes and shapes. A few number of lysosomes (~6 per cell) were also identified. BF1 at the concentration 10 μM caused apoptotic destructive changes in lymphoma cells, such as a shrinkage of cell, deformed and decrease the nucleus size and destruction of plasma membrane. The number of mitochondria were increased compare to control. It was found that the ratio of the area of nucleus and cytoplasm (N/C ratio) of control NK/Ly cells was 0.6. BF1 decreased this ratio by 46%. The complexes Th4 and Th14 also led to a cell shrinkage, fragmentation or even loss of the nucleus, blabbing and destruction of the integrity of the plasma membrane and changes in the shape of mitochondria compared with control NK/Ly cells. It was found, that both of investigated complexes increased the number of mitochondria and lysosomes and decreased N/C ratio compare to control by 49% and 52%, respectively. Th4 also decreased the area of mitochondria to 38% vs control cells.

Conclusions. Studied thiazole derivative in complex with polymeric carrier at concentrations of 10 μM leads to apoptotic and necrotic like changes in the ultrastructure of lymphoma cells. Complexes Th4 and Th14 significantly reduce the nuclear cytoplasmic index, increase the number of lysosomes and mitochondria, Th4 also increased the area of mitochondria. Thus, studied complexes of thiazole derivative and PEG-containing polymeric carrier can be used for further investigations as potential antitumor drugs.

MODIFICATION OF NUTRIENT MEDIUM COMPOSITION FOR *IN VITRO* CULTURE ESTABLISHMENT OF PAULOWNIA TOMENTOSA

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Background. *In vitro* culture establishment is one of the most important stages of microclonal propagation of plants. At this stage, problems with the release of oxidation products, phenolic compounds and other toxic metabolites often occur when the initial explant contacts with the nutrient medium. This issue is crucial for most wooden plants species and can potentially lead to death or a significant delay in the growth of explants *in vitro*. To prevent it, using of ascorbic acid as an antioxidant could be possible. The aim of the study is to determine the effect of adding different concentrations of ascorbic acid to Murashige and Skoog medium (MS) on the survival rate of *Paulownia tomentosa* explants, the time of first proliferation and the number of formed shoots.

Methods. The stock material of paulownia was selected from donor trees grown in open ground. The prepared plant material was surface-sterilized according to the protocol, then explants were planted in vials with MS medium. The medium was prepared according to standard composition, but with the addition of different concentrations of ascorbic acid after autoclaving. Medium variants with 50, 100, and 200 mg/l and a control variant without ascorbic acid were made. For each MS medium variant 30 explants were analyzed, the study was performed in triplicate. After 30 days, the results were evaluated by three parameters. The survival rate was calculated as ratio of the alive explants number to all planted. First proliferation time was estimated as the day cultivation number when the growth of new vegetative organs from meristems on the explant was recorded for the first time. The number of formed shoots was counted as the average quantity of meristems that reproduced new shoots on an explant during observation.

Results. The addition of all experimental concentrations of ascorbic acid showed certain changes compared to the control. The survival rate of explants with the addition of 50 mg/l, 100 mg/l and 200 mg/l increased by 33, 30 and 26%, respectively. First proliferation occurred, on average, 0.82 days earlier in explants on a medium with 50 mg/l of ascorbic acid, while in other variants, the proliferation period was approximately equal to the control. The difference between the number of formed shoots in the control and the variant with 200 mg/l was statistically insignificant for paulownia explants. Addition of 50 mg/l and 100 mg/l of ascorbic acid to the MS medium contributed to the formation of 0.73 and 0.65 more shoots from one explant than in the control.

Conclusions. Thus, the addition of ascorbic acid to the nutrient medium had a positive effect on the survival rate, growth and development of the initial explants of paulownia in the early stages of microclonal propagation. According to the results, the concentration of 50 mg/l of ascorbic acid is the most promising for use and can be recommended for modification of MS medium composition for *in vitro* culture establishment of *Paulownia tomentosa.*

FACTORS INFLUENCING SHAPE OF SODIUM ALGINATE MICROCAPSULES

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Aim. Methods for obtaining microcapsules based on polymers of animal and vegetable origin are rapidly developing branch of biotechnology. One of these methods is electrospray or formation of microcapsules under influence of an electrostatic potential. The main advantage of this method is the possibility of obtaining microspheres of uniform size in a wide range from 50 µm to 1 mm. Shape of the obtained microcarriers can be affected by such parameters of electrospray as applied voltage, the distance between the contacts, and the polymer dosing rate. The purpose was to study the influence of electrospray-operating parameters on shape of alginate microcapsules.

Methods. To obtain alginate microcapsules we used an equipment developed in our department. Low viscosity sodium alginate to obtain microcapsules and $CaCl_2$ solution of 2% concentration for their copolymerization were used. The capillary diameter was 200±10 µm, the distance between the contacts was 20±0.1 mm. Evaluation of the shape and size distribution of sodium alginate microcapsules was carried out by analysing the obtained micrographs (confocal microscope Axio Obzerver Z1, Carl Zeiss). The analysis of equivalent circle diameter, ellipticity coefficient and Wadell sphericity coefficient was carried out using the ImageJ 0.52 and Toolbox 1.0 software. All results are given as mean ± standard error.

Results. An analysis of the differential distribution curves by equivalent circle diameter of microcapsules depending on voltage has shown that up to 5000±158 V there is one pronounced peak in the region of 90±4.9 µm. Subsequent increase in voltage leads to the formation of a second distribution peak with a maximum of 240±10.4 µm. The changes in voltage does not affect the microcapsules distribution by ellipticity coefficient. Three main peak on the differential curves of the distribution by Wadell sphericity coefficient (0.76±0.03, 0.84±0.04 and 0.93±0.04) characteristic of all voltage were identified. At voltages above 5000±158 V, the fourth peak appears (0.68±0.03) and a significant decrease in the percentage of microcapsules with Wadell sphericity coefficient of 0.93±0.04 from 89% to 53% is observed. It has been shown that an increase in the dosing rate of sodium alginate from 5±0.1 ml/h to 40±0.1 ml/h leads to an almost 2-fold increase in equivalent circle diameter of the microcapsules. The distribution of microcapsules by ellipticity coefficient and Wadell sphericity coefficient was not affected by the dosing rate.

Conclusions. Voltage above 5000±158 V during the production of microcapsules by electrospray leads to significant changes in distribution of microcapsules by equivalent circle diameter and Wadell sphericity coefficient. Increasing the dosing rate of the sodium alginate leads to a significant increase in equivalent circle diameter, but does not affect the distribution of microcapsules by ellipticity coefficient and Wadell sphericity coefficient.

$GDVO_4:EU^{3+}$ NANOPARTICLES AFFECT PROLIFERATION OF FIBROBLAST CULTURE *IN VITRO*

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Aim. To investigate the effect of gadolinium orthovanadate doped with europium ($GdVO_4:Eu^{3+}$) nanoparticles on the proliferative activity of fibroblast cell culture.

Methods. Fibroblasts were obtained from 20day-old rat embryos. Cells were cultured in DMEM with 10% fetal serum at 5% CO_2 and 37°C for up to 3 passages. These fibroblasts were transplanted into 12well plates at 100,000 cells per well and left for a day to provide adhesion. Thereafter, $GdVO_4:Eu^{3+}$ nanoparticles at concentrations of 0-30-65-130-260-520 μg/ml were added. Following 72 hours, the cells were harvested with EDTA-trypsin solution. The number of cells was determined using a hemocytometer. The doubling time was calculated. Statistical data processing was conducted by performing the Kruskel-Wallis and Dunn's tests.

Results. Fibroblast cultivation with the studied nanoparticles in small concentrations resulted in an increase in the proliferative activity, evidenced by a decrease in the doubling time of cell population. At concentrations of up to 65 μg/ml, the doubling time increased to a greater extent than that of intact cells, while at the concentration of 130 μg/ml, the number of cells decreased following 72 hours. At concentrations of 260 μg/ml and above, it was technically impossible to count cells due to their small number. The data obtained in this study are consistent with our previous observations on the toxicity of these nanoparticles and provide insights for the application of nanoparticles in low concentrations for wound healing. When comparing the evaluation of proliferative activity with other methods used for the assessment of $GdVO_4:Eu^{3+}$ nanoparticle toxicity, it can be assumed that this is one of the most sensitive evaluation criteria.

Conclusions. $GdVO_4:Eu^{3+}$ nanoparticles increase proliferation rate of fibroblasts in cell cultures at small concentrations while reducing it at the high ones.

DOMAIN-INDEPENDENT INHIBITION OF CBP/P300 ATTENUATES α-SYNUCLEIN AGGREGATION

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Aim. Neurodegenerative diseases feature compromised neuronal functions and survival, associated with failed proteostasis and accumulation of insoluble protein aggregates. In Parkinson's disease, phosphorylated and aggregated α-synuclein is an integral part of the pathological hallmarks of the disease, Lewy bodies and neurites. The aim of this study is to explore the role of two closely related proteins, CBP and p300, that contain both lysine acetyltransferase and bromodomains and are responsible for the recognition and enzymatic modification of lysine residues, as promising targets for selective attenuation of α-synuclein aggregation.

Methods. To investigate the effect of the series of inhibitors for CBP/p300 (A485, GNE049, and SGC-CBP30), SMARCA2/4 (PFI3), TATA-box binding associated protein 1 (3i5001), and bromodomains and extra-terminal domain family proteins ((+)JQ1) on the accumulation of the phosphorylated at Ser129 α-synuclein in dopaminergic neurons, we induced α-synuclein aggregation in mouse embryonic (E13.5) midbrain cultures with pre-formed fibrils on day *in vitro* (DIV) 8 and treated the cells with above-mentioned compounds on DIV 12. The cells were fixed on DIV 15 and immunostained for tyrosine hydroxylase (TH) and phosphorylated α-synuclein. Lewy -body-like intracellular accumulations in TH-positive dopaminergic neurons were quantified using semi-automated image analysis.

Results. Potent bromodomain inhibitor of CBP/p300, GNE049 at all tested concentrations (0.1, 1, and 10 μM) decreased α-synuclein aggregation, similarly to positive control glial cell line-derived neurotrophic factor (GDNF). Acting through the same molecular mechanism, SGC-CBP30 decreased α-synuclein aggregation at 1 μM; at 10 μM, its effect was similar to positive control GDNF. Also KAT domain inhibitor of CBP/p300 A485 at concentrations of 0.1, 0.5, and 1 μM decreased α-synuclein aggregation to a similar extent as positive control GDNF. Other bromodomain inhibitors of SMARCA2/4 (PFI3), TAF1 (3i5001), and BET family proteins ((+)JQ1) showed little or no effect on α-synuclein aggregation and the number of TH-positive cells, highlighting the selective effect of CBP/p300 inhibition in dopaminergic neurons.

Conclusions. Our findings suggest the potential for the development of α-synuclein aggregation-modulating CBP/p300 inhibitors as disease-modifying therapeutic approach in PD. Thus, further repurposing studies of systemically administered specific CBP/p300 inhibitors, initially developed for cancer therapy, are justified for the treatment of Parkinson's disease.

THE EFFECT OF VITAMIN D_3 ON THE BINDING OF SARS-COV-2 S-PROTEIN TO ACE2 ON THE SURFACE OF EPITHELIAL CELLS

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Aim. Recent studies showed an association between serum vitamin D_3 (VD_3) level and COVID-19-related severity/mortality. However, the role of VD_3 status under SARS-CoV2 remains unclear. The aim was to evaluate the effect of the VD_3 hormonally active form ($1,25(OH)_2D_3$) on the binding of SARS-CoV2 spike (S) protein to angiotensin-converting enzyme 2 (ACE2) on epithelial cells.

Methods. Genetic construct for the production of recombinant receptor-binding domain (RBD) of SARS-CoV2 S-protein in a prokaryotic system was created using pET28b plasmid with a polyhistidine tag (His-tag). Recombinant RBD was purified by an immobilized metal affinity chromatography (IMAC) on the column with Ni^{2+}NTA agarose. The purity of the protein was analyzed by SDS-PAAG electrophoresis. Kidney epithelial cells of African green monkey (MA-104) and Swine testis (ST) cells with ACE2 overexpression were treated with $1,25(OH)_2D_3$. Metabolic activity after $1,25(OH)_2D_3$ action (0.0004-400 nM, 24 h) was measured by MTT assay. The binding of fluorescently labeled RBD to ACE2 was determined by flow cytofluorimetry (FC) and visualized by confocal microscopy.

Results. Genetic construct pET28b-mNG-RBD encoding RBD (amplified from pET28a-SARS-CoV2-S-RBD-entero plasmid) with fluorescent protein mNeonGreen (mNG) (amplified from pLVX-IRES2-mNG plasmid) was developed based on prokaryotic pET28b vector. pET28b-mNG-RBD allowed expressing recombinant RBD with His-tag in *E. coli* BL21 Rosetta (DE3) strain (with 1 mM IPTG). The final concentration of RBD-mNG (M.W. 51 kDa) was 0.5 mg/L. Based on the FC data, the optimal concentration for RBD-mNG binding to ACE2 on the MA-104 and ST-cells was 70 μg/10^6 cells. Using MTT assay, we showed that the metabolic activity of MA-104 cells was reduced by $1,25(OH)_2D_3$ in a range of 0.78-50 nM in a dose-dependent manner with the minimum at 0.78 nM (by 25,26% lower vs. untreated cells). Interestingly, $1,25(OH)_2D_3$ in the range of 1.56-400 nM did not change the metabolic activity of ST-cells, and it was inhibited only at 0.78 nM by 15.31% compared vs. control. Finally, $1,25(OH)_2D_3$ increased the RBD-mNG to ACE2 binding on MA104 cells based on the percentage of RBD-mNG+ cells (25 nM – 73.7±3.3%, 50 nM – 70.2±4.2%, 100 nM – 69.3±4.8% compared vs. 65.5±4.1% in control); while $1,25(OH)_2D_3$ did not affect the RBD-nMGmNG-ACE2 complex binding on ST-sells, and the percentage of RBD-nMGmNG+ cells was 10.3±1.0% in control, 9.7±0.9% at 25 nM, 9.3±0.7% at 50 nM, 7.9±0.8% at 100 nM.

Conclusions. $1,25(OH)_2D_3$ enhanced binding of RBD- mNG nMG-ACE2 complex with a peak at 25 nM on MA-104 cells, while ST-cells were less sensitive to $1,25(OH)_2D_3$ action based on the unchanged metabolic activity and RBD-mNG nMG-ACE2 binding. Our data suggest an ineffectiveness of $1,25(OH)_2D_3$ at the stage of SARS-CoV2 cell entering. However, the further studies of the mechanisms of $1,25(OH)_2D_3$ action using recombinant RBD will allow to uncover the possible pharmacological efficacy of VD_3 in the context of COVID-19.

SPECTROSCOPY STUDIES OF ERYTHROCYTE MEMBRANES AND OXIDATIVE PROCESSES IN PATIENTS WITH CONTROLLED AND TRUE RESISTANT ARTERIAL HYPERTENSION

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Aim. The purpose of the study is to explore the condition of erythrocyte membranes and oxidative processes in patients with controlled (CAH) and true resistant (TRAH) arterial hypertension.

Methods. The study included 40 patients (Department of Hypertension, NSC "Strazhesko Institute of Cardiology", NAMS of Ukraine,) male and female aged 45 to 65 years with CAH (arterial hypertension, stage II) and TRAH. The number of men (52%) and women (48%) did not differ significantly. The microviscosity of erythrocyte membranes was assessed by spin probes using a nitroxyl radical based on adamantane. The final probe concentration in the samples was 5×10^4 M. EPR spectra were recorded using a Varian E3 Xband spectrometer (9 GHz). Activity of enzymes (myeloperoxidase, paraoxonase1, superoxide dismutase, catalase), content of lipid and protein oxidation products and reduced glutathione were determined spectrophotometrically. Statistical processing was performed using the software package for biometric studies WinPEPI.

Results. In patients with CAH and TRAH there is an impairment of oxidative status towards the development of oxidative stress, as evidenced by a significant ($p<0.05$) increase in myeloperoxidase activity, accumulation of lipid and protein oxidation products, decreased antioxidant enzyme activity (arylesterase activity of paraoxonase1, superoxide dismutase and catalase activity) and reduced glutathione content. At the same time, patients with TRAH by certain indices (activity of myeloperoxidase, superoxide dismutase and catalase, the content of reduced glutathione) have deeper changes in redox balance compared to patients with CAH. The structural-dynamic changes of erythrocyte membranes in patients with CAH and TRAH by the method of spin probes with the use of nitroxyl radical AdTEMPO (bis(1oxyl-2,2,6,6tetramethylpiperidinyl-4) ether 5,7dimethyladamantane-1,3dicarbonic acid ester) are studied for the first time.

Conclusions. The obtained results may indicate an impairment of the structural organization of the lipid layer and its integrated proteins, which is expressed in an increase of τ_{eff} (correlation time of rotational diffusion – inversely proportional to the rotation rate of the radical, the higher the value, the slower the rotation) in patients with CAH and TRAH compared to practically healthy individuals. The difference in the intensity of the residual signal of spin probes for the studied groups indicates an impairment of the erythrocyte antioxidant system, which correlates with the obtained biochemical parameters. Changes in the functional activity of the erythrocyte membrane surface are also evidenced by a decrease of sorption capacity of erythrocytes in patients with CAH and TRAH. Demonstrated structural and dynamic changes in erythrocyte membranes in patients with CAH and TRAH may be caused by impairment of redox status in the direction of oxidative stress, as the latter is one of the common mediators underlying the pathophysiological processes of hypertension.

IN SILICO STUDY OF COMPLEX FORMATION OF CHEMICALLY MODIFIED NUCLEIC BASES

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Aim. To study *in silico* in a fragment-to-fragment approach the possibility of the formation of stable [Het-BioM]-complexes with chemically modified nucleic bases by the π-stack mechanism and due to hydrogen bonding [HB]-complex.

Methods. The main characteristics of the electron structure of the studied compounds were calculated by DFT [wB97XD/6-31G(d.p.)] method (package GAUSSIAN 09).

Results. The introduction of fluorine in position 5 of uracil almost does not affect its affinity, does not change the nature of the S0-S1 transition, on the contrary, and reduces the S0-S2 transition energy (0.2 eV), increasing the possibility of [Het-BioM] complex formation by the π-stack mechanism and thymidylate synthase is blocked. The replacement of the fluorine atom by the chlorine atom in the uracil molecule (3) led to a change in the nature of the S0-S1 transition (n-pi* changed to pi-pi) and a decrease in the energy of this transition to 0.34 eV. Therefore, such a compound is primarily focused on the [Het-BioM] complex formation by the π-stack mechanism. The introduction of the electron-accepting sulfo group in position 5 of uracil (2) increases its electron-accepting properties without changing the nature of the S0-S1 transition (n-pi), increasing the probability of overlap of electron density with the second component of the [HB]-complex by 8 times. The S0-S2 (pi-pi*), transition energy remains approximately the same as in compound 1. Although the acceptor atoms of fluorine and chlorine have an inverse effect on the basicity of uracil, fluorine slightly increases the stability of the complex at 1.8 kcal/mol, and sulfo group stabilizes [HB]-complex at 2.5 kcal/mol. It can be assumed that such a replacement in the uracil molecule will have better therapeutic properties in the fight against cancer. The introduction of chlorine into the uracil molecule shows the low energy of stabilization of the [HB]-complex at 6.1 kcal/mol, because such a replacement will change the nature of the first electronic transition in molecule 3, and change the probable mechanism of complexation.

Conclusions. Thus, a detailed *in silico* study in the fragment-to-fragment approach shows that nitrogen π-conjugate molecules can form stable [Het-BioM] complexes with nucleic bases through π-stack interaction, as well as due to hydrogen bonding between LEP in twocoordinated nitrogen atom and proton of the functional groups of the nucleic bases. The possibility of complex formation by different mechanisms depends on the following quantum-chemical descriptors: parameter φ_0 of both components, as well as on the nature of their first electronic transitions. The introduction in the uracil of the acceptor sulfo group stabilizes the [HB]-complex at 2.5 kcal/mol, and the introduction of the chlorine atom at the same position changes the nature of the S0-S1, S0-S2 electron transitions so that such a molecule is characterized by the formation of a [π,π]-complex instead of [HB]-complex.

INFLUENCE OF CAFETERIA DIET ON LEVEL OF LIPID PEROXIDES IN LIVER AND ADIPOSE TISSUE OF MICE

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Background. Hypercaloric diets are characterized by a large amount of fried, fatty products, sweets and a small fraction of fruits, vegetables, cereals. This diet increases the risk of obesity and type 2 diabetes development. In a cafeteria diet delicious but harmful in excess, unhealthy, hypercaloric human foods are consumed by animals for the modeling of obesity and diabetes. Bioactive components of nutrition are considered as a tools of minimizing and preventing the development of these disease conditions. One of main characteristic that illustrates changes in metabolism is the development of oxidative stress. The major effect of this condition is damage of molecular and cellular structures. One of the markers of oxidative stress is the level of lipid peroxides, which increases as a result of the interaction of reactive oxygen species with unsaturated fatty acids.

Aim. The purpose of the work was to determine the level of lipid peroxides in the liver and adipose tissue of mice which consumed a cafeteric diet and a solution of α-ketoglutaric acid.

Methods. 8month old C57BL/6J mice were divided into 4 groups with 4 individuals in each for 8 weeks period. The first group (control) consumed basic feed. The second group consumed a 1% solution of α-ketoglutaric acid in drinking water. The third group consumed cafeteria diet, consisting of human diet products: cheese, ham, sausages, sweet and salty peanuts, corn sticks, chocolate, crips. The fourth group consumed cafeteria diet with a solution of α-ketoglutaric acid in drinking water. The determination of lipid peroxides was carried out by the ability of oxidized ferum (III) to bind to the xylenol orange with the formation of a colorful complex. The intensity of the color of the complex depends on the level of lipid peroxides and is determined spectrophotometrically.

The **results** of the study showed increased level of lipid peroxides by 60% in liver of mice that consumed α-ketoglutaric acid compared to the control group. In the group with a cafeteria diet, this index was increased by 30% compared to the control group. In a group with a cafeteric diet and αketoglutaric acid, this level was increased by 9% compared to control. Consumption of experimental diets did not significantly affect the level of lipid peroxides in adipose tissue.

As a **conclusion**, the consumption of α-ketoglutaric acid and a cafeteric diet increases the level of lipid peroxides in liver and does not affect their production in adipose tissue.

A BIOMIMETIC 3D MODEL TO REPRODUCE WOUND-ASSOCIATED BIOFILM-RELATED INFECTIOUS PROCESS *IN VITRO*

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Aim. It is renowned that biofilm-associated infections are challenging to treat because of their increased tolerance to antimicrobial agents. However, traditional *in vitro* bacterial sensitivity assays do not often resemble that of biofilms seen *in vivo*. To improve translatability from laboratory to clinic, *in vitro* models must better reproduce the host environment. Therefore, we aimed at the development of a biomimetic 3D model to reproduce the biofilm-related would-like infectious process of *K. pneumoniae in vitro*.

Methods. The 3D scaffolds were prepared by freeze-drying a solution of bovine atelocollagen I in 0.05M acetic acid. 105 HEK293 cells suspended in the DMEM medium containing 10% FBS were seeded on the scaffolds 72 hours before inoculation of the hospitally-isolated *K. pneumoniae* UHI 1667 strain. The two day-old biofilms were analysed using CLSM and CFU were assessed.

Results. 3D scaffold made from collagen as a growth matrix was performed to reproduce wound-like environment *in vitro*. To better mimic the damaged tissue environment, the scaffold was seeded with epithelial-like HEK293 cells and fetal bovine serum was added as a source of blood proteins. There were three different modifications of the liquid phase contents performed: №1: 90% DMEM/high glucose, 10% FBS; №2: 89,9% DMEM/high glucose, 10% FBS, 0,1% Bacto proteose Peptone; №3: 49,9 % DMEM/high glucose, 50% FBS, 0,1% Bacto proteose Peptone. After inoculation of *K. pneumoniae* UHI 1667, the microcosms with the models were placed in hermetic chambers with high carbon dioxide content and incubated stationary at 37^{0}C. Under such conditions, the pH in the cultures increased and was 7.5-8.9, which better mimics the wound-like environment. All three models demonstrated differences in bacterial culture behaviour. The CLSM imaging revealed strong biofilm formation and HEK293 cell destruction when biofilms formed. Interestingly, the production of functional amyloids by *K. pneumoniae* was detected only in variant №1 contained the lowest content of protein nutrients. The CFU of free-swimming subpopulations in all models confirmed that infection developed intensively. However, bacterial growth in model №1 with the lowest protein content was 10^7 CFU, the lowest microbial growth was observed in model №2 – 10^5 CFU and 10^8 CFU per ml was observed in model №3. All three models were used to assess the anti-biofilm effects of two antibiotics – colistine methansulfonate and azithromycin applied solely and in a combination.

Conclusion. The biomimetic 3D model was developed to reproduce the wound-associated biofilm-related infectious process *in vitro*.

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STUDY ON UBIQUITINATION OF DELETION MUTANTS OF MRPS18-2 IN MAMMALIAN CELLS

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Background. The MRPS18-2 protein belongs to a family of three mitochondrial ribosomal proteins MRPS18. All these proteins are encoded by the human genome. We have shown earlier that MRPS18-2 is involved in cell cycle regulation through interacting with a retinoblastoma associated protein, RB. Moreover, MRPS18-2 can induce malignant transformation and a stem cell phenotype. It is known that the ubiquitin–proteasome system regulates most cellular processes by controlling protein stability. We have found that the MRPS18-2 half-life is quite long, i.e., protein is characterized by a high stability. Moreover, we have shown that, unlike MRPS18-1 and MRPS18-3, MRPS18-2 show a high degree of co-localization with ubiquitin in the cell nucleus. However, what part of the MRPS18 2 protein is responsible for such phenomenon remains an open question. To study this question, we decided to prepare the deletion mutants of the MRPS18-2 and study its relation with ubiquitin *in vivo*, in mammalian cells.

Aim. The **aim** of this study is to monitor cellular localization and ubiquitination pattern *in vivo* of MRPS18-2 deletion mutants.

Methods. Constructs, encoding the GFP-tagged deletion mutants of the MRPS18-2 protein were created, namely GFP-MRPS18-2-NT (N-terminus of MRPS18-2, amino acids 1-89), GFP-MRPS18-2-CT (C-terminus of MRPS18-2, residues 170-248) and GFP-MRPS18-2-MP (a middle part of MRPS18-2, residues 90-180). These plasmids and the control plasmid, encoding GFP-MRPS18-2 full size, as well as plasmid HA-Ub, were introduced in the MCF7 cell line as an experimental model. Immunostaining and fluorescence microscopy was used to assess cellular localization of proteins.

Results. GFP-MRPS18-2 was expressed at high levels mainly in the cytoplasm, and a tiny fraction was also localized in the nucleus, as was shown by us before. Co-localization of GFP-MRPS18-2 and HA-Ub was detected in both, the cytoplasm, and the nucleus, although to a much lesser extent in the nucleus. Surprisingly, the GFP- MRPS18-2-MP deletion mutant co-localized with ubiquitin exclusively in the nucleus. In contrast, cells expressing high amounts of the GFP-MRPS18-2-CT mutant protein, showed cytoplasmic localization of the mutant protein. Moreover, such cells demonstrated characteristic signs of dying cells.

Conclusions. The study found that MRPS18-2 deletion mutants have a characteristic behavior in cell and also different co-localization pattern with ubiquitin. We propose that functional consequences of MRPS18-2 ubiquitination shall be studied further.

ENDOCYTIC PROTEINS ITSN1/2 FORM A COMPLEX WITH TAU

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Background. Alzheimer's Disease (AD) is an incurable age-related neurodegenerative disorder that affects approximately 44 million people worldwide with a constantly growing tendency of occurrence. Overexpression and accumulation of misfolded microtubule-associated protein Tau consider being one of the main hallmarks of AD. Multiple studies suggest that protein-protein interactions of Tau with numerous partner proteins may contribute to the development of AD and other tauopathies. Proline-rich motifs in the Tau structure enable its interactions with SH3 domain-containing proteins. Intersectins (ITSNs) are a conservative SH3 domain-containing scaffold protein family (consisting of ITSN1 and ITSN2 in mammals) with established functions in clathrin-mediated endocytosis, membrane trafficking, and actin cytoskeleton reorganization. While ITSN1 is overexpressed in AD with a still unclear role in the development of the disorder, there is no evidence of the involvement of ITSN2 in neurodegeneration. We hypothesize that ITSNs can form protein complexes with Tau.

Aim. To characterize Tau-ITSN complexes, revealing a possible role of intersectins in tauopathies including AD.

Methods. Full-length human MAPT (Tau) sequence was cloned in pCMV-Tag2 vector for overexpression in HEK293 cell line followed by *in vitro* GST-binding assays with GST-tagged SH3A-E domains of ITSN1 or ITSN2. Lysates of 293 cells expressing endogenous ITSN1 and ITSN2 were used for the precipitation of GST-tagged full-length Tau. Co-localization of overexpressed Tau and ITSN1 in MCF-7 cells was analyzed by laser scanning confocal microscopy.

Results. Endogenous ITSN1 and ITSN2 form complex with full-length Tau. Further analysis revealed the association of human Tau with SH3-domains of ITSNs. Ubiquitously expressed SH3A and brain-specific SH3A domains of ITSN1 equally bind full-length Tau, while only SH3C domain of ITSN2 precipitated human Tau *in vitro*. Immunofluorescence data confirmed co-localization of Tau and ITSN1 in MCF7 cells.

Conclusions. ITSNs were identified as novel protein partners for Tau *in vitro*, which, alongside the overexpression of ITSN1 in AD brains, may suggest a particular role of ITSN1 in the pathology of AD. Moreover, ITSN2 can be involved in AD development as well, despite the absence of evidence for ITSN2 to be differentially expressed in neurodegeneration. Considering the role of ITSNs in membrane trafficking and the fact of the enlargement of early endosomes in AD with the involvement of endolysosomal impairments in the aggregation and propagation of Tau, we suggest possible roles of ITSNs in Tau pathology, which can serve as a basis for the further studies.

A CASE OF DOUBLE (16/19) ALLELES AT DYS576 LOCUS

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Aim. To describe a case of double (16/19) alleles at the DYS576 locus and to clarify if it is an inherited mutation or a case of somatic mosaicism.

Methods. Samples of buccal epithelium were taken from three persons of the same family (grandfather-father-son). DNA was extracted by Chelex and quantified with "Quantifiler® Human Plus DNA Quantification Kit" and "7500 Real-Time PCR Systems" (AB). The isolated DNA was typed using the polymerase chain reaction method with a standard kit for the identification "PowerPlex Y23 System" (Promega) and the GeneAmp PCR System 9700 amplifier (AB). Fractioning and detection of fluorescently labeled amplified fragments were performed with 3500 Genetic Analyzer (AB) in POP4 polymer medium with the length of capillary 36.0 cm and run time of 42 minutes. Evaluation of the lengths of amplified fragments and the establishment of allele numbers were performed by the internal size standard WEN 500 and kit-specific allelic ladder, using the software package GeneMapper ID-X v1.5.

Results. In forensics, Y-STRs are known to be especially informative for male DNA fraction detection in samples involving male/female mixtures. In complex cases (multiple rape cases, mass graves, etc) Y-STRs are often used to clarify the number of contributors. Detection of additional Y-linked alleles in a profile is usually interpreted as the presence of material of more than one contributor. But many reports have already shown two alleles found for a single Y-linked STR locus (Kayser et al,1997, 2000; Kurihara et al, 2004; Çakir et al, 2004). In most such cases for a single male haplotype double alleles were detected just in one locus. While Bosch and Jobling (2003) have demonstrated three cases of Y-STR multiple alleles resulting from AZF region duplication followed by mutations in DYS389 II and/or in DYS439. Diederiche et al (2005) described cases of double alleles at three Y-STR loci (DYS437, DYS439, and DYS389 II). Evaluation of frequency of mutated Y-linked STR in forensic genetic analysis allows escaping misinterpretation of multiple peaks presence as mixed profiles. Here, we describe a case in which double peaks (16 and 19 repeats) in the DYS576 locus alleles range were detected in a male sample from Kharkiv (Ukraine). The same was also found neither in his father`s nor in his son`s samples (both of them possessed 19 repeats alleles only). The most likely explanation for the additional allele presence is somatic mosaicism resulting from primary mutational mechanism leading to changes in microsatellite length – polymerase template slippage, – due to the gain or loss of a single repeat as it is described in the stepwise mutation model (Valdes, Slatkin,Freimer, 1993).

Conclusion. A case of double (16/19) alleles in the DYS576 locus was fixed in a male sample from Kharkiv (Ukraine). It was proved to be noninherited but resulted from somatic mosaicism.

COMPARATIVE ANALYSIS OF EPILEPTIC ACTIVITY CAUSED BY BLOOD - BRAIN BARRIER LEAKAGE WITH NEURAL ACTIVITY INDUCED IN LITHIUM–PILOCARPINE EPILEPSY MODEL

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Background. The blood-brain barrier is a key structure that maintains the stability of the neuronal environment and ensures the transport of micro- and macromolecules from the capillary lumen to the brain parenchyma. Increased permeability of the barrier can be caused by trauma, infection, or hemorrhage which disrupts its transport function and increases the penetration of serum proteins, ions, and other substances into the brain with a further increasement in the seizures risk. Studies of epileptic-like activity which is caused by increased permeability of the blood-brain barrier are relevant today. Also, it is important to develop models convenient for further screening of possible therapeutic agents aimed at restoring barrier integrity.

Aim. This study aimed to examine the effect of increasing the hippocampal blood-brain barrier permeability on the development of epileptic activity and to compare the results with those obtained in the lithium-pilocarpine model of epilepsy.

Methods. To imitate the leakage of the blood-brain barrier during the registration of electrophysiological activity, the artificial cerebro-spinal solution was replaced with a solution of plasma ionic composition, followed by the addition of thrombin. The resulting epileptiform activity was compared with neuronal activity obtained in the lithium-pilocarpine model, which also leads to an increasement in the blood-brain barrier permeability. In our study, we recorded extracellular potentials from pyramidal neurons of the CA1 hippocampal area under conditions of the blood-brain barrier leakage. Also we registered the activity of pyramidal neurons in the acute phase of the lithium-pilocarpine epilepsy model. For our experiment we used male Wistar rats aged 19-21 days (35-45 g).

Results. We found that the replacement of the ACSF solution with a solution of plasma ionic composition causes seizure-like activity, the amplitude and frequency of which increase significantly when serum protein thrombin is added. The amplitude and frequency of epileptic activity in the acute phase of the lithium-pilocarpine model are higher than activity obtained under conditions of plasma ionic composition solution application and lower than with the addition of thrombin. Therefore, the results of this study indicate that the amplitude and frequency of epileptic activity in the acute phase of the lithium-pilocarpine model of epilepsy are significantly higher than an activity that occurs when using a solution of plasma ionic composition without the addition of proteins. When thrombin is added, the amplitude of epileptic activity is higher than in the acute lithium-pilocarpine model of epilepsy. Proteins and thrombin are transported to the brain parenchyma as a result of severe barrier damage and are unlikely to cause sufficient changes in the rat brain during 12 days after induction of the lithium-pilocarpine model.

THE EFFECT OF THIOSULFONATES ESTERS ON THE GLUTATHIONE LINK OF ANTIOXIDANT PROTECTION IN RATS.

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Aim. Our study **aim**ed to determine the effect of thiosulfonate esters, general formula of R-S(O)2S-R': S-ethyl-4-aminobenzenethiosulfonate (ETS), S-allyl-4-aminobenzene thiosulfonate (ATS), S-allyl-4 -acetylaminobenzenethiosulfonate (AATS), which is characterized by a broad spectrum of biological action, on the glutathione link of antioxidant protection in the tissues of rats.

Methods. The studies were performed on male white laboratory rats of the Wistar line, weighing 190–200g. The animals were divided into four groups of 5 animals each: I - control, II, III, IV - experimental. Animals in the control group were added 0.5 cm3 of oil to the diet once a day; II, III, IV groups - 0.5 cm3 of oil solution ETS, ATS, AATS (concordantly) at the rate of 100 mg/kg body weight. The experiment lasted 21 days. Tissue samples from brain, kidney, spleen and skeletal muscle were taken for analysis and tissue homogenates were prepared. In the homogenates determined levels of reduced glutathione (GSH), glutathione peroxidase (GP), glutathione reductase (GR).

Results. The studies showed an increase in the concentration of GSH in the kidney homogenates of rat kidneys in II, III, and IV experimental groups by 19%, 44%, and 19%, respectively, and a decrease in GP and GR activities by 23%, 30%, 27% and 16%, 28%, 30%, respectively. In the brains of rats of group II, which consumed ETS, we observed an increase in the level of GSH, GP, and GR, while in other experimental groups - the indicators probably did not change compared to controls. In the spleen, there was an increase in GP activity in rats of group IV by 48%, and in groups II and III, this enzyme's activity decreased by 35% and 22%, respectively. GR activity did not change, and GSH content decreased by 46%, 54%, and 54%, respectively, in II, III, and IV experimental groups. No significant changes were observed in rat muscle homogenates of all experimental groups compared to controls.

Conclusions. Analysis of data on the activity of the glutathione system in the tissues of rats of experimental groups indicates certain effects caused by ETS, ATS, and AATS. The decrease in GH activity in the kidneys and spleen of rats of the experimental groups can be explained by a reduction in NADPH level, which is synthesized in the glucose-6-phosphatedehydrogenase reaction of pentosephosphate shunt under the action of the studied compounds. While the increase in GSH levels in the kidneys of rats of all experimental groups may be due to the intensification of its synthesis de novo with the participation of γ-glutamylcysteinesynthetase. And the multidirectional changes in the activity of GP in the tissues under the action of the studied compounds indicate their different effect on the activation of the antioxidant system's protective mechanisms of the glutathione link.

MESENCHYMAL STROMAL CELLS STORAGE AT AMBIENT TEMPERATURE

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Background. Mesenchymal stromal cells (MSCs) possess high proliferative potential, the ability to multilinear differentiation, low immunogenicity and immunomodulatory properties. MSCs are increasingly used in various fields of biology, tissue engineering, etc. Short-term storage at ambient temperatures may both solve problem of cell injury associated with cryopreservation and simplify transportation between clinical centers.

Aim. To study viability, metabolic activity, and cell cycle of MSCs under culture and during storage at ambient temperature in form of monolayer, suspension, and encapsulated in alginate microspheres (AMS).

Methods. The experiments were performed on human dermal MSCs derived after adult donors informed consent. Storage was performed in sealed containers at 22°C in alfaMEM supplemented with 10% of fetal bovine serum. Viability (FDA/EB dual staining), metabolic activity (Alamar blue-test) were assessed on 0, 3, 7, 10 and 14 days of storage. For cell cycle analysis the FUCCI Cell Cycle Sensor was used, live-cell imaging of cell cycle progression of MSCs cultured in monolayer or in AMS was performed with confocal laser scanning microscope. Abcam Cellular ROS Assay Kit (Deep Red) was used for assessment of ROS level, basal and induced by incubation with hydrogen peroxide.

Results. Viability by FDA/EB decreased during MSCs storage in form of monolayer for 70%, in suspension for 40% at 7 day compared with initial viability. Metabolic activity reduced even more sharply. During storage of MSCs in the form of a suspension spheroid formation was revealed at times. On 7th day of MSCs storage encapsulation supported the viability and metabolic activity of 85% and 55% of initial indexes, correspondently. Metabolic activity of cells after 24 hr of culture in AMS decreased 40% compared to MSCs in monolayer. Cell cycle analysis showed that MSCs were completely arrested in G1 phase in 48 hrs after encapsulation. ROS sensor fluorescence which directly reflected real-time intracellular ROS level was 32.8±5.2 and 2.32±0.25 RFU/cell in intact monolayer and AMS, respectively. After 2 hrs incubation with 3 mM hydrogen peroxide the fluorescence of ROS sensor was 154.5±7.8 RFU/cell in monolayer and 11.78±0.44 RFU/cell in AMS. Therefore, encapsulation of MSC in AMS decreased both basal, and induced ROS level.

Conclusions. Encapsulation of MSCs in alginate microspheres was shown as benefit approach for short-term storage and transportation under ambient temperature. Changed metabolic activity rate, cell cycle reversible arrest, and increased oxidative stress resistance of encapsulated MSCs were revealed as factors of resistance to storage at ambient temperature.

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INTERPLAY BETWEEN LOCAL CELL MORPHOLOGY AND KINETICS OF HIPPOCALCIN CALCIUM-DEPENDENT INSERTION

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Background. Hippocalcin (HPCA) is a neuronal calcium sensor protein that regulates neuronal functions in the hippocampus. Binding of calcium ions to HPCA induces conformational changes and highly heterogeneous insertions in the cellular membrane. Prior research demonstrates that the pattern of HPCA insertion in neurons corresponds to certain conservative regions. Although, observed biophysical properties of this process frequently vary among different studies. Due to the complex spatial structure of neurons it is possible that factors other than the HPCA's own biophysical properties influence the calcium-dependent behavior of the protein.

Aim. This study examines how HEK 293 cell morphology affects calcium-dependent HPCA behavior.

Methods. HEK 293 cells were cultured in a 12-well plate covered with 18-mm coverslips at 37°C and 5% CO2. Cells were incubated in a Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 0.25% gentamicin. On days 3-4, lipofection with a chimeric HPCA-TagRFP gene-encoding plasmid (1 ug of DNA per well) was performed. Transfected cells were loaded with the calcium chelator NP-EGTA and the calcium dye Fluo-4 prior to imaging. 24-36 hours after transfection, images were acquired using the Olympus FV 1000 confocal system. Three repetitive laser-induced uncaging from NP-EGTA were used for stepwise calcium levels increase. Python scripts were used to perform semi-automatic image analysis (code is available in GitHub repository github.com/wisstock/trans_scripts).

Results. Obtained data indicates that the insertion intensity is proportional to the local cytoplasm volume, which we define as the distance between the nucleus and the corresponding plasma membrane regions. It has been observed that incorporation is greater in regions of the plasma membrane, which correspond to lower cytoplasmic volumes (short distance from nucleus to membrane), and diminishes gradually with increasing distance from the nucleus. Conclusion. Our findings indicate that, despite the conservative insertion regions, the kinetic properties and maximal values of the HPCA insertions are significantly different along the plasma membrane. We hypothesize that the insertion process is strongly influenced by the local cell morphology via diffusion time of HPCA calcium-bound form. Differences in the distances that protein overcomes before insertion into the cellular membranes affect the kinetics of this process. And in regions where insertion occurs rapidly and consistently, the volume of cytoplasm is negligible. Therefore further research on HPCA signaling should consider not only the local morphology and composition of the membranes but also the neuron morphology diversity.

AZITHROMYCIN REVEALS BIOFILM–INHIBITORY ACTIVITY AND POTENTIATES NON-BACTERICIDAL COLISTIN METHANESULFONATE AGAINST KLEBSIELLA PNEUMONIA

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Background. Gram-negative antibiotic-resistant infections have become a global health problem. An intrinsic capability of many pathogens to form a biofilm underlies persistence, survival, and resistance to the immune system and antibacterial therapy, with biofilms generally providing phenotypic resistance when genetic resistance might be restricted. However, this may not always be the case, as *P. aeruginosa* biofilms are susceptible to azithromycin (AZM) whereas planktonic (non-biofilm) cells are not. As for *P. aeruginosa*, *K. pneumoniae* infection is associated with biofilm formation and persistence and isolates also share similar antibiotic resistance profiles. Thus, we have hypothesized that some macrolides might possess similar anti-biofilm activity against *K. pneumoniae*.

Methods. *K. pneumoniae* ATCC 10031 and hospital strains isolated from Ukrainian patients were used in the biofilm and plankton assays. Biofilms were cultivated in the 96-well plate. The biofim biomass was measured directly as optical density at 570 nm. Total metabolic activity was measured following 2 hours of incubation with 0.05% MTT, sedimentation and measure of absorption at 570 nm of the DMOS-dissolved sediments.The scaffolds were prepared by freeze-drying of 40 mg/mL solution of bovine atelocollagen I in 0.05 M acetic acid. 105 human embryonal kidney cells (HEK293) suspended in DMEM medium containing 10% FBS were seeded on the scaffolds 72 hours prior to inoculation with 104 MDR *K. pneumoniae* UHI 1667 cells.

Results. We have evaluated anti-biofilm and bactericidal effectiveness of eight macrolides against *K. pneumoniae* ATCC10031 and found that AZM had a significant anti-biofilm effect. Than, ATCC10031 and eleven hospital isolates, including six antibiotic-sensitive, four MDR and one PDR strain were used to study the effects of AZM and colistin when applied solely and in a combination. Although colistin, an antibiotic of choice for carbapenem-resistant *K. pneumoniae*, showed low effectiveness against both planktonic and biofilm cultures, we found the combination of colistin and AZM led to a synergistic effect, decreasing the MIC of colistin up to serum C_{max} levels which could increase the effectiveness of colistin therapy in treating *K. pneumoniae* infections for sensitive, NDR and PDR strains. This effect was also demonstrated in the biomimetic 3D models of wound-associated infection. Specifically, low anti-biofilm effectiveness was demonstrated for colistin when stronger anti-biofilm affects were noticed for AZM. The combination of AZM and colistin demonstrated the highest effectiveness in suppression of *K. pneumoniae* infection (Poster presented by Pokholenko et al.).

Conclusions. Novel antibiotic combinations may act synergistically to inhibit the growth of multidrug-resistant bacterial pathogens but predicting which will be successful is difficult, and standard antimicrobial susceptibility testing may not identify important physiological differences between planktonic free-swimming and biofilm-protected

surface-attached sessile cells. A combination therapy using anti-biofilm AZM with a specific antibiotic such as colistin might be used to combat MDR *K. pneumoniae* infections; the use of AZM potentiates colistin providing an effective solution for PDR and MDR infections.

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DNA DOUBLE STRAND BREAKS IN ACUTE MYELOID LEUKEMIA BLASTS OF DIFFERENT MATURITY

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Background. DNA double-strand breaks caused by various exo- and endogenous genotoxic factors may lead to various pathological processes in cells including malignant transformation. Recent data suggest that DNA damage in hematopoietic stem cell may be involved in leukemogenesis, although such evidence is limited and controversial.

Aim. To evaluate the level of DNA double-stranded breaks in cells of patients with acute myeloid leukemia (AML) regarding the expression of stem cell and differentiation antigens on blast cells.

Methods. The study was performed on bone marrow samples of 31 patients with AML (38.7% of men and 61.3% of women, mean age 62.1±2.6 years). The content of blast cells exceeded 70% in all samples included in the study. DNA double-strand breaks level was detected with a neutral version of the DNA comet assay by calculating the percent of DNA in the tail (% tDNA). FAB classification was used to assess the differentiation status of blast cells. Expression of CD34, MPO, CD117, CD11b, and CD14 antigens was analyzed by flow cytometry.

Results. According to the FAB classification, the studied cases belonged to the following subtypes: M0 (6.5%), M1 (16.1%), M2 (16.1%), M3 (6.5%) and M5 (54.8%). The immunophenotype analysis revealed approximately the same number of patients with positive and negative status of stem cell marker CD34 on blast cells (45.2 and 48.4% of patients, respectively). MPO and CD117 were expressed in 48.4% of patients, and in 19.3% of cases the majority of cells were more differentiated ($CD11b^+CD14^+$ phenotype). The level of DNA double-strand breaks varied in the range of 4.4-30.4% tDNA with an average of 12.5±1.5% tDNA. Overall, the level of DNA damage was not significantly different in blast cells of various FAB subtypes of AML. However, there was a tendency towards an increase in the level of DNA double strand breaks in $CD34^+$ compared to $CD34^-$ cells (16.9±1.6 and 11.2±2.1% tDNA, respectively, p=0.08). Nevertheless, the DNA damage level in MPO^+CD117^+ cells was approximately 1.7 times higher compared to cells with the $CD11b^+CD14^+$ phenotype (15.5±1.8 and 8.9±1.6% tDNA, respectively, p=0.04).

Conclusions. Blast cells of AML with immunophenotype markers of stem cells and early maturity are characterized by an increased level of DNA double-strand breaks regardless of FAB classification. Obtained results may suggest an involvement of this type of DNA damage in pathogenesis of AML.

LACTIC ACID BACTERIA AS VECTORS IN CONTROLLED DELIVERY OF DRUGS

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Aim of the abstract is to present opportunities and prospects for the use of lactic acid bacteria for controlled drug delivery.

Background. There are three fundamentally different strategies for using lactic acid bacteria (LABs) as vectors.

1. Live bacteria act simultaneously as a cell factory and a delivery system for pharmacologically active proteins that are drugs / prodrugs or immunomodulatory agents. One of the problems is the limited control associated with gastrointestinal colonization. The most interesting thing in terms of the use of live bacteria is bactofection – a process in which genes are encoded in the bacterial plasmid, and then the mammalian expression systems are used. 2. Inactivated bacteria are not widely used. They are interesting in terms of antigen delivery in cases where potential adjuvant activity persists. They can also be used as filters by molecular mimics of host receptors that bind toxins. At the same time the immunomodulatory properties of LABs are potentially preserved. 3. Gram-positive enhancer matrix are analogs of bacterial ghosts. The problem is that they can only be created for gram-negative organisms, so this is a fundamentally different model, but also an inanimate cell membrane. The advantage of empty cells is that they do not contain DNA, and therefore are easily regulated and are not genetically modified organisms. Another form of transport is mini-cells – achromosomal products of abnormal cell division. It is possible to pack 110 million molecules of the drug into the cell. Most LABs have S-layer proteins to which biomolecules are easily attached.

It is known that e.g. cancerous tumors are a hypoxic region, so anaerobic LABs easily survive there and are able to move directly to this region. Recently, magnetized nanoparticles have become more widely used. The advantage of bacterial magnetotaxis is that control relies on the induction of magnetic moment in a chain of magnetic particles inside the bacterial cell. Tumor cells also contain nanoparticles of endogenous nanomagnetite. There is also a technique of artificial attachment of magnetite to the cell surface, followed by attachment of biomolecules. It is worth mentioning that LABs have been successfully used to deliver IL10 for the treatment of intestinal diseases, they are the basis for models of oral vaccines and including those to combat diabetes and allergies.

Conclusions. There is interest in creating vectors for the delivery of LAB-based drugs. This technology involves the use of both live bacteria capable of producing therapeutic proteins *in situ* and inanimate membranes capable of presenting antigens. Among the non-classical methods of directing are magnetotaxis, but its use is somewhat limited. The safety of live bacteria creates prospects for antigen-based vaccines or DNA vaccines.

A NOVEL ENZYMATIC WOUND BURN DRESSING GEL OF CARBOPOL

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Background. Serratiopeptidase (EC 3.4.24.40) is a metalloprotease belongs to the group Serralysin. A wide range of proteolytic activities determines the prospects for the use of enzyme for debridement and stimulation of wound healing of various etiologies. Traditionally gels are a convenient and widely used dosage form. One of the most promising polymers for gels creating is carbopol.

The **aim** is to develop and characterize a gel of carbopol with immobilized serratiopeptidase, D-panthenol as perspective wound burn dressing.

Methods. Serrata® tablets were used to isolate the enzyme, and SDS electrophoresis was used to determine the purity of the enzyme. The proteolytic and collagenolytic activities of enzyme were determined spectrophotometrically using casein and collagen as substrates, respectively. Protein was determined by the Lowry-Hartree method. Immobilization of active ingredients was carried out by the method of inclusion in the gel. The evaluation of the wound healing effect of the gel was carried out *in vivo* experiments on simulated rat skin burns.

Results. The biochemical characteristics of the enzyme were determined: homogeneity, protein content 2 mg/cm^3, proteolytic activity 614 mg tyrosine/mg protein/min, collagenolytic activity 725 nmol/mg protein/min. The preservation of proteolytic activity is 74.3%. The quality gel form of serratiopeptidase was assessed by microbiological parameters. It has been shown that the gel is sterile and inhibits the growth of the *Pseudomonas aeruginosa* test strain. The formula of the gel formulation demonstrates good spreading ability and full release of active ingredients. The optimum pH of the free and immobilized enzyme has the close values (8.0-9.0). After six months of storage, enzymatic gel retains 70.6% of its initial proteolytic activity. The efficacy of the gel was evaluated in comparison with "Iruxol". The gel was placed at the burn area and the wound closure was monitored for 21 days. The remaining wound area's percentage was calculated according to wound diameters at the definite time points (121 days after operation). On the 15th day, the carbopol gel group exhibited higher wound burn closure rate in comparison with the "Iruxol" group, which can be attributed to maintaining the proteolytic, collagenolytic, anti-inflammatory activity of serratiopeptidase, the regenerative effect of D-panthenol, as well as maintaining a moist environment in the wound.

Conclusions. A new wound burn dressing gel based on carbopol has been developed. The composition of the wound healing gel formulation based on carbopol, serratiopeptidase (0.05%), D-panthenol 5%, an antiseptic agent, plasticizers providing wound healing, anti-inflammatory, antibacterial, stimulating tissue regeneration processes has been substantiated.

DRUG TRANSMEMBRANE TRANSPORT FACILITATION BY PENETRATION ENHANCING AGENT: MODEL STUDY OF INTERMOLECULAR INTERACTIONS OF DIMETHYL SULFOXIDE WITH THE DRUGS AND MEMBRANE PHOSPHOLIPID MOLECULES

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Aim. The aim of the current study is to reveal molecular mechanisms of action of dimethyl sulfoxide (DMSO) as known agent for transmembrane and transdermal drug transport facilitation in medical practice. One of the effective ways to promote drug molecules administration into the target biological system is to use a penetration enhancing agent whose molecules form associates with the drug to be delivered. To reach the aim formulated above we decided to examine the formation of stable noncovalent complexes of DMSO with a number of antibiotics and anticancer drugs along with the study of intermolecular interactions of DMSO with the molecules of membrane phospholipid dipalmitoylphosphatidylcholine (DPPC).

Methods. We used the electrospray ionization mass spectrometry (ESI MS), which provides the possibility to reveal the clusters formation between DMSO and drugs molecules in the model systems, containing DMSO and the following drugs of different structures: antibiotic levofloxacin (LEV), anti-tuberculosis agent cycloserine (CYS), antimicrobial chemotherapeutic preparation decamethoxinum (DEC) and anticancer drugs referred to the class of mercapto-derivatives of nucleobases, 6-mercaptopurine (MCP) and 2-mercaptoadenine (MCA), dissolved in a polar solvent.

Results. Formation of stable noncovalent complexes of DMSO with the molecules of a number of studied drugs, such as: LEV, CYS, MCP and MCA in the polar solvent methanol has been revealed by the ESI MS probing of the model binary systems. At the same time ESI MS investigation of the similar system containing DMSO and DEC has shown that no peaks of the noncovalent complexes between DMSO and this antimicrobial drug are recorded in the mass spectra that points to the dependence of DMSO-drug complexation peculiarities on the drug's structure. The data of ESI MS examining of DMSO+DPPC model system reveal that the DMSO molecules also do not form stable noncovalent clusters with DPPC in the polar surrounding.

Conclusions. The results of our study as to formation of stable noncovalent complexes of DMSO with some antimicrobial and anticancer drugs may serve as a proof of suggested molecular mechanism of the facilitating role of DMSO as a vehicle in the drug transmembrane and transdermal transport. The dependence of the DMSO role as a drug carrier on the transported drug molecule structure is shown.

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EXPRESSION PATTERN OF MRPS18 FAMILY OF GENES IN EMBRYONAL BRAIN TUMORS

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Embryonal brain tumors (EBTs) of central nervous system affect predominantly infants and children. They are usually characterized by extremely aggressive course and worse outcome in majority of cases. EBTs is heterogeneous group and include several nosologic forms with different localization, cell origin, molecular profile, and genetic alterations; thus, each of them requires appropriate therapy. However, it should be noted that almost all molecules that showed associated altered expression in one or another EBTs' subtypes are not exclusive markers. These data altogether indicate the importance of further searching the putative markers, that could have the oncogene properties and influence on the course of disease, from one side. From other side, such markers could be used for precision individual diagnostics and therapy. Among the potential molecules we chose the mitochondrial ribosomal proteins MRPS18 family (MRPS181/2/3), based on our earlier data showing overexpression of MRPS182 and MRPS183 in many types of cancer. Moreover, it is known that MRPS182 protein interacts with retinoblastoma-associated protein (RB), maintaining cell stemness. Considering that RB plays a central role in embryonal brain tumorigenesis studying the expression patterns of MRPS18 family proteins within EBTs is one utmost important task.

Aim. The aim of our study was to explore the profile of mRNA expression patterns of *MRPS18* genes in serum and tumor tissue samples obtained from patients with embryonal brain tumors.

Methods. Serum and tumor tissue samples from 14 patients with embryonal brain tumors were collected with further mRNA isolation and cDNA synthesis. Relative mRNA expression levels of target genes were determined using real-time qPCR. Obtained data were normalized to TBP mRNA expression level that served as the internal control. Data were calculated using $2^{-\Delta\Delta Ct}$ method and statistically estimated using nonparametric t-test.

Results. The analysis of sera and tumor tissue samples revealed differences in mRNA expression levels of *MRPS18* genes. In particular, expression level of *MRPS181* in sera was reliably up to 4- and 5-times higher, compared to those for *MRPS183* and *MRPS182* genes. At the same time, no significant differences in *MRPS18* mRNA expression levels were found in tissue samples. The statistical analysis showed that *MRPS181* expressed at the same level in sera and tumor tissues, while relative expression levels of *MRPS182* and *MRPS183* mRNA in tumor tissue were up to 5- and 6.5 fold higher, compared to such detected in patient sera.

Conclusions. Considering the obtained data on differential patterns of mRNA expression of *MRPS18* family genes it is important to continue our research, studying protein expression patterns, aiming to investigate whether these genes could be considered as the putative tumor markers.

POSTTRAUMATIC STRESS DISORDER: BIOCHEMICAL ASPECTS

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Research problem. Posttraumatic Stress Disorder (PTSD) results from single or repeated heavy stress episodes. At initial phases of the stress, animal and human organisms demonstrate commonly known stress responses, but at the last stage organisms demonstrate no capability to adapt and recover from the stress. Especially it is true for the cases of very strong stress and its repeats. Such scenario leads to development of different pathologies one of which has been called PTSD. It is a very common disorder for soldiers taking part in the ongoing war in Ukraine now.

Literature review. Despite nervous system from one hand is strongly protected from influences of environmental challenges, on the second hand it is very sensitive to them because it coordinates general response of the organism to the stress. Earlier I noticed that negative environmental challenges may cause accelerated aging (Lushchak, 2021). With mouse model we have found that substantial changes take place at middle age and are related with reorganization of homeostasis of reactive oxygen species and energy provision in the brain (Bayliak et al., 2021).

Results and conclusions. Disturbances of brain homeostasis may result in number of brain pathologies such as accelerated (premature) aging, Alzheimer's and Parkinson's disease, epilepsy, etc. Relatively recently PTSD was recognized as specific disease. Importantly, at general biochemical and psychological levels all listed above pathologies share common features due to which in many cases it is problematically to differentiate them. However, last years some clues to discrimination between the mentioned neurologic disorders have been identified. Neuroactive steroids are promising perspective in this field. Potential relationships between brain energetic status homeostasis of reactive oxygen species and neuroactive steroids will be covered.

INVESTIGATION OF BIOCOMPATIBILITY AND OSTEOGENIC POTENTIAL OF BIOMATERIALS BASED ON HYDROXYAPATITES *IN VITRO*.

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Aim. To evaluate the biocompatibility and osteoinductive properties of hydroxyapatites (HAPs) with various chemical modifications in mouse mesenchymal stem cells culture.

Methods. Adipose-derived mesenchymal stem cells (ADSC) were isolated from six-week-old male mice of Balb/c strain and cultivated under standard conditions. ADSCs were analyzed for surface antigens CD73, CD90, CD105, CD34, and CD45 by flow cytometry and immunohistochemistry. To estimate toxicity and biocompatibility of hydroxyapatites towards ADSCs, we performed microscopic analysis of live cells, cells viability and MTT test, adhesion assay. Osteoinductive properties of HAPs were tested on ADSCs cultured in Control and Osteogenic media. Osteogenic differentiation was evaluated with alkaline phosphatase (ALP) and Alizarin Red (ARS) stainings. Osteopontin (OP) levels were measured by ELISA. Mitochondrial analysis was done with MitoTracker Green and Rhodamine123 supravital stainings, followed by light and fluorescence microscopy. Statistics were analyzed using Prism7 software (GraphPad, USA).

Results. All analyzed HAP crystals have high adhesive capacity – two times higher compared to control cells. We did not observe HAPs toxicity where survival rate of ADSCs was close to 100%. MTT test demonstrated a two-fold reduction of mitochondrial metabolic activity in ADSCs after 72h of cultivation with HAPs, compared to controls. Silver-ligated HAPs caused high lethality of ADSCs after cultivation together for 5-6 days. Next we analyzed the possible osteoinductive properties of HAPs and found strong positive ALP staining of cells, cultivated with HAPs in Control and Osteogenic media. A greatly increased number of mitochondria were found in ADSCs cultivated with HAPs in osteogenic conditions, compared to cells cultivated with HAPs in Control media, but mitochondrial membrane potential was lowered in both variants, what points cells differentiation. Strong ARS staining for calcium deposition was visualized in cells grown on HAPs in Osteogenic and Control media for 17 days, as a result we noted that the HT-1 series of hydroxyapatites have great osteoinductive properties without extra stimulus. We observed increased levels of secreted OP as well as OP levels in cells lysates when ADSCs were cultivated with HAPs under osteogenic conditions, compared to control without HAPs. However even in non-osteogenic conditions we found high levels of secreted OP in variants where cells were cultivated with HAPs.

Conclusions. In result we managed to single out the most biocompatible and osteoinductive hydroxyapatite samples (HT-1, HT-1/H_2O, HT-1/H_2O_2), that can be further used for bone tissue bioengineering.

T CELL RESPONSE IS ASSOCIATED WITH CYTOKINE AND MIRNA LEVELS IN PATIENTS WITH COVID-19

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Aim. The adaptive immune response is a critical factor in COVID-19 clinical outcomes. T-cell responses appear early and are accountable for protection while associated with lymphopenia and the cytokine storms' consequences. Here we focused on how the dynamic changes in lymphocyte subpopulations in the peripheral blood correlate with cytokine and miRNA levels in individuals with severe COVID-19.

Methods. This study included 14 patients with verified COVID-19 and 17 age-matched non-COVID-19 volunteers. Flow cytometry was used to determine the percentage of lymphocyte subsets in the peripheral blood, ELISA was used to identify cytokine levels, and real-time PCR was used to quantify miRNA expression. Data were collected for four weeks at different time points: on the day of the patient's admission (day 0), the 7th, 14th, and 28th days of the study.

Results. During the first two weeks of hospitalization, the content of CD4 T cell subsets (memory, effector, activated, senescent, senescent memory, and exhausted CD4 T cells) and CD8 T cells subpopulations (effector, activated, senescent, senescent memory, and exhausted CD8 T cells) increased and remained elevated, indicating the activation of an adaptive immune response. In addition, the number of regulatory CD4 and CD8 T cells grew dramatically from day 0 to day 7, demonstrating that Tregs in patients with COVID-19 are highly proliferative compensating for the lymphopenia identified on admission day. The most prominent correlation between exhausted subsets of CD8 cells and 27, 126, and 146 miRNA was observed. miR126 also correlated with the CD8 senescent, activated, and Treg cells. High contents of TNF-α, IL-6, and MCP-1 in the plasma of patients were observed throughout the research while G-CSF decreased gradually from day 0 to day 28. We revealed a negative correlation between G-CSF and CD4 Treg cells as well as senescent and exhausted CD8 T cells. TNF-α content correlated positively with activated CD4 T cells. MCP-1 content in plasma of patients with COVID-19 correlated with senescent and senescent memory in both CD4 and CD8 T cells subsets.

Conclusion. According to the results of our correlation research, the following subsets of CD8 cells: senescent, regulatory, and exhausted had the greatest impact on the activation of immune response and pro-inflammatory cytokine and miRNA levels.

DOSE DEPENDENCE OF HEPATOPROTECTIVE ACTIVITY OF MESENCHYMAL STEM CELLS EXOMETABOLITES

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Background. Mesenchymal stem cells (MSCs) are a self-sustaining population of cells that have the ability to divide and differentiate. They are located in many organs and are necessary for regeneration of damaged organs. Their biological activity allows them to be used in regenerative medicine. The most widely used MSCs from bone marrow, adipose tissue and cord blood. MSCs practically do not cause an immune response and tumors, but nevertheless, there are risks from the use of living cells. They secrete into the internal milieu or culture medium biologically active metabolites containing soluble peptides and lipid vesicles with microRNAs called a secretome. Both individual components and the whole secretome are being actively studied to obtain new pharmaceutical compositions. It is known about their immunomodulatory effect, as well as their stimulation of regional stem cells in many organs, including the heart, the CNS organs, pancreas, liver, etc. An important step towards the practical application of MSCs EMs in pharmaceutical biotechnology is the study of the effect of different doses of secretome or its different fractions on various organs and systems.

The **aim** of the research was to study the hepatoregenerative effect of three whole secretome concentrations of xenogeneic MSCs in an *in vitro* model.

Methods. MSCs EMs were obtained from the bone marrow of a healthy donor dog. Under aseptic conditions, the biopsy material was resuspended, fractionated by centrifugation, and inoculated on culture plastic to a growth medium (90% DMEM, 10% fetal bovine serum), the floating fraction was removed, and cells were incubated (37°C, 5% CO_2). At the 3rd passage, the secretome was collected. The growth medium without cells cultivation was a control. The study model was an organotypic culture of rat liver. Cylindrical fragments were excised from the liver using and inoculated into a culture medium (90% DMEM/F12, 10% fetal bovine serum), into which secretome samples were added. After 48 hours of incubation, the area index was taken into account, i.e. the intensity of the formation of the “zone of eviction and growth” by the cellular elements of the liver. Statistical analysis was carried out using the Scheffe method.

Results. Doses of 0 (control), 30, 60 and 120 µl/ml were studied. A significant increase of the area index experimental cultures by 1.25-2.30 times in comparing control was shown. At the same time, an almost linear dose dependence of the stimulating effect was observed in the studied dose range.

Conclusion. Thus, it has been shown that the whole secretion of xenogenic MSCs has hepatoregenerative activity in the range of 30-120 µl/ml. The dependence of the biological effect on the dose is almost linear. In the future, additional *in vitro* and *in vivo* studies are planned to elucidate the mechanisms of the observed effects.

PRUSSIAN BLUE NANOCOMPOSITE COUPLED WITH CARBON MATRIX AS ARTIFICIAL PEROXIDASE IN GLUCOSE BIOSENSOR

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Aim. The **aim** of our research is to develop amperometric biosensors (ABS) to determine glucose using glucose oxidase (GO), nano-scaled hexacianoferates of transition metal (HCFs) as artificial peroxidases (POs) and carbon materials as potential platforms for immobilization of HCF. Nanomaterials with enzymatic activity or nanozymes (NZs), due to their high stability and low cost of production, are the effective mimetics of natural enzymes. That is why NZs can substitute the natural enzymes in biosensors.

Methods. To select the most effective PO-NZ, various HCFs, including CoHCF, NiHCF, MnHCF, ZnHCF, Fe/FeHCF, Fe/CuHCF, Fe/CoHCF, Fe/NiHCF, were synthesized by mixing of $K_4Fe(CN)_6$ with the appropriate salts solutions. All potential PO-NZ s were characterized for their PO-like activities in solution using the colorimetric method, with o-dianisidine as a chromogenic substrate in the presence of H_2O_2. One unit of PO-like activity was defined as the amount of PO-NZ releasing 1 μmol H_2O_2 per 1 min at 30°C under standard assay conditions. Carbon materials (CM), namely, graphene (GF), nanotubes (NTs), and hemin (GM) were screened for PO-like activity in a solution too, as potential carriers for immobilization of the best PO-NZs. For selection of the most effective nanocomposites of CM with PO-NZ, Fe/CuHCF и Fe/FeHCF were chosen as the best PO-NZs. As a biorecognition element in glucose biosensor, commercial GO was used.

Results. GF and NT were shown to possess a higher PO activities compared to GM. The combination of PO-NZs with GF and NTs led to an increasing activity of nanocomposites. On the basis of the best PO-NZs, amperometric sensors for the analysis of H_2O_2 were constructed, and their electrochemical characteristics were studied using CV approach. Cu/FeHCF-NT and Fe/FeHCF-NT-based electrodes were shown to have the higher sensitivities to H_2O_2 (351 and 211 $A\times M^{-1}\times m^{-2}$, respectively) and wider linear ranges if compared with other electrodes, which were described here. Thus, these chemosensors were chosen as the platforms for development of biosensors. As a result, the biosensors for glucose determination were developed, namely, GO/Cu/FeHCF-NT and GO/FeHCF-NT-based, which demonstrated the improved analytical characteristics.

Conclusions. A novel amperometric biosensor for glucose analysis based on GO and Cu/FeHCF-NT was created and characterized. This biosensor has a rather high sensitivity to glucose (380 $A\times M^{-1}\times m^{-2}$) and a low limit of detection (8 μM), so it can be promising for determining glucose content in real samples of foods and biological fluids.

LABORATORY CHARACTERISTICS AMONG PATIENTS WITH TYPE TWO DIABETES (T2D) WHO WERE HOSPITALIZED WITH SARS-COV-2 INFECTION DURING PERIODS OF DELTA AND OMICRON VARIANT PREDOMINANCE IN THE TRANSCARPATIAN REGION OF UKRAINE

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Aim. To establish laboratory features of patients with T2D and COVID-19 during periods of delta and omicron variant predominance

Methods. Retrospective patient data collected from medical records available in the electronic health information system were used. A total of 65 subjects were eligible for the study, among which 7 with Delta and 26 with Omicron variant were comorbid with T2D.

Results. The average procalcitonin levels in T2D patients with Delta variant of SARS-CoV-2 infection were higher than in the same group with Omicron variant (0.606 vs. 0.45, mg/L) and showed a significant difference (P = 0.003). The two groups showed no significant differences in creatinine (P = 0.798), granulocytes count (P = 0.449), CRP (P = 0.751), D-dimer (P = 0.157), WBC (P= 0.406) and lymphocyte counts (P = 0.528). Patients with COVID-19 and TD2 who took insulin had higher levels of granulocytes than patients who didn`t takes insulin (p = 0.022 respectively). Patients taking metformin had lower levels of CRP (p = 0.046).

Conclusions. Haematological parameters in patients with T2D and COVID-19 were somewhat similar in the period of omicron and delta waves. However, the question remains concerning the cause of elevated procalcitonin levels in patients with type 2 diabetes and coronavirus infection during the omicron wave.

PFKFB3 AS A TARGET OF COMPENSATORY CELL SIGNALING IN RESPONSE TO EGFR INHIBITION IN NON-SMALL CELL LUNG CARCINOMA

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Background. Lung tumor development and aggressiveness is are largely driven by different oncogenes including epidermal growth factor receptor (EGFR). Non-small cell lung carcinoma (NSCLC) patients whose tumors harbor EGFR activating mutations respond favorably to treatment with EGFR tyrosine kinase inhibitors (TKIs). However, few three generations of TKIs (erlotinib, osimertinib) have failed to show any significant overall survival benefit due to the developing of resistance and relapse within 2 years of treatment. Clinically, responses to EGFR-TKIs are evaluated by positron emission tomography using 2[^{18}F]-fluoro-2-deoxy-glucose (^{18}FDG-PET) as tumors consume significantly more glucose compared to adjacent normal tissue *in vivo*. Increased glycolysis and cell proliferation in NSCLCs require the presence of an active 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) enzyme. The product of PFKFB3, F2,6BP, regulates glucose metabolism by activating PFK1, which in turn facilitates glycolysis to meet the elevated tumor demands for precursors needed for survival and proliferation – the functions that confer resistance to targeted therapies.

Aim. The aim of the study was to investigate the potency of PFKFB3 inhibition using a small molecule inhibitor (PFK-158) developed by our group to increase the efficacy of EGFR-TKIs in NSCLCs *in vitro*.

Methods. H522, H1437, PC9, HCC827 lung cancer cell lines were treated with erlotinib +/- PFK-158. PFKFB3 expression, glycolytic flux, cell viability, growth rates, levels of autophagy flux and tumor colony formation were measured.

Results. We demonstrate that exposure to erlotinib modulates PFKFB3 expression in NSCLC cell lines. In PC9 cells, EGFR inhibition for 48h results in 70% decrease in PFKFB3 expression. At the same time, compensative reactivation of the MAPK pathway promotes 4-fold increase in PFKFB3 mRNA expression via recruiting of the cyclic AMP response element binding (CREB1) to the CRE site on the *PFKFB3* promoter. Elevated PFKFB3 mRNA expression is required to compensate for TKI-induced PFKFB3 protein degradation. Dual inhibition causes only minor changes in glycolytic flux when compared to individual treatment. Moreover, we identify PFKFB3 as a mediator of erlotinib-induced autophagy in NSCLCs. Finally, dual treatment with PFK-158 and erlotinib improves the efficacy of EGFR inhibitor in NSCLCs. Taken together, our findings suggest that non-metabolic function of PFKFB3 limits the usefulness of EGFR-TKI *in vitro,* and described approach can be utilized for optimization of the clinical efficacy of EGFR-TKIs in lung cancer treatment.

Conclusions. Our studies provide evidence that targeting PFKFB3 may be essential to improve responses and alleviate resistance to the EGFR TKIs in NSCLCs with different EGFR mutation status.

MOLECULAR BACKGROUND OF POST TRAUMATIC STRESS DISORDER (PTSD)

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Aim of this thesis is to provide a brief overview of the current knowledge within neurobiology, epigenetics and epigenomics in relation to PTSD.

PTSD is a disorder affecting approximately 8% of the world's population during their lifetime, and typically develops after exposure to a traumatic event.

Trauma is as a response to a deeply distressing event that overwhelms an individual's ability to cope and diminishes their sense of self along with their ability to feel the full range of experiences. There are no preventive measures to minimize the impact of traumatic stress on health, and PTSD treatment options are limited.

There are a lot of evidence as for epigenetic factors critical role in PTSD, by mediating the impact of environmental exposures to trauma on the regulation of gene expression. DNA methylation, or the methylation of cytosine residues – one of the best-characterized epigenetic mechanisms in the mammalian genome involved in long-term persistent alterations. It also contributes to the adaptive capacity of the transcriptional response to dynamic alterations in environmental factors throughout life. Other epigenetic mechanisms are also being studied, including histone modifications and miRNAs.

Evidence for its role in PTSD can be viewed from different aspects: molecular aspect, including genetic, ligand–receptor and metabolic alterations, regional alterations, and specific clinical symptoms.

Many studies have reported an increase of oxidative stress during PTSD. Stress is a risk factor for PTSD development and triggers a sustained growth in nitric oxide synthase activity. The oxidation of nitric oxide produces a peroxynitrite, which is extremely toxic to neurons. Observations of high levels of peroxynitrite and its predecessor nitric oxide are known for patients with PTSD.

Epigenetic changes can be acquired over the lifespan and mediate environmental effects on gene expression. In the brain, epigenetic regulation is vital for basic cellular processes involved in aspects of neuronal function, such as synaptic plasticity, and for complex behaviours, involved in learning and memory.

Studies discovered 3989 genes that were significantly upregulated in patients with PTSD. Also was noticed an upregulation in olfactory function and immune system gene expression. Other studies also noted downregulation in immune system gene.

Conclusions. Better understanding of epigenetic modifications would be of great benefit. DNA, ctytosyne, methylation are one of the best-characterized epigenetic mechanisms in the mammalian genome and is involved in long-term more volatile changes induced by environmental exposures. For all of these, in recent years, approaches have moved from targeted gene or genomic region-based ones to genome-wide explorations looking for integrated regulatory patterns or mechanisms in the field of epigenomics.

DYSFUNCTIONAL ADIPOSE TISSUE AND CHANGES IN THE BLOOD ENZYME SYSTEMS OF OVERWEIGHT COLORECTAL CANCER PATIENTS

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Background. It is well-known that obesity is a risk factor for the occurrence and adverse course of the number of tumors. Dysfunctional and inflamed adipose tissue is one of the factors that form the tumor microenvironment and the aggressive phenotype of the tumor, which contributes to its invasion and metastasis.

Aim. The levels of superoxide radicals generation (SRG), activity of matrix metalloproteinases 2 and 9 (MMP2 and 9) or gelatinases, ribonucleases (RNAses, as well as double-stranded RNAses – dsRNAses), ornithine decarboxylase (ODC) in tumor, adipose tissue and peripheral blood, the number of tumor-associated adipocytes (TAA) in the tumor of overweight CRC patients were investigated.

Methods. Samples of the tumor tissue, the tissue adjacent to the tumor (TAT), the distant adipose tissue (DAT), and the blood samples of 28 patients with CRC were studied. The rate of SRG was determined by EPR spectrometry, gelatinase and RNAase activity was determined by zymography, immunohistochemical, spectrophotometric, statistical methods were used.

Results. In overweight patients with CRC (BMI>25 kg/m^2), compared to non-obese patients (BMI≤25 kg/m^2), both tumor and TAT are characterized by severe mitochondrial dysfunction resulting in a high rates of SRG, which led to the corresponding changes in the blood of the patients. In particular, in overweight patients with CRC, the RNAses and ODC activity in TAT was 2.1 and 2.8 times higher respectively ($p<0.05$) than in non-obese patients. Additionally, in obesity 1.7 times higher total RNAses activity was detected in TAT, but significantly lower (37 times) of double-stranded RNAses activity compared to the distant adipose tissue (DAT); the levels of gelatinase and ODC activity in TAT were 2.3 and 4 times higher than in DAT, which indicates the tumor influence on the formation of dysfunctional state of the adipose tissue under conditions of its excess weight. CRC in obesity is characterized by a 6.7 times higher frequency of the tumor-associated adipocytes (TAA) in the tumor, which is due to the influence of TAT on the tumor microenvironment. It has been shown that the levels of neutrophil SRG and the MMP2 activity in the blood serum of obesity CRC patients are 1.7 and 2.8 times respectively higher than in normal weight patients.

Conclusion. Obesity in patients with CRC is associated with the signs of dysfunction of the adipose tissue and its interaction with the tumor microenvironment, as well as corresponding changes in the functioning of several enzyme systems studied in blood. The studied indicators can be used as a basis for searching of course markers in overweight CRC patients.

ANALYSIS OF BIOCHEMICAL POLYMORPHISM OF MEDITERRANEAN MUSSELS FROM THE BLACK SEA

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Aim. To investigate the genetic polymorphism of mussels from the Black Sea using protein markers associated with antioxidant enzyme systems. To determine the activity of the studied enzymes in different organs of the mollusk.

Material and methods. The material of the study were mussels caught in the Gulf of Odesa (the North part of the Black Sea) at geographical coordinates N: 46°26'28"/ E: 30°46'20". The following mussel organs were selected for analysis: hepatopancreas, ktenidia, mantle, leg and adductor. Aggregate material consisting of organs of 6-10 individuals used for the experiment. Biochemical analysis of mussels was performed using 9 protein markers (enzymes): superoxide dismutase (SOD, EC 1.15.1.1), NADH oxidase (OXN, EC 1.6.3.3), NADPH oxidase (NOX, DIA (NADP), EC 1.6.99.6), glutathione peroxidase (GPx, EC 1.11.1.9), glutathione reductase (GSR, EC 1.6.4.2), ferroxidase (CP, EC 1.16.3.1), catalase (CAT, EC 1.11.1.6), peroxidase (PER, EC 1.11.1.7), peroxyredoxins (PRX, EC 1.11.1.15). Analysis of antioxidant enzymes was performed in polyacrylamide gel (PAGE) electrophoresis (Toptikov et al., 2017). The enzymes in various organs of mussels were detected using the methods of Manchenko (2003), Meijer & Bloem (1966) and Lloyd (1967). Detection of multiple molecular forms (MMFs) of enzymes after separation in PAGE was performed according to the method (Toptikov et al., 2002). The computer program AnaIS (Rybalka & Podzharsky, anaispro@ua.fm) was used to analyze electrophoregrams. Research results were processed using Microsoft Excel software.

Results. In the studied organs of mussels up to 6 MMFs of SOD, CP, PRX and OXN were detected. The highest value of specific activity (Am, un.act./mg) of SOD was observed in hepatopancreas (26.83), CP – adductor (8.65), PRX and OXN – mantle (9.15 and 31.16 respectively). The lowest value of Am SOD was in ktenidia (1.91), CP – hepatopancreas (4.78), PRX – adductor (0.81), OXN – leg (4.41). Up to 8 MMFs were detected for NOX. The highest value of Am NOX was observed in the mantle (23.8), the lowest – adductor (5.43). GPx is represented in the hepatopancreas and mantle by two MMFs, in other organs – by one MMF. The highest value of Am GPx was observed in the hepatopancreas (4.67), the lowest – ktenidia (0.05). Up to 11 MMFs were detected for PER, the highest Am was observed in the mantle (17.12), the lowest – hepatopancreatic (5.60). CAT was detected by three MMFs in all organs, the highest Am was observed in the hepatopancreas (3.67), the lowest – mantle (0.55) and adductor (0.66).

Conclusions. All studied enzymes were polymorphic. The highest amount of MMFs was found in PER, the lowest – in GPx. Mussel organs differed in the level of activity of antioxidant enzymes. This provides a basis for their use as protein markers to study the population-genetic properties of different groups of mussels.

HORMETIC EFFECT OF HYDROGEN PEROXIDE IN PRECONDITIONING OF THE WHARTON JELLY-DERIVED MSCS FROM DIFFERENT DONORS

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Background. The efficacy of mesenchymal stem cell (MSC) transplantation depends on the survival of the cells in damaged tissues. These injured sites are characterized by high levels of inflammatory and oxidative stress mediators. High reactive oxygen species (ROS) levels induce oxidative stress, leading to cellular damage and dysfunction. Simultaneously, a low basal level of ROS is required in order to maintain cellular functions. It was shown that preconditioning of MSCs, which is based on the biological concept of hormesis, through mild oxidative stress increases their survival and activates signaling pathways that promote resistance of the cells to more severe stresses. However, a number of studies *in vitro* and clinical trials have demonstrated varied outcomes. We proposed that preconditioning parameters and donor selection could promote the survival of transplanted cells.

Aim. This study focuses on the estimation of an extent of variation of the individual responses of WJ-MSCs to non-toxic levels of H_2O_2 and preconditioning effect of H_2O_2 on the resistance of WJ-MSCs under high concentration of H_2O_2 treatment.

Methods. MSC were obtained by the explant method and cultured according to standard methods. Oxidative stress was caused by treating cells with different concentrations of hydrogen peroxide. The metabolic activity or viability of MSCs was evaluated using the MTT test.

Results. MTT test indicates both a biphasic dose–responses of the H_2O_2-treated WJ-MSCs as one of the hormetic features and a bilinear responses. Survival of WJ-MSCs from each donor in presence of 6,25–100 μM H_2O_2 for 24 h either increased or did not change significantly as compared to untreated MSCs. The exposure of WJ-MSCs to more high concentrations (>100 μM H_2O_2) for 24 h decreased their viability up to 34,9%±2,8% (under 440 μM H_2O_2). The maximal hormetic effect was observed at concentrations of 12.5 μM and 25 μM depending on the donor. The viability of the H_2O_2-preconditioned WJ-MSCs (at 12,5 or 25 μM H_2O_2) are changen in a donor-dependent manner. The conditioning doses 12,5 or 25 μM H_2O_2 induced a cell-survival adaptive responses in some individual to the challenge dose (300 μM H_2O_2), indicating a benefit of preconditioning. For example, 25 μM H_2O_2-conditioning treatment affected a relative increase of the protective response from 56 – 58% to 92% for individual MSCs. However, the correlation between hormetic response to low concentration of H_2O_2 and high resistance to challenge H_2O_2 concentration was not observed.

Conclusions. The present study revealed that hWJ-MSCs have donor-dependent individual differences and do not respond to oxidative stress identically. Taken together, our findings suggest that oxidative preconditioning cannot be applied as an enhancement strategy for some individual WJ-MSCs and inter-individual MSC variability should be taken into account.

PECULIARITIES OF THE FREE RADICAL PROCESSES IN THE KIDNEYS UNDER THE CONDITIONS OF DIFFERENT PROTEIN AND SUCROSE CONTENT IN THE DIET

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Introduction. Nutrient imbalance is one of the factors that causes disruption of antioxidant balance and excessive production of reactive oxygen species, in particular superoxide (O_2•) and hydroxyl (OH•) radicals. Due to their high oxidative potential, the reactive oxygen species (ROS) are capable of damaging cell structure and inducing a number of chain reactions leading to uncoupling of integrative functions in the organism. Excessive formation of ROS is considered as one of the factors contributing to the pathogenesis of a number of chronic and acute kidney diseases.

Aim. The aim of the present study was to measure the rate of ROS generation in the kidneys under the conditions of different protein and sucrose content in the diet.

Methods. The experiments were conducted on white rats of 130-140 g body mass aged 2.5-3.0 months. The rate of superoxide anion radical generation in mitochondrial fraction was measured with nitro blue tetrazolium test (NBT). The rate of hydroxyl radical generation was measured in incubation medium was as follows: 20 mmol deoxyribose, 1 mmol H_2O_2, 20 mmol sodium-phosphate buffer (pH 7.4) and extinction was measured at $\lambda = 532$ nm.

Results. The results of the studies showed that in the mitochondria of the kidneys of rats, which were kept on a low protein diet, there is a preservation of the intensity of the generation of OH• and superoxide-anion of the radical at the level of control values. At the same time, the intensity of O_2• generation increases by 5 times, and the hydroxyl radical increases by 3 times compared to the control indicators. Probably, under conditions of excessive flow of sucrose, the work of the electron transport chain of mitochondria is increased due to increased flow of substrates. It should be noted that in animals that were kept on a low-protein/high-sucrose diet, the intensity indicators of the superoxide-anion radical do not significantly differ from the indicators of the HS group. At the same time, under these experimental conditions, the most pronounced production (5 times) of the hydroxyl radical was recorded compared to the indicators of control. Given that the predecessor of the most reactive OH• is hydrogen peroxide, it can be assumed that the obtained fact indicates a violation of of neutralization O_2• or H_2O_2.

Conclusions. According to our findings, excessive consumption of sucrose leads to intensive generation of reactive oxygen species in the mitochondria of the kidneys of rats, which may result in increased damage and modification of biomolecules in mitochondria, which may be considered as a prerequisite for impaired functional activity of the kidneys.

OBTAINING AND CHARACTERIZATION OF RECOMBINANT FLUORESCENT RECEPTOR-BINDING FRAGMENT OF SARS-COV-2 SPIKE PROTEIN

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Aim. The creation of fluorescent fusion proteins is a popular and convenient approach to studying the aspects of the expression of various proteins in different hosts, receptor-ligand binding dynamics, or intracellular internalization. Also, the fluorescent part can serve as an auxiliary indicator of the efficiency of folding the target protein. The ability to create a structure whose reporter parts will not interfere with the processes of synthesis, post-translational modification, and secretion is a critical factor to pay attention to. The main goal was to create a genetic construct containing the RBD domain of the SARS-CoV-2 coronavirus protein fused to the green fluorescent protein mNeonGreen, to express it in the prokaryotic system and to purify and evaluate biological activity.

Methods. Amplification of required nucleotide sequences was performed by PCR using specific primers. The actual construction of fusion fluorescent protein was accomplished using standard molecular biology techniques. For expression, transformed *E. coli* cells of the Rosetta strain were cultured in an LB medium with the addition of an IPTG. Purification of the protein was performed by metal affinity chromatography on Ni-NTA agarose. The binding of the fluorescent fusion protein to receptors on the surface of eukaryotic cells was assessed by flow cytofluorimetry.

Results. To create genetic constructs encoding the fluorescently labeled receptor-binding domain of the SARS-CoV-2 virus receptor protein, we decided to combine RBD sequences and the mNeonGreen gene in a single reading frame in a prokaryotic pET28b expression vector in different combinations. It was found that the correct folding occurred only for protein with mNeonGreen at the NH2-terminus. A functional variant of fusion fluorescent protein expressed in the Rosetta strain of *E. coli*. mNeonGreen-RBD formed only in an insoluble form (as bacterial inclusion bodies), so refolding was conducted *in vitro* to return the correct conformation. Finally, we performed the saturation ligand binding assay with Vero and MA104 cell lines. According to the flow cytometry data, a dose-dependent increase in the fluorescence intensity of cells stained with mNeonGreen-RBD was observed.

Conclusions. Thus, we developed a recombinant fluorescently labeled fragment of the coronavirus spike protein in the prokaryotic expression system - mNeonGreen-RBD. The total yield of renatured protein was in the range of 0.5 to 1 mg/L. RBD-mNeonGreen expressed in prokaryotic culture was efficiently bound to receptors on MA104 and Vero cells.

ROLE OF VITAMIN D_3 IN MODULATION OF OSTEOCLAST NUCLEAR RECEPTORS FUNCTIONING IN SECONDARY OSTEOPOROSIS ASSOCIATED WITH EXPERIMENTAL TYPE 2 DIABETES MELLITUS

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Background. Growing evidence suggests vitamin D deficiency as one of the risk factors for type 2 diabetes mellitus (T2DM) and its complications. In particular, prolonged decompensated T2DM leads to the occurrence of secondary osteoporosis due to disturbances in bone remodeling, excessive activation of osteoclasts and impairment of osteoblast-mediated bone formation. The molecular basis of impaired bone remodeling may involve the downstream signaling through nuclear receptors. Therefore, the **aim** of our study was to evaluate the contribution of vitamin D receptor (VDR), receptor activator of nuclear factor κB (RANK) and peroxisome proliferator-activated receptor gamma (PPAR γ) imbalance to T2DM secondary osteoporosis and to indicate potential curative effects of vitamin D3.

Methods. T2DM was induced in male Wistar rats by combination of high-fat diet and i.p. streptozotocin (STZ) injection (25 mg/kg of b.w.). The animals were divided into 3 groups: control, T2DM and diabetes treated with vitamin D_3 (780 IU/kg, orally, 30 days).

Results. We found 2.6-fold decrease in 25(OH)D content in blood of T2DM animals, accompanied by a decrease in total Ca^{2+} and phosphorus concentration (1.2-fold vs. control). In contrast, activity of alkaline phosphatase (AP), total and bone-specific isoform, was significantly higher (1.6-fold vs. control). In bone tissue of T2DM animals we revealed elevation of mRNA and protein levels of RANK and PPAR γ, and down-regulation of VDR, which indicates the disturbance of bone remodeling and shifting the balance towards osteoclastogenesis. Vitamin D_3 treatment led to normalization of vitamin D bioavailability, Ca^{2+} and phosphorus concentration and a decrease in AP activity in blood. Improvement of 25(OH)D content resulted in restoration of nuclear receptors balance.

Conclusion. Thus, we can conclude than normalization of vitamin D-status of T2DM animals can prevent secondary osteoporosis development through normalization of bone remodeling processes.

THE PROTEIN-NUCLEIC ACID STRUCTURAL DATABASE WITH INFORMATION ON ACCESSIBLE SURFACE AREA (PROTNA-ASA): UPDATED VERSION

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The rapid growth of information accumulated by the scientific community has led to the creation of diverse life science databases, in which analysis is the main way to delve into the tiny biomolecular organization. Among such databases is our Protein-Nucleic Acid Structural Database with Information on Accessible Surface Area (ProtNA-ASA: http://www.ire.kharkov.ua/ProtNA-ASA) [1]. The main objective of the ProtNA-ASA is to facilitate the study of protein-DNA binding and recognition mechanisms. Polymorphism and deformation of DNA during protein-DNA complexation is a problem of direct biological significance, as protein recognition of nucleic acid sequences is used at all stages of the implementation of genetic information. ProtNA-ASA is regularly updated and contains structures obtained by X-ray crystallography and NMR. All structures have a double DNA helix with more than four nucleotides in each chain; only structures with unmodified bases were selected. It contains 973 protein-DNA complexes 129 structures of naked A- and 403 B-DNA taken from the Nucleic acids Data Bank. Each entity and calculated parameters are available in separate files for viewing and downloading. Each record of the ProtNA-ASA database is marked with the PDB identifier and contains the following information: crystallographic coordinates of structures in pdb format and their components (protein, DNA, water); conformational DNA parameters for each nucleotide calculated with the 3DNA/CompDNA program; the accessible surface area of each DNA atom calculated with the modified Higo and Go algorithm in minor and major grooves separately [2]; and electrostatic potentials calculated using the DelPhi software package [3]. The new version of the ProtNA-ASA database is implemented by advanced search, which allows finding structures not only by PDB/NDB ID but also by citation; length and sequence of protein or DNA chain; type of structure; by the method and resolution of X-Ray. All these queries can be used in different combinations with and/or statements. This makes the use of the database sonorous and efficient. The combination of structural information and physical characteristics from the ProtNA-ASA database is particularly useful for studying the mechanisms of protein-DNA recognition.

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HIGHLY SELECTIVE CHIP-BASED FLUORESCENT SENSOR SYSTEMS FOR IN-FIELD ANALYSIS OF MYCOTOXINS

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Aim. The aim of this work is design and development of the highly selective chip-based plasmon-enhanced fluorescent (PEF) sensor system for in-field aflatoxin B1 (AFB1) and zearalenone (ZON) analysis in cereals.

Methods. The proposed highly-selective chips were based on thin molecularly imprinted polymer (MIP) membranes with embedded *in situ*-synthesized Ag nanoparticles (AgNPs) immobilized on the surface of glass slides. The MIP membranes were synthesized according to a dummy template-based approach. To obtain AgNPs embedded in MIP structure, $AgNO_3$ was added to the initial monomer mixtures that were used for the synthesis of MIP membranes, selective towards mycotoxins. Spherical AgNPs (30–70 nm in diameter) were formed *in situ* during the pre-heating step and further UV-initiated polymerization procedure (λ=365 nm; 3.4 Wm−2 conducted during 30 min). The covalent immobilization procedure for MIP membranes on the surface of the glass chips was based on the application of γ-methacryloxypropyltrimethoxysilane. The MIP membranes immobilized on glass surfaces were used as sensing elements of the PEF-based sensor systems for analysis of AFB1 and ZON contamination in spiked (maize and wheat flour from different manufacturers) and naturally contaminated cereals samples (Aflatoxins in corn, Zearalenone in corn, Romer Labs). Sensor signals of mycotoxins that were selectively adsorbed on the surface of glass chips coated with thin MIP membranes were registered with standard laboratory spectrofluorimeter. Transmission electron microscope was used to investigate AgNPs embedded in the MIP structure.

Results. Sensor chips coated with MIP membranes embedded with AgNPs which provide selective binding of mycotoxins and enhanced fluorescence signal from the analyte molecule according to plasmon-enhanced fluorescence were used as sensing elements of the PEF sensor systems. The morphology and size of AgNPs formed in the MIP membranes structure as well as the influence of $AgNO_3$ concentration on the PEF phenomenon were investigated. The thin MIP membranes immobilized on glass slides reported significantly higher selectivity towards AFB1 and ZON. AFB1-selective sensor chips can successfully discriminate between AFB1 and others mycotoxins (aflatoxin B2, G2, ochratoxin A). ZON-selective sensor chips can successfully differentiate analyte of interest (ZON) from its close structural analogues (zearalenol, 17-β-estradiol, bisphenol A, resorcinol). The detection limit of AFB1 sensor chips was established at 0.3 ng/mL and the linear dynamic range was 0.3-25 ng/mL. ZON-selective sensor chips demonstrated low limits of ZON detection (5 ng/mL), and the linear detection range comprised 5-25 ng/mL. The proposed AgNPs-containing MIP-based sensor chips were successfully used for evaluation of AFB1 and ZON contamination in spiked and naturally contaminated cereals samples.

Conclusions. The developed highly selective and sensitive chips based on thin MIP films with embedded AgNPs can be used as sensing elements for reliable AFB1 and ZON analysis based on the PEF phenomenon. The proposed sensor chips can be used with a laboratory spectrofluorimeter and with a low-cost portable fluorimeter for in-field fast AFB1 and ZON analysis.

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THE MOLECULAR CHAPERONE TRAP1 IN CANCER: A MASTER METABOLIC SWITCH OF TUMOR CELLS

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Aim. Many neoplastic cells undergo a dramatic metabolic shift in order to cope with harsh environmental conditions encountered during tumor growth. We have investigated the role played by TRAP1, the mitochondrial component of the Hsp90 family of molecular chaperones, in these metabolic changes of tumor cells.

Results. We have previously shown that TRAP1 down-regulates the activity of succinate dehydrogenase, an enzyme placed at the crossroad of oxidative phosphorylation and tricarboxylic cycle, buffering ROS generation and increasing the intracellular levels of the oncometabolite succinate. This establishes a pseudohypoxic phenotype that favors tumor growth, especially in neoplastic models endowed with hyperactivation of the Ras/ERK pathway, as ERK induces TRAP1 activity by phosphorylating it. Here we demonstrate that TRAP1 protects cancer cells by noxious insults through inhibition of the permeability transition pore, a mitochondrial channel whose opening prompts cell death. In accord with a pro-neoplastic role of TRAP1 activity, a set of highly selective TRAP1 inhibitors that we have recently developed has inhibitory effects on tumor cell proliferation.

Conclusions. Taken together, these observations pave the way for a better comprehension of the metabolic adaptations of tumor cells and for their targeting as a novel anti-tumor strategy.

MOLECULAR DOCKING OF PENICILLIN G – HUMAN SERUM ALBUMIN

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Background. A serious problem in practical medicine is the hypersensitivity caused by the toxicity of the drugs. Human serum albumin (HSA) is the major plasma protein with the function of carrier for many drugs. Because of medical allergy to beta-lactam antibiotics (about 35%) accompanied by skin pathology, anaphylaxis, and fatality, the binding interaction between HSA and penicillin's G main and minor determinants was explored in this study. Penicillin G is a chemical hapten that does not exhibit immunogenic properties. Only metabolic rearrangement of penicillin molecule into immunologically reactive main and minor antigenic determinants, which easily form covalent bonds with free amino groups of HSA can induce an immune response.

Aim. The aim of this research was to describe of structures of penicillin G determinants-albumin complexes and find out favorable binding sites by analyzing the Gibbs free energy of these complexes and root mean square deviation (RMSD) from data of X-ray crystallography.

Material and methods. The molecular docking method (AutoDock Tools 1.5.7, AutoDock Vina 1.1.2) was used to understand the penicillin G–HSA interaction. The visualization of docking results was done in PyMol 2.5. The crystallized structure of HSA; PDB code: 1ao6 acquired from Protein Data Bank, was used for docking studies (www.rcsb.org). The ligands – penicilloyl, penicillanic acid (main determinants), penicillamine, penilloic acid (minor determinants) were used the identification energetically most favorable binding sites on the HSA molecule (www.pubchem.ncbi.nlm.nih.gov).

Results. Gibbs free energies of binding for penicilloyl–HSA complex were in the range -7.7 … -7.1 kcal/mol; RMSD lower bound (l.b.) values were in range 2.98 Å…39.23 Å; van der Waals interactions were registered between O-13 and LEU-115 (3.7 Å); O-8 and VAL-116 (4.0 Å). Free energy of complex penicillanic acid–HSA was in the range -5.2…-5.5 kcal/mol; RMSD l.b. values were in range 1.89 Å…6.53 Å; van der Waals interactions were registered between O-12 and ARG-186 (3.5 Å); O-13 and LEU-182 (3.8 Å). Free energy of complex penicillamine–HSA was in the range -3.9…-3.5 kcal/mol; RMSD l.b. values were in range 1.9 Å…32.0 Å; van der Waals interactions were registered between N-4 and LEU-182 (3.8 Å); S-1 and VAL-116 (5.5 Å). Free energy of complex penilloic acid–HSA was in the range -5.5…-5.0 kcal/mol; RMSD l.b. values were in range 1.74 Å…38.93 Å; van der Waals interactions were registered between N-4 and GLU-294 (4.0 Å); O-8 and TYR-138 (3.4 Å).

Conclusions. We performed molecular docking of 4 antigenic determinants of penicillin G related to HSA. The most favorable binding site was in penicilloyl–HSA complex with the lowest binding energy. According to a set of RMSD l.b., the best-scored ligand is penicillamine without beta-lactam and thiazolidine ring, and also penilloic acid, that has an open beta-lactam ring: both ligands shift the crystal structure of HSA within 2 Å RMSD.

CORRECTION OF MICROBIOME AND HOMEOSTASIS USING PROBIOTIC TOOL: ACTUAL DIRECTIONS

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Background. Due to biotechnology's success, the probiotic field has been exponentially expanding over the recent years with an increasing newest research. The **aim** of the review is to systematize recent developments in probiotic's biotechnologies.

Methods. Publications on the multidimensional activities of probiotics were reviewed on the NCBI website databases PubMed, Springer Nature, and Science Direct (Elsevier).

Results. A number of new results obtained through the use of a systematic holistic approach based on the introduction of the concept of the human microbiome as an important specific physiological system that interacts with all other physiological systems of the body are briefly presented in this review. Engineered probiotics. Engineered probiotics are modified original probiotics using gene editing with the newest tools and technologies. By means of gene editing, probiotics have a variety of beneficial properties and treat specific diseases, such as inflammatory bowel disease, bacterial infection, tumor, and metabolic illnesses. In the past few years, there have been some advances in the development of engineered probiotics that will benefit people [1]. Psychobiotics. Nowadays considerable attention has been received to the gut-brain axis, and the "psychobiotics" concept indicates that probiotics have a potentially positive effect on mental health in patients and animals. Psychobiotics control the functioning or actions of the central nervous system conciliated by the gut-brain axis through neural, humoral, and metabolic pathways to ameliorate gastrointestinal activity as well as an anti-depressant and anxiolytic capacity. The formulation of new psychobiotic-based therapeutics is in the spotlight [2]. Probiotic anti-age correction. The organism aging based on cellular senescence is an intrinsic aging process that has recently been associated with microbial imbalance, as differences in the composition of certain bacterial species in the human multicomponent (6-7) microbiome have been identified between the elderly and the young. It has been suggested that the manipulation of the microbiomes would be an innovative strategy [3].

Conclusions. Microbiome correction despite the use of new probiotics to promote human longevity and well-being, an effective use of probiotics in clinical practice are limited and need further research in mentioned above fields.

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DEVELOPMENT OF SMALL MOLECULE OF HUMAN PROTEIN KINASE C BETA INHIBITORS AS A KEY ENZYME FOR NEUTROPHILIC GRANULOCYTES ACTIVATING WITH THE FORMATION OF NEUTROPHIL EXTRACELLULAR TRAPS (NETS)

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Aim. The main pathogenetic mechanism of acute respiratory distress syndrome (ARDS) triggering in COVID-19 is the activation of neutrophilic granulocytes with the formation of neutrophilic extracellular traps (NETs). Protein kinase C beta (PKCβ) is a key enzyme for neutrophilic granulocytes activating with the formation of NETs. Therefore, PKCβ is considered as an attractive molecular target for the treatment of COVID-19-related ARDS. The **aim** of this study was to develop novel inhibitors of PKCβ to open up prospects for the development of a new method of ARDS treatment.

Methods. Virtual screening of compounds was performed with pharmacophore modeling and molecular docking. The effectiveness of selected compounds was evaluated *in vitro* both in experiments with enzyme-linked immunosorbent assay using the PKC Kinase Activity Kit (ab139437) and in experiments on isolated neutrophilic rabbit granulocytes (the method of counting cells with double staining with fluorescent dyes was used to assess the activity of the formation of neutrophilic extracellular traps).

Results. Several classes of inhibitors have been already identified. We developed and validated ligand-based PKCβ pharmacophore models based on the chemical structures of the known inhibitors. Two of the most accurate pharmacophore models, which correctly predicted the activity of compounds in the test set by more than 60%, were used for pharmacophore screening. We have used a combination of pharmacophore modeling and molecular docking approaches for the virtual screening of compound collection containing about 150,000 compounds. According to the cross-results of both virtual screenings, we have selected 48 compounds for in vitro testing. To estimate the inhibitory activity of tested compounds toward PKCβ we used the PKC Kinase Activity Kit. The effectiveness of active developed PKCβ inhibitors on the formation of neutrophil extracellular traps (NETs) was determined and the dose-dependence of their activity was evaluated.

Conclusions. Three promising compounds were identified. Their effectiveness was estimated on experimental models of ARDS in rats. According to *in vivo* studies these compounds prevented NETosis in experimental animals and therefore can be valuable candidates for further preclinical investigations.

EVOLUTION OF SPECIES-SPECIFIC *ALUJ_MIM* REPEAT OF *MICROCEBUS MURINUS* IN PRIMATES

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Aim. The aim of the study was to trace the evolutionary history of the species-specific *AluJ_Mim* replication of *Microcebus murinus* in orthologs of the primate *MGMT* gene.

Methods. The homology between nucleotide sequences has been determined by BLAST 2.6.1 program. The results of the search and identification of mobile genetic elements have been obtained from CENSOR program.

Results. *Alu*-repeat *AluJ_Mim* is a species-specific repeat of *Microcebus murinus*. *Microcebus murinus* belongs to the order of Strepsirrhine primates from the superfamily Lemuroide. Repeat *AluJ_Mim* has a length of 317 bp. It is present in the *MGMT* gene mainly in introns 1 and 2 only in representatives of the superfamily Lemuroide (*Microcebus murinus*, *Prolemur simus*, *Propithecus coquereli*) among strepsirrhine primates, and it has not been identified in *Otolemur garnettii*, a representative of the superfamily Lorisoidea. Repeat *AluJ_Mim* has also not been detected in the Tarsiiformes and New World monkeys. but it is present in *MGMT* gene orthologs of the Old World monkeys, in members of the subfamily Colobinae (*Colobus angolensi spalliatus* and *Piliocolobus tephrosceles*) within intron 1. In addition to truncated sequences with partially deleted 5 ‘or 3’ ends, monomeric repeats of FRAM and FLAM, *AluJ_Mim* undergoes to deletion degradation (64 bp fragments identified with coordinates 18-81) in the intron sequences of the studied *Microcebus murinus* gene. Only monomeric FRAM repeats have been detected in the *MGMT* gene sequences of the Colobinae subfamily. And the sequence of the same monomer has been identified in intron 1 of the representative of apes - *Nomascus leucogenys*.

Conclusions. The evolutionary history of *Alu*-repeat *Microcebus murinus AluJ_Mim* covers the time of the formation of Strepsirrhine, Old World monkeys and small apes.

THE REDUCTION OF SULFATE OF GYPSUM BY SULFIDOGENIC MICROBIOME FOR THE PRECIPITATION OF TOXIC METALS

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Background. The problem of treatment of polymetallic wastewater is globally relevant. An approach based on microbial sulfate reduction to precipitate metal cations via sulfide is promising for the removal of a wide range of metals from wastewater. Dissimilatory sulfate reduction is based on the use of sulfates by microorganisms as a terminal electron acceptor. Hydrogen sulfide or sulfide anions are the end products of sulfate reduction, which are universal reagents for the precipitation of a wide range of metals. Divalent metal cations precipitate to insoluble CdS, CuS. High-potential oxidizing metals (CrO_4^{2-}) are reduced to insoluble compounds by low-potential sulfides. For example CrO_4^{2-} with $E_0' = +555$ mV is reduced to insoluble $Cr(OH)_3 \cdot nH_2O$ by S^{2-}, SH^-, H_2S with Eh = -150…-200 mV. However, this technology is still not used, because in high concentration sulfides are hazardous to the environment. The solution to this problem is the use of a sparingly soluble source of sulfates, namely gypsum ($CaSO_4 \cdot nH_2O$).

Aim. The aim of our work was to determine the actual solubility of gypsum to confirm its prospects of application in wastewater treatment processes.

Methods. Two types of samples were used to determine the solubility of gypsum: gypsum powder (LLC LOGICLABGROUP) and rock extracted from the caves of gypsum quarries in the Western Podillya. Gypsum rock was crushed into particles of 3-5 mm. Samples of gypsum of 5 g were placed in flasks and poured with 200 mL of distilled water. Two types of treatments were conducted: with and without mixing. The temperature was 30°C. The degree of solubility of gypsum was determined by the concentration of SO_4^{2-} ions in the solution. The concentration of SO_4^{2-} anions was determined colorimetrically based on the precipitation reaction with Ba^{2+} cations. Calibration curve was built by a series of solutions of Na_2SO_4 with concentrations of 10, 20, 30, 40, 50 and 60 mg/L SO_4^{2-}. Sulfate anions were precipitated with a 0.1% solution $BaCl_2$. Observations were performed for 4 days.

Results. The gypsum was shown to have a very low solubility, only 40-60 mg/L. In this regard, microorganisms are assumed to reduce sulfate very slowly. This means that the release of H_2S will be in very low in safe concentrations than 10-20 mg/L. However, it is sufficient to precipitate a wide range of metals (Co_2^+, Ni_2^+, CrO_4^{2-}, VO_4^- etc.). In comparison with other sources of sulfates in the processes of sulfate reduction, the gypsum is promising, as it is a super-cheap mineral.

Conclusions. Due to the low solubility of gypsum (40-60 mg/L) it was shown to be potentially promising to provide slow sulfate reduction in order to develop the approach for metals removal from solution. This approach is promising to be the basis for the development of new environmental biotechnologies.

INFLUENCE OF MELATONIN ON ROS GENERATION BY CELLS OF ROOTS OF WHEAT SEEDLINGS AND THEIR HEAT RESISTANCE

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Aim. Melatonin, well known as the main animal hormone of the pineal gland, a regulator of the circadian rhythm of all living organisms, is currently being actively studied as an important bioregulator of many functions of plant organisms. One of the most important components of the stress-protective effect of melatonin is the reduction of stress-induced oxidative damage to cellular structures. This effect is due, in particular, to the direct antioxidant action. However, ROS, primarily hydrogen peroxide, are important signaling molecules involved in both the transduction of stress signals and the effects of various regulatory molecules, including some phytohormones. The possible involvement of ROS in the appearance of the stress-protective effect of melatonin on plants has been very poorly studied. The aim of the investigation was to establish the participation of ROS in the melatonin-induced resistance of wheat seedlings to potentially lethal heat stress.

Methods. Etiolated seedlings of wheat (*Triticum aestivum L.*) of Doskona variety were used for experiments. Three-day-old seedlings were incubated in medium with melatonin at the concentration of 1 μM during 24 hours. To clarify the possible role of hydrogen peroxide, generated with the participation of NADPH oxidase, in the implementation of the effects of melatonin, an H_2O_2 scavenger dimethylthiourea (DMTU – 150 μM) or an NADPH oxidase inhibitor imidazole (10 μM) were added to the medium of the corresponding variants. After the incubation on the appropriate solutions, the seedlings were subjected to the damaging heating in water ultrathermostat (45°C, 10 min) and their survival was evaluated after 3 days.

Results. Incubation of seedlings in melatonin solution led to a transitory increase in the content of hydrogen peroxide in the roots with a maximum in 1 h. But at the end of incubation (24 h) there was a decrease in the content of H_2O_2 compared to control. The melatonin-induced effect of increase of hydrogen peroxide content in seedling roots was eliminated by the H_2O_2 scavenger DMTU and the NADPH-oxidase inhibitor imidazole. Both DMTU and imidazole almost completely eliminated the protective effect of melatonin on seedlings, exposed to heat stress.

Conclusions. The obtained results show that ROS, generated by seedlings during the treatment with melatonin, are necessary mediators in the implementation of its protective effect under heat stress. A probable reason of increased ROS generation by plant cells during melatonin treatment is increase in NADPH-oxidase activity.

BIOGAS PRODUCTION IN LABORATORY CONDITIONS

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Aim. The situation in connection with the aggression against our country, the largest supplier of natural gas, Russia, requires an active search for alternative energy sources. Biogas is a renewable fuel, an analogue of natural gas, consisting of methane, carbon dioxide and impurities formed during anaerobic fermentation of biomass by methane-forming bacteria. Biogas has great energy potential, which will give Ukraine energy independence. It should be produced from residues to be disposed of, such as poultry manure, waste from sugar and alcohol factories. The main raw material for biogas production in the study is molasses bard (vinasse). Vinasse – the residue after distillation of alcohol from the brew. Vinasse is a waste that pollutes nature. However, in sugar beet vinasse, the high nitrogen content makes the C/N ratio below the optimal range for biogas production. Therefore, carbon-rich materials should be added. Lignocellulosic raw materials such as bagasse or sugar sorghum stalks can serve as this.

Methods. Microbiological (cultivation of biogas producers *in vitro*). Chemical, biochemical, physico-chemical: study of the composition of bioethanol production waste for biogas production (sorghum bagasse, beet molasses bard); determination of products of metabolism of microorganisms-producers during methane fermentation of these wastes of bioethanol production; study of indicators of residues formed after methane fermentation – potential fertilizers. Mathematical (statistical processing of research results, optimization of methane fermentation parameters).

Results. After a series of studies of methane fermentation of vinasse in the laboratory, the following results were achieved: Methane fermentation of molasses bards was studied in a laboratory installation in mesophilic conditions, with periodic loading. The biogas plant consisted of a methane formation bioreactor; receiving flask for biogas with liquid displacement (saline solution to prevent adsorption of gas by water); receiving flask for liquid. Fermentation was carried out at temperature 30°C, 37°C, 40°C (in a thermostat), as well as stirring with a magnetic stirrer or shaker. As a raw material, to compare the yield of biogas, served pure vinasse and with the addition of sugar sorghum stalks of different types of processing.

Conclusions. The best result on the yield of biogas showed a sample with the addition of ground stalks of sugar sorghum. Fermentation was much faster and more complete than other samples. The combined use of bard and plant biomass is expected to increase the amount of active microflora in the reactor and increase productivity, increase the load on the installation. Methane fermentation of vinasse will reduce greenhouse gas emissions. Digestate, a product after fermentation and biogas production, can be used as a fertilizer.

GENETIC CONTROL AND FACTORS INFLUENCING HUMAN INTELLECTUAL ABILITIES IN POST-EMBRYONIC ONTOGENESIS

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Aim. Intellectual activity is one of the most complex systems for genetic analysis and control, but despite this, genetic factors affect the intellectual development of each person, which can be inherited and acquired. Intelligence is the ability of a person to solve various mental problems of various forms of complexity. The formation of the so-called elite depends on the intellectual abilities of each subject, and then on the society itself.

Methods: I actively used methods of bibliographic, statistical, system-structural, comparative nature and methods of observation.

Results. As part of the work, two groups of people were analyzed, namely, one family-parent couple and their monozygotic twin children. We have already noted, in genetic formation, intelligence and mental abilities depend primarily on hereditary factors. Thus, monozygotic twins have a high level of correlation in IQ. Indicators of heredity were indeed very significant, and the aspectual impact of the environment and environmental factors - insignificant. I would like to note that due to the ecological changes that are relevant today, the percentage of impact of environmental elements, as well as natural physical and chemical factors on the IQ on organic life forms in our case, is increasing. This applies to the molecular genetic level of the biological system, namely the influence of these factors on the haploid cells of the germ, can be mutagenic all determined at the macromolecular and cellular levels of the human biological system. In particular, mutagenesis can be reflected in the indicators of intellectual abilities in certain subjects. The results of IQ correlation were confirmed in genetic studies of foster children. In addition, the relationship between IQ between children and their biological parents was significantly higher than between foster parents and the degree of kinship that was absent from these children. This proves that the genetic factor in the formation of mental abilities in a new person of the human species plays a significant role.

Conclusions. According to the research, the following conclusions can be drawn. The role of environmental elements in the genetic formation of human intelligence at different levels of the biological system is insignificant, but today ecological catastrophe and global environmental problems force to study the influence of chemical and physical factors on human intelligence at different stages of ontogenesis in higher detail. The formation of IQ in each person really has a hereditary genetic character - this was shown by the results of studies conducted on children between their biological parents and foster parents. The results of the comparative characterization indicate that the connection of intellectual level indicators between children and biological parents is higher than with persons who are unrelated. Intelligence can also be gradually acquired and improved during the whole post-embryonic ontogenesis.

DETERMINATION OF CONTAMINATION OF DNA SAMPLES USING MITOCHONDRIAL DNA MARKERS

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Background. The paper describes the study of the use of PCR- RFLP method as an analytical tool for establishing the mitochondrial genome of hybrid pigs (Large White × Landrace) × Maxgro. This method was used to detect transfected DNA fingerprints in the form of contamination of the studied samples by the amplification of the variable section of the D-loop with a length of 428 base pairs and synthesis of PCR components of TasI endonuclease products.

The aim. The purpose of the work was to establish the cause of contamination of the samples under study. To define the impact of the contamination on the validation of hybrid pigs mitochondrial genome.

Methods. The release of DNA from the bristles of pig's ears was carried out according to the method of Serhii Korinnyi using Chelex -100 ion exchange resin. The samples of epithelial tissue were treated with flame being fired. Afterward, extracted using a DNA-sorb-B kit for the isolation of nucleic acids from LLC "InterLabService-Ukraine". PCR amplification of fragment D–loops mitochondrial genome, carried out on the amplifier TERTSYK-2 (DNA-Technologies) using a set from (Thermo Scientific™) and oligonucleotide primers: forward-MITPRO2F (CATACAAATATGTGACCCCAAA) and reverse –MITPROR (GTGAGCATGGGCTGATTAGTC). Alikvot of PCR product (4 μL) was hydrolyzed with TasI endonuclease (Thermo Scientific™). DNA amplification and hydrolysis products were analyzed in 8% polyacrylamide gel in an electrophoretic device in the 1×TBE buffer. As a marker of molecular weight, pBR322 DNA/MspI and pUC19 DNA/Msp I. Visualization of amplification and restriction products was carried out by painting with bromide ethidium and photographing on a transilluminator in UV.

Results. Samples in quantity (n=9) isolated by ion exchange resin were contaminated which made it impossible to determine the true haplotypes of the studied hybrid pigs. Decontaminated samples by flame being fired on epithelial tissue as well as the extraction of samples by the sorbent method made it possible to determine the true cause of contaminated DNA. It was found that the objects under study (pig ears from a meat processing plant "Globyno", in particular) were contaminated while samples selection. Decontaminated fragments of the studied samples were characteristic to determine the mitochondrial haplotypes of hybrid pigs (n=30). This technique made it possible to obtain haplotype C - Landrace, Hampshire, Wild pig (Ukraine, Poland). - 7; 5 pigs with haplotype G - Wales, Wild pig (Italy); 10 pigs with haplotype O – Landrace, Wild pig (Sweden); 8 pigs with haplotype N – Large White (Asian type).

Conclusions. Therefore, an important factor determining the variability of the profile is not so much the method of DNA extraction is a true pure sample of the host to establish a clear examination of the mitochondrial genome.

IMPACT OF DIETARY PROTEIN DEFICIENCY ON THE STATE OF THE GLUTATHIONE SYSTEM IN THE LIVER OF RATS OF REPRODUCTIVE AGE UNDER TOXIC INJURY WITH ACETAMINOPHEN

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Introduction. Nowadays, the course of many nutrient-dependent diseases is complicated by the indiscriminate use of a wide range of over-the-counter drugs, in particular paracetamol (acetaminophen, APAP). It is known that taking APAP in supra-therapeutic dosages leads to hepatotoxicity, the development of which largely depends on the functioning efficiency of the glutathione system.

Aim. The aim of this study was to evaluate glutathione redox status, glutathione-S-transferase (GST) and glutathione peroxidase (Se-GPx, non-Se-GPx) activities in cytosolic liver fraction of rats at the reproductive age under acetaminophen-induced liver injury against the background of alimentary protein deprivation.

Methods. Throughout the experiments, we focused on four groups of animals aged 140-150 days, which is equivalent the developmental stage of the human body at the age of 24-25: C − control; LPD − animals kept on a low-protein diet; TI − animals exposed to simulated acute APAP-induced liver injury; LPD/TI − animals exposed to simulated APAP-induced lesion under alimentary protein deprivation.

Results. The results of these studies demonstrated a significant decrease in the redox index (RI) and redox potential (Eh) of glutathione in the liver of the rats of reproductive age in all study groups (LPD, TI, LPD/TI) as compared to the control (C). The changes were probably due to a decrease in the amount of reduced glutathione (GSH) on the background of the maximum increase of its oxidized form (GSSG) in protein-deficient rats with APAP-induced liver injury. At the same time we recorded a decrease in glutathione S-transferase activity in the liver of all the studied groups of animals as compared with the control (C), $p<0.05$. The mechanism of APAP hepatotoxicity is realized by accumulation of the reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), through N-oxidation by the CYP450 system, as confirmed by our earlier studies, and is spontaneously conjugated to glutathione and/or in the glutathione S-transferase reaction. Thus, a surplus of NAPQI leads to a depletion of the GSH pool serving as a GST substrate. Moreover, there was also observed a activity decrease in Se-GPx in the liver of TI and LPD/TI groups of animals of reproductive age under study, while the activity of non-Se-GPx remained at the control level, which may be accompanied by disruption of H2O2 and organic hydroperoxides neutralization with the development of oxidative stress.

Conclusions. Thus, APAP-induced liver injury against the background of alimentary protein deficiency is accompanied by a decrease both in glutathione reduction potential and activities of glutathione-dependent enzymes, glutathione S-transferase and Se-dependent glutathione peroxidase, in rat of reproductive age liver cells.

PARTICIPATION OF A SIGNAL MOLECULE H_2S IN INDUCTION OF WHEAT SEEDLINGS HEAT TOLERANCE

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Aim. Hydrogen sulfide (H_2S) is one of the key molecules-gasotransmitters in both plant and animal cells. The positive effect of H_2S donors on resistance to high temperatures has been established for plant species. The role of endogenous hydrogen sulfide in plants' adaptation to high temperatures is much less studied. The possible role of hydrogen sulfide as a mediator for increasing plant heat resistance to the short-term effects of damaging temperatures remains unexplored. The **aim** was to establish the possible participation of endogenous hydrogen sulfide in the formation of heat resistance of wheat seedlings under the influence of short-term heating.

Methods. The object of the study was etiolated seedlings of winter soft wheat (*Triticum aestivum L.*) variety Doskonala. Three-day seedlings of the respective variants of the experiments were transferred for 24 h to hypotaurine (300 μM) – hydrogen sulfide scavenger solutions or sodium pyruvate (300 μM) – an inhibitor of L-cysteine desulfhydrase. After treatment with the test compounds, the seedlings were subjected to 1-min hardening heating in an aqueous ultrathermostat at a temperature of 42°C. The heat resistance of seedlings was assessed 24 h after exposure to hardening temperature. For this, the samples were subjected to damaging heating in an aqueous thermostat at a temperature of 45°C for 10 min. The H_2S content in the roots was determined using the reaction with 5,5'-dithiobis-2- nitrobenzoic acid (Li et al., 2014).

Results. After a 1-min exposure to a temperature of 42°C in roots of wheat seedlings, a transient increase in hydrogen sulfide with a maximum of 1.5 h after heating was observed. At the same time, 24 h after exposure to high temperature, the H_2S content in roots decreased to the control level. The effect of increasing the content of hydrogen sulfide caused by the action of the hardening temperature did not manifest under the treatment of seedlings with H_2S scavenger hypotaurine and the inhibitor of L-cysteine desulfhydrase sodium pyruvate. Damaging heating of seedlings caused an increase in the content of lipid peroxidation (LPO) products in root cells and the subsequent death of a significant part of the seedlings. The preliminary hardening heating significantly increased the heat resistance, decreasing the LPO intensity and the level of seedling death. At the same time, their treatment with the H_2S scavenger hypotaurine and the inhibitor of L-cysteine desulfhydrase sodium pyruvate largely neutralized the development of heat resistance caused by hardening heating.

Conclusions. The results obtained suggest the participation of endogenous hydrogen sulfide in the formation of the heat resistance of wheat seedlings after short-term exposure to hardening temperature. One of the components of this effect could be an H_2S-mediated modification of the antioxidant system involved in the protection of root cells from oxidative damage caused by higher temperatures.

EVALUATION OF CHANGES IN BIOLOGICAL ACTIVITY OF PLACENTA EXTRACTS UNDER LOW-TEMPERATURE EXPOSURE

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Aim. Erythrocytes are a unique model for assessing the intensity of processes that destabilize membranes. A good knowledge of erythrocyte structural and functional state makes it possible to use them to identify targets of action of bioactive compounds and to assess the potentially damaging effect of various factors on the revealed drugs' bioactivity. The purpose was a comparative study of the effect of low temperatures on the antioxidant and anti-inflammatory activity of human placenta extracts (HPE) and their fractions on erythrocytes as a model.

Methods. The research objects were aqueous-saline extract and its individual fractions obtained from human placentas as well as the suspension of erythrocytes. HPE fractions were obtained by gel chromatography. The extract antioxidant activity was evaluated on erythrocytes subjected to oxidative stress caused by sodium nitrite or hydrogen peroxide. The anti-inflammatory effect of HPE and their fractions were evaluated by their effect on erythrocyte thermal stability. Thermal stability was assessed by the level of thermal hemolysis of erythrocytes, exposed to 55°C as well as by a cytosol dynamic state and a barrier function of the erythrocyte membrane for small ions (ferricyanide) at hyperthermia. The methods of spectrophotometry and EPR spin probes were used.

Results. It was shown that after freezing the placenta to -20°C and -196°C, the antioxidant and anti-inflammatory effects of HPE remained at the control level. As in the case of HPE from the fresh placenta, for HPE from the frozen placenta, the greatest effect was observed for the 7-13 kDa fraction. Placenta storage at moderately low temperatures (-20°C), provided that the bioactivity of HPE is preserved at a sufficient level, is limited to up to 3 months. Storage of the placenta at -196°C ensures the preservation of antioxidant and anti-inflammatory activity almost at the control level for more than 6 months. Wherein, the impact of low temperatures on isolated extracts leads to a decrease in their beneficial effects. An analysis of the obtained results suggests that the target of the HPE action most probably is the weak interactions between cytoskeleton, cytosol, and membrane, the molecular dynamics of which determines the physiology of erythrocytes.

Conclusions. Erythrocytes are an adequate and accessible model for a rapid *in vitro* assessment of the potential biological activity of natural substances, in particular, their antioxidant and anti-inflammatory properties. RBCs are also promising for evaluating the level of preservation of the biological activity of extracts during long-term storage.

SKIN MICROBIOTA AND PHAGOCYTIC ACTIVITY OF PERIPHERAL BLOOD CELLS

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Aim. The purpose of the work is to reveal associative relationships between the parameters of the skin microbiota and the phagocytic activity of neutrophils and monocytes in peripheral blood.

Methods. Two cohorts of practically healthy individuals were examined. The first cohort was formed by university students, and the second group – was meat and egg production personnel. The survey was conducted in compliance with the norms of bioethics, the participants gave written consent to participate in the research. Skin microbiota parameters were determined by washing and growing on nutrient media, followed by Gram staining of microbiological preparations. Blood sampling was carried out by medical specialists. The phagocytic index and the phagocytic number of neutrophils and monocytes were determined by the ability to absorb yeast cells, followed by smear staining according to Romanovsky-Giemsa.

Results. When assessing the microbiota, special attention was paid to sanitary-indicative microorganisms: bacteria of the *Escherichia coli* group (Coliform bacteria); *Staphylococcus spp.*; *Enterococcus spp.* (Group D Streptococcus (GDS)). Representatives of the *Escherichia coli* were found in 12% of the examined from the control group and 10% of the examined from the experimental group. Representatives of the *Staphylococcus spp.* group were found in 72% of the surveyed from the group of university students and 68% of the personnel in the production of meat and egg products. The MAFanM and phagocytic activity indicators were within the normal range, there is no statistically significant difference between the groups under consideration. There was a tendency to increase the indicators of phagocytic activity of monocytes in carriers of *Staphylococcus spp.* A positive correlation was found between the MAFanM indices and the phagocytic activity of peripheral blood monocytes ($P<0.05$).

Conclusions. Despite the absence of a direct connection between the skin microbiota and peripheral blood phagocytes, the presence of individual sanitary-indicative representatives of the microbiota affects the phagocytic anti activity of monocytes. The mechanisms of such an association require further research.

ROLE OF NON-SPECIFIC PHOSPHOLIPASES C AND DIACYLGLYCEROL KINASES IN STEM GRAVITROPISM OF *ARABIDOPSIS THALIANA*

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Background. Gravitropism is an important directional growth response toward gravity vector. Since the role of phospholipases and signaling lipids was proposed in plant gravitropism, the role of non-specific phospholipase C (NPC, that hydrolyzes membrane phospholipids producing diacylglycerol) and diacylglycerol kinases (DGK, that further phosphorylates diacylglycerol generating signaling phosphatidic acid) in plant gravitropism have not been investigated to date.

The **aim** of this paper was to investigate the participation of NPC and DGK in plant responses to gravity, gravitropism of inflorescence stems of *Arabidopsis thaliana* intact plants, and knockout mutants of different NPC and DGK isoforms genes (*npc* and *dgk*).

Methods. It was studied by horizontal stem orientation for 180 min and CorelDraw program analysis.

Results. It was observed that stem gravitropism was dramatically reduced in mutants *npc3*, *npc4*, and *npc6*, in comparison to *npc1*, *npc2*, and *npc5* plants. Gravitropism of *npc2* mutants was weakened only in later stages, whereas in *npc1* and *npc5* mutants, it was partially reduced in all time-points of the gravitropic response. The reduction of gravitropism of the mutants was also highly expressed in later time-points of gravitropic response (especially in *npc3* mutants). In DGK mutants, stem gravitropism was reduced in all analyzed plant lines, in comparison to wild-type plants. In *dgk1*x*dgk2*, *dgk3*x*dgk7*, and *dgk4* mutants, stem gravitropism was highly reduced in all investigated stages of the response, whereas in *dgk5*x*dgk6* mutants, the gravitropism reduced in the early stages of the response was restored to the wild-type level only in later time-points.

Conclusion. The results suggest that NPC4, NPC6, and especially NPC3 play an important role in all stages of *Arabidopsis thaliana* stem gravitropism, whereas NPC1, NPC5, DGK1-4, and DGK7 play a partial role in the response. Also, NPC2 plays a partial role only in later stages, whereas DGK5 and DGK6 – are in the early stages of plants' stem gravitropism.

EVALUATION OF GENOTOXICITY OF SILVER NANOPARTICLES *IN VIVO*

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Aim. The **aim** of the study was to investigate the safety *in vivo*, namely the genotoxicity and mutagenicity of silver nanoparticles (AgNPs) of medium size 30 nm, obtained by the method of "green" synthesis.

Methods. Experimental AgNPs were synthesized at the F.D. Ovcharenko Institute of Biocolloidal Chemistry of the NAS of Ukraine by the method of "green" synthesis. AgNPs are a sterile brown aqueous dispersion with spherical silver nanoparticles of medium size 30 nm and a concentration of 0.8 mg/ml per metal. The *in vivo* genotoxicity of AgNPs was assessed by alkaline DNA under alkaline conditions and mutagenicity by micronucleus test in polychromatophilic erythrocyte cells of the bone marrow of laboratory mice involving adult females of white laboratory mice of the BalB/C line. The manifestation of the mutagenic effect of AgNPs was also investigated by the anaphase method of counting chromosomal aberrations in the cells of the apical meristem of the onion *Allium cepa*.

Results. Comet assay showed that the studied AgNPs at concentrations of 2 mg/kg and 4 mg/kg did not show genotoxic effects *in vivo* on uterine and ovarian cells of female mice when administered intravenously 10 times. Indicators of DNA-destructive activity - "IDNA" were at the level of a similar indicator of the control group of animals. Testing of AgNPs mutagenicity *in vivo* by micronucleus test in polychromatophilic erythrocyte cells of the bone marrow of laboratory mice showed that the incidence of cells with micronuclei, both in the control group and in the experimental groups of animals intravenously 10 times 2 mg/kg and 4 mg/kg was about 0.17%, which corresponds to the spontaneous frequency of micronuclei formation. A study of the manifestation of the mutagenic effect of AgNPs by anaphase method of counting chromosomal aberrations in cells of the apical meristem *Allium cepa* showed that AgNPs in the concentration range of 0.8 - 0.01 mg/mL of metal caused a slight increase in the percentage of aberrant cells (up to 0.3%) compared with *Allium cepa* plants of the control group. Such indicators may be due to the intensification of the mitotic process in the treated cells with these nanoparticles and do not indicate their mutagenic activity.

Conclusions. Experimental *in vivo* studies have shown no genotoxic effect in the "green" synthesis of 30 nm "AgNPs" in the cells of the reproductive organs of laboratory mice – ovaries and uterus. The absence of mutagenic action of these nanoparticles in the test for the formation of micronuclei in polychromatophilic erythrocytes of the bone marrow of laboratory mice is also shown. No mutagenic effect of such AgNPs on the plant organism has been reported. It is concluded that the low level of ecotoxic action of AgNPs with an average size of 30 nm – are products of "green" synthesis.

EFFECT OF ALPHA-KETOGLUTARATE ON PRO-/ANTIOXIDANT STATUS IN MIDDLE-AGED *DROSOPHILA MELANOGASTER*

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Background. Recent studies have shown that alpha-ketoglutarate (AKG), an intermediate of the tricarboxylic acid cycle (TCA), possesses both prooxidant and antioxidant properties. Being TCA intermediate, AKG is involved in NADH supply for mitochondria, thereby activating mitochondrial respiration followed by increased production of reactive oxygen species as by-products. Antioxidant properties of AKG is realized due to non-enzymatic interaction of this alpha-keto acid with hydrogen peroxide with formation of succinate, water and carbon dioxide. Indirect antioxidant effects of AKG are connected with the stimulation of endogenous antioxidant defense, perhaps due to activation of transcription factor Nrf2. It has been shown recently that AKG as a dietary supplement can delay aging and increase lifespan of several model organisms, including *Drosophila melanogaster*.

Aim. To elucidate whether these effects of AKG could be connected with modulation of redox processes, we studied the effects of dietary AKG on pro-/antioxidant status of middle-aged *D. melanogaster* flies.

Methods. *D. melanogaster* Canton S strain was used in the experiments. Adult 4-day-old female flies were divided into five groups, which were kept for next 21 days on standard medium (control) and media supplemented with 0.01, 0.1, 1 and 10 mM disodium salt of alpha-ketoglutarate. In 25-day-old flies, activity of antioxidant enzymes (catalase and glutathione-S-transferase) and markers of oxidative stress (levels of oxidized lipids and thiol-containing compounds) were measured.

Results. The level of lipid peroxides was unaffected in flies consuming diets with AKG. Similarly, the content of protein thiols was not affected any diet type. In contrast, the content of low-molecular mass thiols, presented mainly by glutathione, remained unchanged at low AKG concentrations (0.01 and 0.1 mM) but was reduced by 25% and 26% in flies consumed diets with 1 and 10 mM AKG, respectively. Activity of catalase decreased by 42% in flies fed with diets containing 10 mM AKG, whereas food with lower AKG concentrations did not affect catalase activity. Activity of glutathione-S-transferase was unaffected by AKG-containing diet.

Conclusions. Feeding with low concentrations of dietary alpha-ketoglutarate does not affect redox homeostasis in middle-aged Drosophila, but at higher concentrations AKG seems to display pro-oxidant properties supported by decreased low-molecular mass thiol content and lower catalase activity. One may suppose that development of mild oxidative stress can be one of the mechanisms of beneficial effects of AKG observed in earlier studies.
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EFFECTS OF HIGH-CALORIE DIET AND ALPHA-KETOGLUTARATE ON THE CONTENT OF LIPID PEROXIDES IN MOUSE TISSUES

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Aim. Reactive oxygen species (ROS) are continuously generated as a result of redox reactions in response to endogenous and exogenous factors. If the rate of ROS formation exceeds the capacity of antioxidant systems, cells are subjected to oxidative stress. The intensity of oxidative stress can be assessed by various markers, one of which is the content of lipid peroxides (LOOH) which represents the intensity of lipid oxidation. The development of obesity is also associated with the intensification of oxidative stress. It was shown recently that alpha-ketoglutarate (AKG), a key intermediate of the tricarboxylic acid cycle, has a mitigating effect on oxidative stress and can act as an antioxidant in mammalian cells. Therefore, the aim of our work was to investigate the effect of dietary AKG on the content of lipid peroxides in various tissues of mice fed basic and high-calorie diets.

Methods. C57Bl/6J male mice were used in the experiments. Five-month-old animals were randomly divided into four groups, control, and three experimental ones. Control mice were fed a standard diet (10 kcal% fat). Mice of the first experimental group were fed a high fat and high fructose diet (HFFD, 45 kcal% fat). The second group received a standard diet supplemented with 1% disodium salt of AKG in drinking water (AKG group), and the third one received HFFD with 1% AKG in drinking water (HFFD + AKG group). Mice were kept on an appropriate diet for the next 8 weeks.

Results. In the mouse liver, the content of lipid peroxides was 42% and 85%, higher in the AKG and HFFD groups respectively, compared with the control group. HFFD increased lipid peroxides by 90%, 83%, and 85% in the cortex, adipose tissue, and liver, respectively, and these changes were not corrected by supplementation with AKG. Lipid peroxides in the heart, muscles, and kidney were not significantly affected by HFFD or HFFD + AKG, except HFFD + AKG increased lipid peroxide level in muscles. Consumption of AKG alone did not significantly affect the content of lipid peroxides in the cortex, heart, muscles, and adipose tissue, but led to 38% and higher lipid peroxide level in the kidneys and liver, respectively.

Conclusions. Thus, a high-fat high fructose diet increased levels of lipid peroxides in many organs of young mice suggesting developing oxidative stress. Heart and muscles were more resistant to HFFD-induced changes. The addition of AKG to HFFD diet with did not show any modulating effects of lipid peroxide levels. AKG alone increased LOOH level in some mouse organs indicating development of mild oxidative stress.

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ERYTHROCYTES DON'T INTERNALIZE $LaVO_4:Eu^{3+}$ NANOPARTICLES

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Aim. To determine the ability of lanthanum orthovanadate doped with europium ($LaVO_4:Eu^{3+}$) nanoparticles of approximately 100 nm in size to enter intact rat erythrocytes.

Methods. Briefly, 13 µl of freshly prepared erythrocyte suspensions obtained from blood of intact WAG rats incubated with $LaVO_4:Eu^{3+}$ nanoparticles for 24 h at 80 mg/L were used. Images of cells were acquired via the Olympus FV10i-LIV scanning confocal microscope supplemented with a 60/1.2 NA water-immersion objective. Post-acquisition images were processed taking into account the analysis of autofluorescence, background subtraction and fluorescence profiles. Europium-enabled luminescent $LaVO_4:Eu^{3+}$ nanoparticles were excited at 391 nm, while the fluorescence was acquired at 618 nm.

Results. In this study, we investigated the ability of large-sized (100 nm) rat erythrocytes to internalize $LaVO_4:Eu^{3+}$ nanoparticles. As demonstrated by scanning confocal laser microscopy, exposure of rat red blood cells to large $LaVO_4:Eu^{3+}$ nanoparticles did not result in their internalization. Instead, the nanoparticles adhered to the cell membranes and were not found in the cytosol of cells. Our findings are consistent with other experimental data on the inability of large-sized nanoparticles to enter erythrocytes and provide novel insights concerning the mechanisms of biological effects of nanostructured materials.

Conclusions. Rat red blood cells cannot internalize $LaVO_4:Eu^{3+}$ nanoparticles whose size is about 100 nm.

D-GLUCOSAMINE-CONTAINING DERIVATIVES OF ORGANIC DYES AS FLUORESCENT PROBES FOR CELL IMAGING

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Aim. Fluorescent far-red labels and their conjugates with various molecules are widely used in medicine, bioimaging, and drug distribution. An essential condition for using dyes is the absorption and emission of light in the "therapeutic window" (600–800 nm) of biological tissues. It is necessary to study the changes in the spectral properties of dyes depending on the substituent for the successful use of dyes as fluorescent labels. The research **aim**s to synthesize merocyanine and squaraine dye and their glucosamine-containing derivative. The spectral-luminescent properties and cell membrane permeability have been investigated to understand how derivatives affect the properties of the dyes.

Methods. Organic synthesis, fluorescence spectroscopy, UV-VIS spectroscopy, laser scanning confocal microscopy.

Results. The spectral-luminescent properties of studied dyes in the methanol, aqueous solution (0.05 M Tris-HCl buffer pH 7.9), and in the presence of bovine serum albumin (BSA) were investigated. The fluorescence of the studied is observed in the far-red area at 638–682 nm while exciting at the range of 626–662 nm. All these dyes showed similar fluorescence sensitivity with BSA (up to 10 times). The maxima of the fluorescence emission spectra of dyes in the presence of BSA are shifted in the long-wavelength region up to 15 nm. This could point out the binding of the dyes to proteins. A breast cancer cell line (MCF-7) was used to study the ability of the studied dyes to penetrate the cell membrane and their distribution inside the cells. The blue fluorescent standard dye Hoechst binding to nuclear DNA was used for the co-staining. All studied dyes can penetrate through the cellular membrane, stain the cell components in the cytoplasm, and do not accumulate in nuclei as shown by co-staining with Hoechst: no co-localization with nuclear DNA dye is observed. The introduction of glucosamine substituents via the linker into the dye molecule reduces fluorescence intensity in the aqueous solution compared to unsubstituted merocyanine dye. However, this does not affect the ability of the conjugate to visualize MCF-7 cells.

Conclusions. The far-red dyes with different substitutes are suggested as promising far-red probes in fluorescence microscopy for visualization with minimum to no autofluorescence. The substituents do not affect the spectral properties of the dyes making them promising probes for labeling and studying drug distribution.

IMPROVEMENT OF BACILLUS SUBTILIS STRAIN PRODUCER FOR RIBOFLAVIN ACCUMULATION INCREASED

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Background. Riboflavin (vitamin B_2) plays an important role in many cellular functions. Riboflavin is used in medicine, pharmaceuticals, the food industry, and feed. Riboflavin in world industrial production is obtained in three ways: chemical synthesis, microbiological synthesis, and mixed, which includes microbiological synthesis of ribose, followed by chemical modification of ribose into riboflavin. Chemical synthesis of riboflavin takes place in several stages with all possible disadvantages, in contrast to the microbiological method, which allows producing riboflavin in one stage. Organisms of different taxonomic groups for microbiological production of riboflavin are used. Riboflavin from cells of industrial strain producer of fungi *Ashbya gossypii*, *Candida famata* var. *flasheri*, and bacteria *Bacillus subtilis* by the microbiological method is obtained, accumulates amount 15, 20, and 18 g/dm^3, in accordance. The main task for the improvement and development of riboflavin technology is to increase the strain producer biosynthetic capacity by improving the producer, and cultivation conditions, and expanding the range of substrates from cheaper carbon sources.

Aim. The **aim** of the work was to improve *Bacillus subtilis* IMB B-7797 strain producer from "Collection of strains of microorganisms and plant lines for food and agricultural biotechnology" SE "Institute of Food Biotechnology and Genomics NAS of Ukraine" for accumulation increasing of riboflavin through chemical mutagenesis.

Methods. Changes in microorganisms' genome to provide the necessary properties of strain producers through classical selection, mutagenesis, or by genetic engineering are made. Processing of *B. subtilis* IMB B-7797 riboflavin-strain producer by chemical mutagen N-methyl-N-nitro-N-nitrosoguanidine (NTG) to achieve this goal was used. L-agar medium to grow the riboflavin strain producer was used. All colonies grown after mutagenic treatment on solid media were selected for further cultivation on glucose medium for 72 hours in an incubator shaker at 38°C and 240 rpm. Determination of riboflavin accumulation in the culture fluid was performed by spectrophotometric method.

Results. The optimal time (30 min.) of mutagenic treatment at 50 μg/dm^3 NTG concentration to obtain 1% of living cells on L-agar medium was determined. After sowing of living cells, colonies were obtained that differed in morphological characteristics (color, size, shape of colonies) and the ability to accumulate riboflavin. As a result, *B. subtilis* IFBG NTG2 with an accumulation of riboflavin (14.8 g/dm^3) which differed from the original culture was obtained.

Conclusions. *B. subtilis* IFBG NTG2 strain producer was obtained by chemical mutagenesis, it produced riboflavin in the amount of 14.8 g/dm^3, which is 9% more than the original producer strain *B. subtilis* IMB B-7797 (13.9 g/dm^3).

ETHANOL ATTENUATES TOXIC EFFECTS OF ARGININE EXCESS IN *DROSOPHILA MELANOGASTER*

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Aim. L-arginine is an essential amino acid for animals. Recently, we have shown both beneficial for health and toxic effects of L-arginine in the fruit fly, *Drosophila melanogaster*. The toxic effects of arginine could be connected with the production of nitric oxide (Bayliak et al. 2018). Ethanol, a frequent component of *D. melanogaster* natural food, can exert similar and opposite effects to L-arginine (Bayliak et al. 2016). Therefore, we have questioned whether these nutrients, L-arginine and ethanol, with partially opposing effects will quench or reinforce each other.

Methods. *D. melanogaster* w1118 flies were used in the experiments. Three-day-old adult flies were divided into three groups, which were further maintained for 14 days on standard yeast-sucrose food (control) and foods supplemented with 10% ethanol, 50 mM arginine, or their mix.

Results. Consumption of food supplemented with 50 mM arginine by adult flies decreased their climbing activity and resistance to starvation and heat stress. At the same time, ethanol treatment increased resistance to starvation in both sexes. Food with 50 mM arginine or 10% ethanol sensitized *D. melanogaster* males to sodium nitroprusside (SNP) whereas the mix of ethanol and arginine reduced the negative impact of these supplements added separately. Females fed with arginine or arginine plus ethanol survived better after SNP treatment than flies in the control group. Females fed with food supplemented with ethanol had similar survival as control ones. Males fed with arginine had 28% lower catalase activity compared with the controls. A similar trend in catalase activity was observed for males which consumed food containing ethanol or a mix of ethanol and arginine. Males fed with arginine alone or in combination with ethanol had 60% lower glucose-6-phosphate dehydrogenase (G6PDH) activity than the controls. A slight decrease in the activity of G6PDH was also observed for females on food supplemented with arginine alone but not in females fed with arginine and ethanol. In males, arginine reduced glutathione S-transferase (GST) activity by ~ 60% and increased lipid peroxide levels by ~ 40%. These effects were canceled when flies were fed with a mix of arginine and ethanol. Respective groups of female flies showed a similar pattern of changes in GST activity as male ones, although GST activity in females was a more variable parameter than in males.

Conclusions. Our findings show how fruit flies respond to the natural components of their food provided in excess. Arginine excess causes oxidative stress and decreases resistance to other stresses in a sex-specific manner. Ethanol is able to improve the survival of arginine-fed flies, making them more resistant to nitrosative stress, heat shock, and starvation. It would be dare to assume that fruit flies seek food sources that contain moderate amounts of ethanol to attenuate the toxicity of protein-rich sources when feeding on them.

PARP INHIBITION PROTECTS AGAINST OVARIAN INJURY IN MICE WITH EXPERIMENTAL MODEL OF IMMUNE COMPLEX DISEASE

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Background. Various immune-mediated inflammatory diseases are associated with the formation and tissue deposition of immune complexes (ICs). As we showed earlier, ICs can induce reproductive function disturbances. Molecular mechanisms involved in the development of IC-mediated ovarian pathology require clarification. In this work, we focused on the study of poly(ADP-ribose)polymerase (PARP1) involvement in the pathogenesis of IC-induced ovarian injury.

Aim. To determine the effect of the PARP1 inhibitor, 4-hydroxyquinazoline (4HQ), on genomic integrity and viability of granulosa cells, and oocyte maturation, as well as to investigate the change in expression of *TNFα*, *TNFRI*, and *TNFRII* in granulosa in mice with IC-induced ovarian injury.

Methods. A model of IC-mediated pathology was induced by immunization with BSA (intravenously 6 times every 7 days according to the scheme: 1) 150; 2) 175; 3) 200; 4) 225; 5) 250 and 6) 275 mg of BSA per kg of body weight).

Results. The introduction of BSA caused severe DNA damage and genotoxic stress in granulosa cells, which contributed to a significant reduction in granulosa cell viability, as well as disruption of oocyte meiotic maturation. We assessed PARP activity in granulosa by immunocytochemical (ICC) detection of PAR. ICC score for PAR level was significantly elevated in BSA-treated mice (Me, min-max: 0.31, 0.17-0.41) as compared with control (0.08, 0.04-0.19, $P<0.001$). Also, we revealed changes in the expression of *TNFα*, *TNFRI*, and *TNFRII* in granulosa cells. It was found that mRNA levels of *TNFα* and *TNFRI* were higher in granulosa from the follicles of immunized mice. However, the expression of *TNFRII* mRNA was decreased. Numerous studies support the role of TNFRI in mediating cell death signaling and inflammation, while TNFRII signaling may be important in mediating signals for cell survival. Injections of 4HQ to immunized mice decreased ICC score to 0.12 (0.06-0.21, $P<0.05$). These results indicated that PARP1 is activated in mice with IC-induced ovarian injury. 4HQ treatment decreased *TNFα* and *TNFRI* gene expression in granulosa cells. In contrast, the expression of mRNA for *TNFRII* was enhanced. It is predicted that the inhibition of PARP1 may attenuate the cell death and inflammation associated with TNFα and TNFRI while sparing or potentiating the protective effects of TNFRII signaling. Treatment with 4HQ significantly reduced genotoxic stress and death of granulosa cells, as well as improved oocyte meiotic maturation.

Conclusion. Thus, the administration of 4HQ was effective in substantially preventing PARP1 activation in this pathology. It is important that the inhibition of PARP1 contributed to a significant decrease in the number of cells with such severe DNA damage that cannot be repaired but leads to necrotic cell death. So, PARP1 inhibition interrupted destructive proinflammatory connections, favored protection against genotoxic stress, and led to the prevention and weakening of the pathological process in ovaries.

CARBON MONOXIDE AND THEIR DONOR (CORM-2) AFFECT ON SKIN WOUND HEALING THROUGH ACTION ON AQP3-CHANNELS

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Background. Damage to the skin can lead to pathological conditions and death. After a wound is formed, the complex processes associated with the death of the damaged cells begin. Recently, much attention has been paid to the study of gas transmitters, among these gas transmitters, carbon monoxide (CO) is singled out. However, an important role in the healing process is played by the relatively recently discovered water and glycerin-aquaporin (AQP) transporters. These are important channels that take part in the transport of water and glycerol into the cell, in the processes of embryogenesis, angiogenesis, and oncogenesis.

Aim. The purpose of the study determines how CO and CORM-2 affect the expression of aquaporin-3 in the dermis and the wound healing rate in laboratory mice.

Methods. With the help of biopsy, stylet mice have applied 2 full-layer skin wounds. 1st group, after forming skin wounds, 2 sterile polyethylene bubbles were glued above with the help of medical adhesive. After that, pure CO was injected into the cavity of the left bubble, and atmospheric air was injected into the cavity of the right one (control). In the case of the second group (without polyethylene bubbles) one wound was treated with a solution containing carbon monoxide donor CORM-2 (50 μm/l). The wounds were photographed daily. After that, the area of the wound surface was calculated. For PCR study of the quantitative expression of AQP-3 mRNA on the 5th and 21st days, skin biopsy was taken from the animals.

Results. In the group of animals with wounds treated with air, the healing process was observed from day 5. The wounds treated by CO on the same animals began to heal after the 15th day. Meanwhile, a massive crust was formed which covered the wound. From the 19th day on, the wounds treated with CO begin to decrease sharply in size. Wounds washed with physiological solution began to heal after the 15th day, and were treated with CORM-2 – from the 9th. No complications, such as a wound infection or fluid collection, occurred in either group. In comparison with control, AQP3 expression in the epidermis of the group treated with CORM-2 decreased on the 5th day and increased on the 21st day. After CO treatment, AQP3 expression was also maximally reduced and increased at the end of healing. In wounds treated with saline, AQP3 expression to the contrary was increased, at the end of the experiment - decreased, but was at the level of the control group. In the control group, the expression was initially increased but slightly decreased at the end of healing.

Conclusions. Wound treatment with CO results in a dry crust. Treatment with CORM-2 did not lead to the formation of a crust. CO or CORM-2 led to a decrease in the expression of AQP-3 mRNA at the beginning of the wound healing process. At the end of healing, expression increased in a compensatory way. Changes in the wound healing rate are associated with changes in AQP-3 mRNA expression under the influence of CO.

QSAR MODELING AND *IN VITRO* EVALUATION OF ISOXAZOLE-CONTAINING SULFONYLAMIDES AGAINST *ACINETOBACTER BAUMANNII*

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Aim. Gram-negative bacterium *Acinetobacter baumannii* in the last years became one of the pathogens that cause the greatest problems of antibiotics resistance, sickness, and mortality in medical settings in the whole world. Since 2017 *A. baumannii* has topped the priority pathogens list in urgent need of new antibiotics [1]. The aim of this work was to study new effective isoxazole-containing sulfonyl amides against the resistant strain of *A. baumannii* using *in silico* modeling and *in vitro* testing.

Methods. The prediction of the antibacterial activity of the isoxazole-containing sulfonyl amides against *A. baumannii* was performed using previously developed and published on the OCHEM server QSAR models [2]. The data set consisted of 1078 inhibitors of *A. baumannii*. QSAR models were developed using Transformer Convolutional Neural Network, ASsociative Neural Network, Scalable and Flexible Gradient Boosting methods and descriptor packages such as AlogPS, CDK 2.0 (constitutional, topological, geometrical, electronic, hybrid), E-state, and Dragon7. The antibacterial activity of selected compounds was estimated against MDR (ampicillin-, oxacillin-, and ceftriaxone-resistant clinical isolate) *A. baumannii* by disc diffusion method in a Mueller–Hinton agar [3]. The inoculum concentration was 1×10^5 colony-forming units per mL. The compound content on a disk was 5.0 μM.

Results. The regression QSAR models with the best performances were used to predict the anti-*A. baumannii* activity of new isoxazole-containing sulfonyl amides. The cross-validation coefficient q^2 was determined for training and test sets of all models in the range of 0.66-0.79. A virtual set of these derivatives was generated and screened using the consensus regression model. The compounds with the highest predicted activity (up to 100 μM) were retained for synthesis and testing. The antibacterial activity results by measuring the zone diameter of growth inhibition of two synthesized compounds showed that 4-[1-(3-phenyl-isoxazol-5-yl)-cyclopent-3-enesulfonyl]-morpholine and 4-(3-p-tolyl-isoxazol-5-ylmethanesulfonyl)-morpholine demonstrated the highest activity potential against MDR clinical isolate of *A. baumannii*. The diameter of the growth inhibition zone of these compounds was registered as 16±0.3 and 22±0.6 mm respectively.

Conclusion. Overall, tested isoxazole-containing sulfonyl amides showed antibacterial activity and deserve further investigation to ascertain their potential as candidate antibacterial agents.

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"HAIRY" ROOTS OF *ARTEMISIA TILESII* LEDEB. AS A PROMISING CANDIDATE FOR ANTI-INFLUENZA TREATMENT

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Aim. Tilesius' wormwood is a poorly studied perennial herb that can be a promising candidate for the treatment of various diseases. It is known to be used in traditional indigenous medicine as it contains many bioactive substances with anti-inflammatory properties. The present study aimed to investigate the anti-influenza activity of *Artemisia tilesii* "hairy" root extracts.

Methods. *A. tilesii* "hairy" roots were cultivated *in vitro* conditions using hormone-free ½ MS medium for 6 weeks. Roots were lyophilized and powdered, then extracted with 70 % ethanol on the rotation shaker for 36 h. The filtrated solution with a total flavonoid concentration of 0.2 mg/mL was used as the extract No1. For the preparation of extract No2, extract No1 was dried using a rotary evaporator (AES 2010) and resolved in DMSO up to 3 mg/mL total flavonoid concentration. The total content of flavonoids was studied using the standard method with $AlCl_3$. HPLC assay was carried out using Shimadzu LC-20 chromatograph. Anti-influenza activity study was performed using "hairy" roots extract, influenza virus strain A/FM/1/47 H1N1 (infectious titer 10.0 lg ID50), and Madin-Darby canine kidney cell line (MDCK) susceptible to this virus. A daily subcultured MDCK cell line was incubated in a CO2 thermostat for 3 days and monitored with a microscope to note the occurrence of cytopathic effect. Then the culture fluid was collected and the infectious titer of influenza virus was determined by titration in cell culture.

Results. The HPLC chromatogram showed the presence of Kaempferol, Silibinin, Luteolin, Epicatechin, Rhamnetin, Apigenin, Rutin, and Quercetin, with the highest concentration of Epicatechin and Silibinin. These polyphenols are powerful antioxidants that can neutralize reactive oxygen species in cells and serve as antiviral agents having an inhibitory effect on viruses. This effect was proved with the present study: inhibition of infectious titer expressed as the change in lg ID_{50} was from 8.0 lg ID_{50} at the start of co-cultivation to 3.0 lg ID_{50} after 3 days of co-cultivation with DMSO extract of "hairy" roots.

Conclusions. According to the results of the experimental study, *A. tilesii* "hairy" root extracts showed an inhibitory effect on the influenza virus due to the high content of bioactive substances, such as flavonoids, in their composition.

CROSS-LINKED GLYCOSIDASES AGGREGATES FOR OLIGOSACCHARIDES AND FLAVONOIDS HYDROLYSIS

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Background. At the present stage of industrial development, biocatalysis has many advantages over chemical methods: mild reaction conditions, high activity and selectivity, lower energy expenses, and reduced pollution. One of the main disadvantages of commercial enzymes is their high cost, which is a consequence of thermal and pH lability. Chemical immobilization of enzymes makes it possible to increase the time of use of the biocatalyst and ensures the ease of its reuse. One of the methods for obtaining stable enzymes of prolonged action is the production of aggregates by intermolecular binding of protein molecules with bifunctional reagents. Glutaraldehyde is a convenient tool for obtaining cross-linked aggregates (CLA) due to its low cost and availability.

The **aim** of our work is to obtain CLA of α-L-rhamnosidases and α-galactosidases of micromycetes and to investigate the activity and stability of native and glutaraldehyde-modified enzyme preparations.

Methods. α-Galactosidase preparations were isolated and purified from the culture liquid of *Aspergillus niger*, *Cladosporium cladosporioides*, *Penicillium canescens*, *Penicillium restrictum*, extracellular α-L-rhamnosidases — from *P. restrictum*, *Penicillium tardum* and *Eupenicillium erubescens*. Synthetic p-nitrophenyl substrates were used to determine glycosidase activity. 10, 20, 30, 40, and 50 µl of 25% glutaraldehyde solution was added to 1 ml of the purified enzyme solution (8 U/ml). The cross-linking reactions were performed at 0 °C and pH 5.0 for 1 h. The remaining reagent was removed by gel filtration on Sepharose 6B. Thermal stability was measured by preincubation of the enzymes and CLA at the optimum pH at different temperatures (30, 37, 50, 60, and 70 °C) with an exposition time of 300 min.

Results. It was shown that high molecular weight α-galactosidases (range 400-430 kDa) after treatment with glutaraldehyde lost activity by 10-75%. The acceleration of thermodenaturation of all modified α-galactosidases at 60 and 65 °C was noted. No significant differences in activity, optimum pH, and temperature values of all studied α-L-rhamnosidases were recorded after immobilization. It was found that treatment of α-L-rhamnosidases with glutaraldehyde led to thermal stabilization of enzyme preparations at temperatures of 60-75 °C. The half-life of α-L-rhamnosidase-CLA at 70-75 °C in comparison with the native enzyme was increased by 3.5-fold for the enzyme from *P. tardum*, and 5-fold — for *P. restrictum*.

Conclusions. The formation of cross-links between amino groups of a protein with the help of glutaraldehyde can lead to the fixation of the active conformation of the molecule α-L-rhamnosidase and the formation of aggregates with increased stability compared to native enzymes and indicates the possibility of obtaining industrial preparations of prolonged action on its basis.

YEAST ADAPTIVE RESPONSE TO THE VERY HIGH-FREQUENCY ELECTROMAGNETIC RADIATION

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Background. The question of the influence of the very high frequency (VHF) electromagnetic radiation on living organisms is still under discussion.

Aim. The **aim** of the presented study was to determine the effects of VHF electromagnetic exposure on *Saccharomyces cerevisiae* strains and evaluate their possible adaptive response to the stress factor.

Methods. For this purpose, molecular-genetic analysis at the genomic and transcriptomic levels was carried out. *Saccharomyces cerevisiae* haploid and diploid strains were treated by VHF electromagnetic radiation and cultivated in YPD media supplemented with agar for 7 days. Genome variability was detected using PCR with primer to tetranucleotide repeat and the relative expression level of genes encoding the ubiquitin-conjugating enzyme (UBC6), two enzymes of fatty acids synthesis (OLE1 and FAS1), and two flocullins (FLO1 and FLO11) was defined by RT-PCR. Characteristics for both haploid and diploid strains were registered at three-time points: immediately, 24 hours and, 7 days after exposure.

Results. Comparative analysis of amplicon patterns obtained by amplification with primer to short repeat revealed differences in nucleotide sequences in haploid strain only immediately after irradiation. The sets of PCR-fragments of control and exposed samples after 7 days were identical. Results of comparative analysis of genes expression between non-irradiated and irradiated *S. cerevisiae* strains showed that there were no changes immediately after exposure and a slight decrease of genes expression up to 2 times was detected at 24 hours and 7 days after exposure although the increase of expression level of all analyzed genes was observed at 7th day after irradiation.

Conclusions. The results obtained in our research suggest that VHF electromagnetic irradiation can cause genome rearrangement in *S. cerevisiae* haploid strain reflecting possible repair processes but the major changes occur at the gene expression level supposing epigenomic regulation of adaptive process in yeast.

ATP DEPLETION OF ERYTHROCYTES "CANCELS" THE PROTECTIVE ACTION OF SOME AMPHIPHILIC SUBSTANCES UNDER POSTHYPERTONIC SHOCK

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Aim. To study the protective action of trifluoperazine and sodium decyl sulfate under posthypertonic shock of ATP-depleted erythrocytes.

Methods. Rabbit erythrocytes were depleted of ATP by incubation with 2-deoxyglucose (10 mmol/L) for 2 hours at 37°C. Posthypertonic shock (PHS) was performed by transferring red blood cells from 2.0 to 0.15 mol/L NaCl at 0°C. Trifluoperazine (TFP) (150 μmol/L) and sodium decyl sulfate (C10) (600 μmol/L) were added to 0.15 mol/L NaCl. The level of posthypertonic hemolysis was estimated. Statistical processing of the experimental results was performed using Statistica 6.0 software (StatSoft Inc., USA).

Results. We found that the hemolysis rate of the control rabbit's erythrocytes at 0°C was 64 ± 6%, and that of ATP-depleted red blood cells – was 53±6%. TFP and C10 reduce the level of posthypertonic hemolysis of control red blood cells by 55±6% and 66±7% respectively. ATP depletion of erythrocytes does not affect the efficacy of C10, while TFP loses its ability to protect rabbit erythrocytes under PHS. It is known that metabolic depletion of erythrocytes leads to a strong association of the 4.1R-spectrin-actin complex by changing the level of protein phosphorylation. This may be the cause in a certain decrease of erythrocyte hemolysis rate under PHS since the mechanical resistance of the membrane is increased. The protective effect of amphiphilic compounds under PHS is associated with their ability to integrate into the lipid bilayer and increase the surface area of erythrocytes, which allows cells to avoid damage when moving into the rehydration medium. The loss of the ability of TFP to protect erythrocytes under PHS after ATP depletion of erythrocytes can be explained by the peculiarities of its incorporation into the membrane. TFP is a cationic amphiphilic compound. It is mainly distributed in the inner membrane monolayer enriched in anionic lipids. During metabolic depletion of erythrocytes, the levels of some negatively charged lipids in the inner monolayer are reduced, which may lead to a decrease in TFP membrane binding. In contrast, C10 is distributed mainly in the outer monolayer, which according to available data does not change at ATP-depletion of cells. Therefore, this amphiphilic compound retains its protective properties under PHS.

Conclusions. Protective agents' activity under stress conditions depends on the state of the cells. Thus, during prolonged storage of erythrocytes, their energetic status can change and they can be partially or fully depleted of ATP. This fact should be taken into account in the selection of protective agents that can be used in the thawing of cells for protection against PHS.

THE EFFECTS OF THE HORMONALLY ACTIVE FORM OF VITAMIN D3 ON MA-104 CELLS IN THE CONTEXT OF SARS-COV-2 INFECTION

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Aim. The global response to COVID-19 is now facing new challenges including vaccine inequity, the emergence of SARS-CoV-2 variants of concern, and an improvement of medical protocols for COVID-19 prevention/treatment. Given the immunomodulatory effects of vitamin D_3 (VD_3), the **aim** is to elucidate the possible protective mechanisms of VD3 action *in vitro* via modulation of NF-κB-associated signaling pathways under COVID-19.

Methods. African green monkey epithelial cells MA-104 with ACE2 (angiotensin-converting enzyme 2) overexpression were treated with 25 and 100 nM of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$, 24 h). Reactive oxygen species (ROS) were determined by flow cytometry with 2′,7′-DCF-DA. Poly(ADP-ribose)polymerase 1 (PARP-1), glyoxalase 1 (GLO1), total and pNF-κB p65, NF-κB inhibitor (IκB), p38, and p53 levels were measured by western blotting. ACE2 and transmembrane protease, serine 2 (TMPRSS2) mRNAs were detected by RT-qPCR.

Results. Multidirectional dose-dependent effects of $1,25(OH)_2D_3$ on ACE2 and TMPRSS2 mRNAs levels were revealed: a reducing effect at 25 nM (ACE2 – by 3-fold, TMPRSS2 – by 1.6-fold) and an increasing impact at 100 nM (ACE2 – by 8.7-folds, TMPRSS2 – by 1.3-fold) vs. untreated cells. $1,25(OH)_2D_3$ treatment of MA-104 cells led to a decreased ROS production (25 nM – by 15%, 100 nM – 11.2%). Protein levels of enzymes PARP-1 (25 nM – by 28%, 100 nM – by 26%) and GLO1 (25 nM – by 28%, 100 nM – by 50%) was declined after $1,25(OH)_2D_3$ action. Interestingly, a decreased GLO1 level may reflect the reduced ability to utilize methylglyoxal. Methylglyoxal modifies the receptor-binding domain of the S-protein of SARS-CoV-2, which may decrease its ability to bind with ACE2, thus mediating the protective effects of $1,25(OH)_2D_3$. An inhibitory effect of $1,25(OH)_2D_3$ on the NF-κB system was observed: by reducing the content of total (25 nM – by 65%, 100 nM – by 40%) and phosphorylated at Ser536 (25 nM – by 65%) forms of NF-κB p65, and via increasing IκB content by 82 % at a dose of 25 nM compared with control cells. Intriguingly, $1,25(OH)_2D_3$ effects on total NF-κB level were more pronounced at 25 nM compared with 100 nM, while a dose of 100 nM almost did not influence phosphoNF-κB and IκB levels. Finally, this was accompanied by a declined level of p38 (by 27% and 25% respectively for 25 and 100 nM), which is known to be overactivated by SARS-CoV-2. $1,25(OH)_2D_3$ treatment induced a significant elevation of p53 protein content by 95% at 25 nM, and by 73% at 100 nM,-which may reflect activation of the p53 cascade, which may be considered one of the molecular mechanisms of VD_3 protective action in the cytokine storm COVID-19.

Conclusions. In the context of COVID-19, the molecular mechanisms of $1,25(OH)_2D_3$ protective action may appear in a range of aspects: a decrease in ACE2/TMPRSS2, PARP-1, and GLO1 content, diminishing oxidative stress, as well as inhibition of NF-κB system and modulation NF-κB-associated pathways: p38 suppression and p53 activation.

MOLECULAR DOCKING STUDY OF INTERACTIONS BETWEEN INSULIN AMYLOID FIBRILS AND GLOBULAR PROTEINS

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Aim. Protein-protein interactions (PPIs) are involved in a wide variety of fundamental biological processes. The aberrant PPIs between pathological protein aggregates, amyloid fibrils (AF), and endogenous proteins may account for amyloid cytotoxicity. The **aim** of the present study was to elucidate the atomistic details of the interactions between the insulin amyloid fibrils (InsF) and multifunctional globular proteins, serum albumin (SA), and lysozyme (Lz) using the molecular docking approach.

Methods. To predict the most favorable modes of interactions between the insulin amyloid fibrils and proteins, molecular docking studies were conducted using the ClusPro and HDOCK web servers. The web application COCOMAPS was used to analyze the properties of the interfacial region in the fibril-protein complexes by setting a distance cut-off of 5 Å.

Results. Both the employed docking tools, ClusPro and HDOCK, provide evidence for the ability of fibrillar insulin to form complexes with serum albumin and lysozyme. The analysis of the selected highest-score docking structures revealed the following main tendencies: 1) the number of interacting residues of InsF is comparable to the examined proteins despite the differences in their size, amino acid sequence, and physicochemical properties, while the number of interacting residues in protein seems to reflect these differences; 2) the lysozyme-fibril complexes are distinguished by the higher numbers of hydrophilic-hydrophobic and hydrophilic-hydrophilic interactions; 3) the number of hydrophobic-hydrophobic contacts is significantly lower than the number of hydrophilic ones; 4) the size of total interface area is greater for the complex InsF-SA; 5) the fraction of interface area in fibrillar insulin insignificantly differs for the examined systems; 6) the polar buried area exceeds the nonpolar one by a factor of ~ 3 for InsF complexes with SA and by a factor ~ 2 for the system InsF-Lz.

Conclusions. Based on the presented results, the following main conclusions can be drawn: 1) hydrophilic fibril-protein interactions dominate over the hydrophobic ones in the examined fibril-protein complexes; 2) the amounts of insulin fibril residues interacting with globular proteins are similar for serum albumin and lysozyme; 3) the hydrophobic leucine, valine and phenylalanine, polar asparagine, glutamine, histidine, cysteine, and negatively charged glutamic acid prevail among the interacting residues of fibrillar insulin; 4) glutamine and asparagine in fibrillar insulin, lysine in serum albumin and arginine in lysozyme represent the most abundant hydrogen bond forming residues. The results obtained provide a basis for a deeper understanding of the toxic action of amyloid fibrils.

STUDY OF ANALYTICAL CHARACTERISTICS OF BIOSENSOR BASED ON UREASE FOR SILVER (I) DETERMINATION

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Aim. The main purpose of this work was to develop enzyme impediometric biosensor based on urease for detection of ions of silver (I) and study the analytical characteristics of developed biosensor.

Materials and methods. The electrochemical transducers were produced in V.E. Lashkarev Institute of Semiconductor Physics (Kyiv, Ukraine) in accordance with our recommendations. They consist of two identical pairs of gold electrodes made with gold vacuum evaporation onto pyroceramic substrate (5 × 30 mm). Bioselective elements of proposed biosensor for inhibitory determination of silver ions has been prepared by immobilizing of urease with bovine serum albumin using glutaraldehyde on the surface of gold electrodes.

Results. As a result of the work the main analytical characteristics of the developed biosensor were investigated, namely response time, sensitivity, linear and dynamic range of urea and silver determination, signal reproducibility, operational stability and storage stability. The developed biosensor was characterized by high signal reproducibility, the standard relative deviation was not more than 10%. Continuous storage of biosensors during the month in a dry state at a temperature of +4° C did not significantly affect the sensitivity of biosensors to urea. So, the developed biosensors can be stored for a month without loss of activity. The next stage of work was to determine the optimal conditions for inhibitory analysis. It was studied the sensitivity of the developed biosensors to different concentrations (0.01 - 10 μM) of silver (I) ions. It was found that the optimal incubation time of bioselective elements in solution with heavy metal ions was 20-30 minutes. The possibility of reactivation of the developed biosensors by different concentrations of EDTA after incubation with an inhibitor was also evaluated in order to reuse the proposed biosensors for inhibitory analysis of heavy metal ions.

Conclusions. The biosensor for determination of silver ions was developed and the main analytical characteristics of developed biosensor were studied. The biosensor is characterized by high sensitivity to silver (I) and can be reused multiply times due to reactivation by EDTA. The developed biosensor can be used in the future in the monitoring of the presence of silver (I) in aqueous samples.

Acknowledgements. This study is part of a project that has received funding from the European Union's Horizon 2020 research and innovation programme under the grant agreement No 958491, Project Waste2Fresh. Also this work is supported by the National Academy of Sciences of Ukraine "Smart sensor devices of a new generation based on modern materials and technologies".

MECHANISMS OF FORMATION OF BCR-ABL/USP1, BCR-ABL/GLG1 PROTEIN COMPLEXES AND THEIR ROLE IN THE DEVELOPMENT AND PROGRESSION OF CHRONIC MYELOID LEUKEMIA

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Background. Chronic myeloid leukemia (CML) is a clonal myeloproliferative disease characterized by the transformation and proliferation of hematopoietic stem cells. The reason for the development of CML is a reciprocal translocation between chromosomes 9 and 22, which leads to the formation of the Bcr-Abl oncoprotein. A feature of the Bcr-Abl oncoprotein is uncontrolled tyrosine kinase activity, which causes deregulation of signaling pathways and malignant cell transformation. The search for new protein partners involved in malignant cell transformation and target proteins that selectively affect oncodrivers is the key to the development of new therapeutic drugs for the treatment of CML.

Aim. Study of the interaction of Bcr-Abl oncoprotein with USP1, GLG1 proteins and establishment of their role in the pathogenesis of CML.

Methods. K562 cells were cultured according to the recommendations of the American Cell and Tissue Culture Bank (ATCC). The study of protein-protein interactions and the detection of phosphorylated forms of proteins were performed by precipitation of endogenous protein complexes of K562 cell lysate. Visualization of the results was with Western blot. Subcellular localization of proteins was studied with immunofluorescence analysis followed by confocal microscopy.

Results. The interaction of Bcr-Abl oncoprotein with phosphorylated USP1 isoforms in the nuclei of CML cells were identified. The interaction of Bcr-Abl oncoprotein and GLG1 in the Golgi complex of CML cells was revealed, it was shown that the GLG1 protein isoform, which forms a complex with Bcr-Abl oncoprotein, is phosphorylated at tyrosine sites. It has been shown that inhibition of the deubiquitin activity of USP1 by ML323 causes a decrease in the level of Bcr-Abl oncoprotein in CML cells.

Conclusions. The transforming activity of the Bcr-Abl oncoprotein is realized by uncontrolled phosphorylation of its protein partners, we found that one of such proteins is USP1 and GLG1. Deregulation of USP1 and GLG1 functions due to uncontrolled tyrosine kinase activity can disrupt cell genetic stability, ubiquitin-proteasomal oncoprotein degradation of Bcr-Abl, and promote cell migration. The correlation between USP1 activity and Bcr-Abl oncoprotein level identifies USP1 deubiquitinase as a promising therapeutic target for developing a new strategy for CML therapy by modulating Bcr-Abl levels in the cell.

LOCALIZATION STUDY OF NIR PENTAMETHINE CYANINE DYES ON MESENCHYMAL STEM CELLS FROM RAT BONE MARROW *IN VITRO*

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Aim. Near-infrared (NIR) fluorescence imaging has emerged as an attractive non-invasive approach for direct bioimaging, which depends on developing stable, sensitive optical probes with high specificity. Therefore, NIR fluorescent probes offer cell visualization with reduced background from cellular autofluorescence and increased tissue penetration depth. Cyanine dyes have often been considered promising contrast optical agents due to their photophysical properties. Thus, the localization study of NIR-emitted pentamethine cyanine dyes on mesenchymal stem cells from rat bone marrow *in vitro* was performed.

Methods. Confocal microscopy.

Results. Spectral-luminescent properties of these dyes were described before in Aristova D., 2020 [1]. It was shown that pentamethine cyanine dyes have low to moderate fluorescence intensity in a free state but increase it in the presence of serum albumins up to 160 times with a quantum yield value equal to 42%. The study on using these dyes as probes for live-cell imaging was performed on a monolayer of mesenchymal stromal cells of the rat bone marrow third passages with cell viability of 87-89%. The dyes were added to the growth medium in a 1 μM concentration. It was shown that the studied dyes were able to penetrate the plasmatic membrane and stain some structures in the cytoplasm. Vital fluorescent markers on autophagosomes and mitochondria were used to determine the localization of dyes in cell compartments for colocalization analysis [2]. The overlapping signal of compartment-specific markers with the studied dyes was assessed by confocal microscopy (Olympus FV10iLIV) by calculating Pearson's correlation coefficient (Olympus CellSense software). It was shown that NIR pentamethine cyanine dyes have different effects on the studied cells. 1753Sl and 1759Sl accumulate in intracellular vesicles. It was confirmed that the dye 1753Sl is visualized in autophagosomes and can be a marker of cell aging. Furthermore, cyanine dyes 1756Sl and 1872Sl can accumulate in cell mitochondria. The accumulation of 1872Sl does not depend on the depolarization degree of the mitochondrial membrane, and 1756Sl stains only mitochondria with depolarized membrane.

Conclusions. Based on the results of colocalization analysis, we can assume the dyes emitted in the NIR region as potential probes for staining autophagosomes with the detection of aging cells (1753Sl) and for monitoring the state of mitochondria (1756Sl).

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2. Abcam Autophagy Assay Kit ab139484, Abcam JC1 Mitochondrial Membrane Potential Assay Kit ab113850.

AZITHROMYCIN MAY POTENTIATE BACTERICIDAL AGENTS DURING M/PDR KLEBSIELLA PNEUMONIAE-ASSOCIATED NOSOCOMIAL INFECTIONS: CASE SERIES

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Aim. This study aimed to collect clinical cases that might support our *in vitro* findings on the synergy of antimicrobials with antibiofilm and bactericidal activity against M/PDR *K. pneumonia* infections.

Methods. Analysis of clinical cases of M/PDR *K. pneumoniae* infections treated by a combined therapy which included anti-biofilm treatment with azithromycin.

Results. The first case was a 27 year old female patient, 21 days after orthotopic heart transplantation obtaining immunosuppressive treatment. Deep sternal wound infection was diagnosed on admission and MDR *K. pneumoniae* strain was isolated, sensitive only to colistin (MIC=2.0 mg/ml). Antibiotic therapy was applied: imipenem 500 mg every 6 h IV (intravenously), colistin 2000 mg every 12 h IV, azithromycin 1000 mg daily PO (perorally), fluconazole 50 mg daily PO. This completely eradicated the causative microorganism and the patient was discharged after 4 weeks. The second case was an 81 year old male patient with sepsis and hepatic dysfunction 14 days after an inguinal hernia repair. MDR *K. pneumoniae* strain isolated, sensitive only to colistin (MIC=2.0 mg/ml). Antibiotic therapy was applied: imipenem 500 mg every 6 h IV, colistin 2000 mg every 8 h IV, azithromycin 500 mg daily IV, fluconazole 100 mg daily IV. This completely eradicated the causative microorganism and the patient was discharged after 8 weeks. The third case was a 72 year old female patient with an acute cerebrovascular condition complicated by a neurogenic bladder and a history of type II diabetes. A urinary tract infection was diagnosed on admission and MDR *K. pneumoniae* strain was isolated, sensitive only to colistin (MIC=2.0 mg/ml) and chloramphenicol (MIC=4.0 mg/ml). Antibiotic therapy was applied: amikacin 500 mg every 12 h IM, chloramphenicol 1000 mg every 8 h IV, imipenem 500 mg every 6 h IV, azithromycin 1000 mg daily PO, fluconazole 150 mg daily PO. This completely eradicated the causative microorganism and the patient was discharged after 7 weeks. The fourth case was a 67 year old male patient with a recurrent acute cerebrovascular condition. An acute urinary tract infection was diagnosed on admission and PDR *K. pneumoniae* strain was isolated. Antibiotic therapy was applied: ciprofloxacin 500 mg every 12 h PO, imipenem 500 mg every 6 h IV, azithromycin 500 mg daily PO, fluconazole 150 mg daily PO. This completely eradicated the causative microorganism and the patient was discharged after 3 weeks.

Conclusions. The collected case series suggest that azithromycin-based combined therapy where the second bactericidal agent such as colistin or imipenem is applied might be used to overcome M/PDR *K. pneumoniae* infections.

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DEVELOPMENT AND OPTIMIZATION OF THE NOVEL CONDUCTOMETRIC BIOSENSOR FOR DETERMINATION OF ARGININE IN AQUOUS SOLUTIONS

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Aim. The amino acid L-arginine is conditionally essential. It is included in a number of important physiological processes. Therefore, when the amount of arginine and its derivatives in the metabolic pathways decreases, there may be serious problems with the functioning of the human body. There is currently a wide range of methods for quantifying arginine. All methods are effective in use, but they have a number of disadvantages: high cost, long pre-processing of samples, and the difficulty of analysis. To solve these problems, we decided to create a single-enzyme conductometric biosensor based on arginine deiminase for the selective determination of arginine in pharmaceutical samples.

Methods. At work on the development of a monoenzyme biosensor for the determination of arginine, we used conductometric transducers, which consisted of two identical pairs of gold interdigitated electrodes. The system of two pairs of electrodes was required to perform measurements in the differential mode. Work began with the selection of the optimal method of immobilization and selection of the main parameters of this immobilization. After that, we began testing the biosensor to measure the concentration of arginine in pharmaceutical samples. The method of standard additions was used. This method consists in first adding a test sample with an aliquot of the pharmaceutical preparation to the measuring cell. Next, three aliquots of standard concentrated arginine solution were added. Based on the feedback received, a straight line was constructed, according to the equation of which the concentration of arginine in the test sample was calculated.

Results. The optimal parameters of the working buffer solution for the best functioning of the developed conductometric biosensor were determined (5 mM phosphate buffer solution, pH 6.2). Analyzing the stability of the developed biosensor, it was experimentally determined that it is characterized by high reproducibility of results during one day of active work (RSD=6.1%), and is characterized by good operational stability for two weeks. The analytical characteristics of the biosensor based on ADI were analyzed. It was found that the minimum limit of determination of arginine was 2 μm. The linear range is in the range from 20 to 750 μm Larginine. The sensitivity to Larginine was 1927 μS/mm.

Conclusions. A novel arginine-sensitive conductometric biosensor based on ADI from *M. hominis* was developed and optimized for highly sensitive determination of arginine in aqueous solutions. According to the results of the biosensor application in the real sample analysis one may conclude that this biosensor can be successfully used in quality control of the dietary supplements containing Larginine.

5S RDNA INTERGENIC SPACER OF ACONITUM SPECIES: MOLECULAR ORGANIZATION AND APPLICATION FOR BARCODING

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Aim. *Aconitum* (fam. *Ranunculaceae*), a genus of perennial herbaceous plants, includes about 400 species divided into two subgenera: *Aconitum* and *Lycoctonum*. Despite the long history of research, the systematics of the genus *Aconitum* and phylogenetic relationships to the closely related taxa remain uncertain. Due to the significant phenotypic polymorphism associated with the wide distribution of interspecific hybridization, comparison of morphological characters does not allow unequivocal differentiation of species and intraspecific taxa within the genus. The use of DNA barcoding can help to resolve this issue. One of the most popular molecular markers successfully applied for phylogeny reconstruction of low-ranking taxa is the 5S rDNA intergenic spacer (IGS). In this work, we cloned and sequenced the IGS for *A. jaquinii*, a widespread in Ukraine taxon, whose status is questionable.

Methods. DNA was extracted from the herbarium samples using a modified CTAB method. 5S rDNA repeats were PCR amplified and cloned into the pJET 1.2 vector. The presence of the inserts was tested using colony PCR. Purified PCR amplicons of 5S rDNA inserts were sequenced at LGC Genomics (Germany).

Results. A total of five 5S rDNA clones were identified, two of which were sequenced. Analysis of the obtained nucleotide sequences showed that the IGS length is 600 bp and 618 bp for the clones AcJac1 and AcJac34, respectively. These length values are within the known IGS length limits for vascular plants, but are significantly higher than the average values. The IGS sequences of *A. jacquinii* were compared with the IGS of *A. kusnezoffii,* the only sequence deposited in Genbank for the 5S rDNA of the genus *Aconitum*. When analyzing the alignment, a large number of nucleotide substitutions, not only between sequences of different species, but also between the two clones of A. jacquinii was found. In addition, single and oligonucleotide indels were detected in the IGS. They are mostly located at the beginning of the IGS, a short distance downstream of the transcription termination signal. Two of the three sequence motives involved in 5S rDNA transcriptional initiation in angiosperms, namely GC and C, also appeared to be conserved in *Aconitum*, while the TATA-box has a highly altered sequence. The percentage of similarity between the two IGS sequences of *A. jacquinii* is 83.9%, while the similarity between the IGS of *A. jacquinii* and *A. kusnezoffii*, ranges from 76.1 to 76.8%.

Conclusions. The high rate of molecular evolution and significant level of polymorphism make the IGS one of the most promising markers for DNA barcoding in the genus *Aconitum.*

PHOSPHATIDIC ACID AND DIACYLGLYCEROL FORMATION IN RESPONSE TO FLAGELLINE PEPTIDE TREATMENT IN *ARABIDOPSIS* DIACYLGLYCEROL KINASE KNOCKOUTS

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Aim. The **aim** of work to investigate role of isoforms of diacylglycerol kinases genes (*DGKs*) in the formation of lipid messengers - phosphatidic acid (PA) and diacylglycerol (DAG) in response to bacterial pathogen-associated molecular pattern molecule (PAMP) flagellin peptide.

Methods. Tissue labeling of *Arabidopsis thaliana* plant with phosphatidylcholine-BODIPY was performed as in Kocourková method with modifications. Phospholipid extraction have been done according to Bligh method. Quantity analysis of lipid zones in TLC plate was performed with Typhoon FLA7000 laser scanner (Fujifilm, USA). Products were identified following Pejchar method and using lipid standards.

Results. Using *Arabidopsis thaliana* wild type (WT) plants and diacylglycerol kinase knockouts (double mutants - *dgk3dgk7*, *dgk1dgk2*) we observed that in response to flg22 treatment PA content increased significantly in WT plants, on the other hand *dgk1dgk2* and *dgk3dgk7* lines possessed much lower PA content. In contrast, *dgk1dgk2* is characterized with significantly increased DAG level, while in WT or *dgk3dgk7* plants DAG level was relatively the same.

Conclusions. Results of investigation points at critical role of *DGK1, DGK2, DGK3, DGK7* genes in the regulation of intracellular signaling mediated with PA-related pathways. Also, we noticed that “knockouts” in *DGK1* and *DGK2* led to accumulation of another signaling messenger – DAG, which probably indicates cells try to overcome PA-deficiency with increased formation of DAG that server both as substrate for DGKs and as signaling molecule.

CHARACTERIZATION OF INHIBITOR-BINDING POCKET IN ENTEROVIRUSES AND IT'S ROLE IN GENOME RELEASE

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Background. Enteroviruses cause a wide variety of illnesses, ranging from the mild common cold to hand-foot-and-mouth disease, myocarditis, pancreatitis, aseptic meningitis, and encephalitis. Their entry can be inhibited by "classical capsid binders," small molecules targeting a surface-exposed hydrophobic pocket in one of the viral coat proteins (VP1) preventing genome uncoating. However, efficacy, toxicity, the emergence of drug-resistant viruses, and the existence of enteroviruses lacking the VP1 pocket limit their clinical benefit.

Aim. The **aim** of the study was to identify and research druggable site at a conserved interface formed by multiple capsid proteins.

Methods. High-resolution cryo-electron microscopy.

Results. Recently, we identified a new druggable site at a conserved interface formed by multiple capsid proteins, the VP1-VP3 interprotomer pocket. We have now determined structures of coxsackieviruses B3 and B4, complexed with the interprotomer-targeting compounds, CP17 and CP48, respectively. At better than 3-Å resolution, we identified the detailed interactions that facilitate ligand binding. Both compounds target the same network with three conserved pocket residues at the core, each originating from a different polypeptide chain, stabilizing the virion.

Conclusions. Structural comparison of amino acid residues shows that this pocket is important for enterovirus entry and when an inhibitor is bound the viral infection is blocked at an early step. Synergistic development of compounds targeting both pockets, the VP1, and interprotomer pocket, is a promising endeavor in the fight against enteroviruses.

CALCIUM-DEPENDENT HIPPOCALCIN DISTRIBUTION BETWEEN DIFFERENT SUBCELLULAR COMPARTMENTS

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Background. The hippocalcin protein (HPCA) is a neural calcium sensor, which in response to calcium input regulates the signaling pathways in the neurons. Binding of Ca^{2+} to HPCA induces conformational changes that lead to hydrophobic Nmyristoyl domain protrusion and highly heterogeneous insertions both into plasma membrane (PM) and trans-Golgi network (TGN) membranes. It is known that HPCA has a high affinity for the minor phospholipid PIP2, which is involved in many important signaling cascades on the membrane. Since PIP2 is primarily localized on the PM, the composition of the membrane could be crucial in the distribution of the protein.

Aim. Evaluation of the HPCA insertions into different cellular compartments membrane with a stepwise increase in Ca^{2+} concentrations in the cytosol ($[Ca^{2+}]_i$).

Methods. HEK 293 cells were cultured in a 12well plate covered with 18mm coverslips at 37°C and 5% CO_2. Cells were incubated in a Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 0.25% gentamicin. On days 34, lipofection with a chimeric HPCA-TagRFP gene-encoding plasmid (1 ug of DNA per well) was performed. Transfected cells were loaded with the calcium chelator NP-EGTA and the calcium dye Fluo4 prior to imaging. 2436 hours after transfection, images were acquired using the Olympus FV 1000 confocal system. Three repetitive laser-induced uncaging from NP-EGTA were used for stepwise $[Ca^{2+}]_i$ increase. Python scripts were used to perform semi-automatic image analysis (code is available in GitHub repository github.com/wisstock/trans_scripts).

Results. For this study, the most native conditions were used to prevent the influence of external factors on protein distribution. Using confocal microscopy, we observed the nature of the insertions of protein into individual compartments in the dynamics. In response to a stepwise increase in the $[Ca^{2+}]_i$, an increase in the area of HPCA insertion was observed, and the nature of changes in the concentration of fluorescently-labeled HPCA on PM and TGN were different. The regions of the insertion sites always remained conservative, with variability in the area of the inserts depending on the concentration of $[Ca^{2+}]_i$.

Conclusions. During the experiment, we observed conservative insertions of HPCA protein into the plasma membrane and trans-Golgi network. As higher activity was recorded in the plasma membrane, it may be due to the differences in minor phospholipids allocation. It is possible that local cellular membrane composition affects the HPCA distribution patterns and potential site-specific signaling in neurons.

ORCHESTRATING THE TUMOR MICROENVIRONMENT: MIRNAOME DEREGULATION IN BREAST CANCER CELLS CAN FACILITATE OVEREXPRESSION OF CHEMOKINES AND THEIR RECEPTORS RESPONSIBLE FOR RECRUITMENT OF STROMAL CELLS

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Aim. To recruit healthy cells into tumor microenvironment, cancer cells produce a quantity of cytokines and chemokines (chemoattractant cytokines), which facilitate chemotaxis and positioning of target cells in tumor tissue. In addition, cancer cells can express chemokine receptors to receive signals from the microenvironment. Although chemokine expression is initiated by transcription factors, esp. proinflammatory factor NFkB, microRNAs (miRNAs) can silence the expression at the post-transcriptional level. On the contrary, down-regulation of miRNA expression removes restrictions for expression of target genes. This research aims to identify how the tumor-related abnormalities in miRNA signature can lead to overexpression of chemokines and their receptors by breast cancer cells.

Methods. MiRNA targets within gene transcripts were predicted *in silico* using the TargetScan software.

Results. Targets of downregulated miRNAs let7, miR1/206, miR15/16, miR22, miR29, miR31, miR34, miR101, miR124, miR125, miR128, miR129, miR138, miR140, miR141/200, miR143, miR145, miR148/152, miR199, miR204, miR205, miR214, miR218, miR302/520 and miR506 were found in transcripts of genes encoding the chemokines CCL2 (MCP1), CCL5 (RANTES), CCL22 (MDC), CCL4/7/8/11/17/20/25, CXCL1/2/6, CXCL12 (SDF1), IL1B, IL6, CXCL8 (IL8), TNF and TNFSF11 (RANKL) as well as chemokine receptors TNFRSF11A (RANK), CCR1/2/4/5/7, CXCR1/2/3/4 and CXCR7 (ACKR3).

Conclusions. Down-regulation of tumor-suppressive miRNAs can allow overexpression of genes encoding the chemokines and chemokine receptors, thereby providing the breast cancer cells with an advantage to recruit normal stromal cells into tumor microenvironment and to communicate with them. In particular, CCL2, CCL5, CXCL1, CXCL2, CXCL6, CXCL8 (IL8), CXCL12 (SDF1), TNF and IL1B are responsible for the recruitment of tumor-associated macrophages, fibroblasts and mesenchymal stem cells. CCL22 facilitates recruitment of the regulatory T cells responsible for tumor immune evasion. CXCL8/12 and TNF participate in angiogenesis induction.

P85S6K1, P70S6K1 AND P60S6K1 ISOFORMS OF S6K1 KINASE ARE INVOLVED IN SPECIFIC REGULATION OF CD326, CD227 AND CD66E ADHESION PROTEINS EXPRESSION

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Aim. To identify and to characterize the common effect of alternatively translated S6K1 isoforms on the regulation of CD326, CD227 and CD66e adhesion proteins expression.

Methods. Cell culture, immunoblotting, microscopy, statistical analysis.

Results. New evidence linking S6K1 isoforms and regulation of epithelial cancer cell adhesion and motility has been obtained. Analysis of protein expression patterns in CRISPR/Cas9-modified MCF7-based stable cell lines with selectively blocked expression of different S6K1 isoforms (S6K1: p85–/p70+/p60+; S6K1: p85–/p70–/p60+; S6K1: p85–/p70–/p60–) was performed to reveal the effect of each isoform. According to the obtained data, knockdown of p85S6K1 significantly reduces expression of CD326 (to 41,6±12,6% – * – compared to the expression level in unmodified MCF7 cell line) and CD66e (to 64,8±2,2%), but does not affect the expression of CD227. Additional knockdown of p70S6K1 causes further attenuation of CD326 (to 3,2±2,2%) and CD66e expression (to 5,4±1,1%), and, unlike p85S6K1 knockdown, critically attenuates CD227 expression (to 11,7±7,3%). Additional knockdown of p60S6K1 leads to a sharp increase in CD326 expression (to 142,3±31,7%*), reaching a level even higher than in unmodified cell line, but does not affect CD227 and CD66e expression. Based on these data, it was revealed a positive regulatory impact of p85S6K1 isoform on CD326 and CD66e expression, a positive regulatory impact of p70S6K1 isoform on CD326, CD227 and CD66e expression, and a negative regulatory impact of p60S6K1 on CD326 expression. Moreover, knockdown of two isoforms, p85S6K1 and p70S6K1, almost completely blocks the expression of all three proteins. At the cellular level, only cell line with p85S6K1 and p70S6K1 knockdown demonstrates visible adhesion disturbance: the cells are easily detached from the growth surface; do not form compact colonies; and prefer scattered migration instead of the frontal one, which is associated with the malignant phenotype of cultured epithelial cancer cells. Knockdown of p85S6K1 as well as knockdown of all three isoforms does not show any visible effect on cell adhesion.

Conclusions. P85S6K1, p70S6K1 and p60S6K1 isoforms of S6K1 kinase are involved in the specific regulation of CD326, CD227 and CD66e adhesion proteins expression. Regulatory effects exerted by each of the isoforms significantly differ and, moreover, in the case of p70S6K1 and p60S6K1 it can be even antagonistic. Regulatory role of p85S6K1 and p70S6K1 isoforms is critical for CD326 and CD66e expression, and regulatory role of p70S6K1 is critical for CD227 expression. Thus, the alteration of S6K1 isoforms balance can lead to the change of adhesion proteins expression, which can be one of the effective mechanisms of cell adhesion and motility regulation.

TRISTETRAPROLIN IN CANCER: TREAT OR TRICK?

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Background. Breast cancer is a most common type of cancer in the world. In 2020, there were 2.3 million women diagnosed with breast cancer and 685 000 deaths globally. It is highly heterogeneous on molecular level, and despite on decades of research there is still a room for discovering of new potential diagnostic markers. One of potential candidates is tristetraprolin (TTP), an RNA-binding protein that is encoded by ZFP36 gene and regulates its target mRNAs stability. TTP is shown to be significantly dysregulated during tumorigenesis and inflammation, which makes it even more interesting target since inflammation is one of the obligatory events for tumor formation.

The study **aim**ed to investigate the expression levels of TTP in clinical breast cancer samples of different stages and subtypes, as well as methylation levels of ZFP36 human MCF7 cell line under the treatment by most known and used anticancer agents – doxorubicin and temozolomid. We also aimed to create a cell line with stable expression of TTP and to GST-fused TTP for prokaryotic expression system for further experiments.

Methods. In the study we used next methods: bioinformatic analysis, nucleic acid isolation, molecular cloning, qPCR, bisulfite conversion, methyl-specific PCR, cell culture, western-blot analysis, GST-precipitation.

Results. We analyzed expression levels of TTP in breast cancer cells of different types from 53 specimens, as well as from 13 specimens of adjacent tissues and 1 specimen from normal tissue. Surprisingly, we found that TTP expression levels in normal and adjacent tissues were significantly lower than in tumor tissues of different subtypes. The highest expression level was observed in HER2-enriched subtype. Although these results are controversial to current knowledge, we suggest that such expression level might be a compensatory mechanism for tumor suppression since most of the specimens were at stage II. Moreover, according to Eliyatkın N. and collaborators, HER2-enriched subtype accounts only for 15% of invasive breast cancer cases, whilst 50% of cases were diagnosed with luminal A subtype. Our study shows that the least TTP expression level was observed exactly in the samples diagnosed luminal A, which somehow reveals this conundrum. The other finding was that doxorubicin and temozolomid indeed influence ZFP36 methylation status and further studies are needed to establish its role. We also created a stable cell line with stable expression of TTP based on HEK293 line, which is mow used for functional experiments and is very suitable for this purpose since HEK293 cells do not possess endogenous TTP. Also, we found that for expression of GST-TTP in *E. coli* it is necessary to co-transform the cells with chaperon plasmid, since TTP is a toxic protein.

Conclusions. TTPs expression levels are significantly altered in different breast cancer subtypes. Moreover, well-known antitumor agents affect ZFP36 methylation status, which can contribute to its expression alterations.

LYSOSOMAL ALTERATIONS IN ORGANOPHOSPHATE-EXPOSED (ROUNDUP, CHLORPYRIFOS AND THEIR MIXTURES) ZEBRAFISH

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Background. General occurrence of organophosphate residues and their metabolites in water bodies worldwide due to the increasing use of organophosphates in agriculture raises concerns about their toxic effects on freshwater biota including fish, as well as human exposures through water and seafood.

The **aim** of the study was to study the individual and mixture toxicities of common pesticides Roundup and chlorpyrifos in environmentally relevant concentrations to the lysosomes of the zebrafish *Danio rerio* using molecular and biochemical methods.

Methods. Adult zebrafish were exposed to organophosphate pesticides Roundup [15 µg/L (RL) and 500 µg/L (RH)], chlorpyrifos [0.1 µg/L (CL) and 3 µg/L (CH)], and their mixtures (RH+CL and RL+CH) for 14 days. The comprehensive analysis of the markers of cytotoxicity (active cathepsin D and neutral red retention (NRR)) in lysosomes was carried out.

Results. NRR of lysosomes isolated from zebrafish liver tissue (as an index of the lysosomal membrane integrity) decreased by ~48% in all pesticide-exposed groups of fish. The total activity of cathepsin D, the key lysosomal protease, also increased in the zebrafish liver of in all treated groups. The maximum deviation of the cathepsin D activity from the control (by ~27%) was found in the fish exposed with 3 µg/L of chlorpyrifos.

Conclusions. Lysosomal dysfunction is commonly associated with pesticide-induced cytotoxicity in vertebrates including zebrafish (Bodnar et al., 2021). Our present study provides evidence that organophosphate pesticides are able to induce lysosomal membrane destabilization. As the consequence of lysosomal destabilization, the activity of cathepsin D increased in all organophosphate-exposed groups except for the fish exposed with 15 µg/L of Roundup. The effects of Roundup and chlorpyrifos on the lysosomal membrane stability were not concentration-dependent and similar in significance between the groups exposed to individual pesticide and their mixtures. Upregulation of cathepsin activity can induce transition from autophagy to apoptosis (Zhao et al., 2019), which partially explain our findings of apoptosis upregulation in the fish exposed to the high concentrations of Roundup and chlorpyrifos. These findings indicate that unlike the oxidative stress, which was increased consistent value in the all pesticide- and mixture-exposed groups (Falfushynska et al. 2022), lysosomal damage in zebrafish is a less sensitive marker of the pesticide-induced stress under the environmentally relevant Roundup and chlorpyrifos concentrations.

THE RESULTS OF A STUDY OF THE COMBINED EFFECTS OF DOXORUBICIN AND LASER IRRADIATION ON THE SURVIVAL OF MCF7 AND MCF7-DOX CULTURE CELLS

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Aim. To study the effect of laser radiation with a wavelength of 660 and 810 nm on the survival of tumor cell cultures MCF7 and MCF7DOX in the presence of doxorubicin.

Materials and methods. Tumor cells of MCF7 and MCF7DOX cultures cultured in DMEM medium (Biowest, France) with 10% fetal calf serum (FST) (Biowest, France) and 40 μg/ml gentamicin (Sigma, USA). Doxorubicin was added to the cells to a final concentration of 1 μg/ml. Cells were irradiated with a laser (Photonica-Plus, Ukraine) with a wavelength of 660 and 810 nm (irradiation time – 5 min, power density – 50 mV/cm^2, irradiation dose 15 J/cm^2). The results were recorded using a multi-well spectrophotometer (Labsystems Multiskan PLUS, Finland). Photomicrographs of cells were taken using a Carl Zeiss microscope, Germany.

Results. The most pronounced cytotoxic effect on resistant cells of MCF7-DOX culture was caused by an infrared laser with a wavelength of 810 nm. Infrared laser light due to greater penetrating power in combination with the toxic effects of doxorubicin creates the conditions for apoptosis of tumor cells.

Conclusions. The results of the study indicate the best antitumor efficacy of the combination of infrared laser with a wavelength of 810 nm with doxorubicin.

REVIEW OF TNF–ALPHA ROLE IN OSTEOCLASTS DIFFERENTIATION AND ACTIVITY

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Aim. By analysis of publications to review TNF–α role in osteoclasts differentiation and to compose a simple and clear scheme of this signalling.

Methods. Information search, analysis, synthesis.

Results. The immune system as an integral part of the normal functioning body not only provides its protection but also manage and adjust tissue homeostasis, in particular balances osteoblasts and osteoclasts activity. The balance of bone tissue resorption and its restoration is guided by the sum of affecting factors of all functional body systems and by the involvement of biologically active substances (BAS) and the immune system cell lines (Faienza et al., 2015). Among the affectors are hormones, cytokines and other BAS. One of them is TNFα. TNFα is a cytokine with highly pleiotropic action in a variety of tissues (Robineau-Charette et al, 2020; Hannan et al, 2018). Its effect on bone tissue is in a complex mechanism of stimulation of osteoclastogenesis. It acts both directly and indirectly, inducing the production by osteoblasts the regulators of osteoclast genesis. There are at least two decisive factors: MCSF (CSF1) and RANKL (TNFSF11) (Wang et al, 2017). Both are synthesized by osteoblasts with receptors located on osteoclasts. The expression of cFms (CSF1R, op) for the monocyte/macrophage line cells is necessary for normal development into osteoclasts (Dai et al, 2016). Mice homozygous for op//op suffer from osteopetrosis due to the complete absence of osteoclasts (Marahleh et al, 2019; Saleh et al, 2018). CSF1CSF1R signal transduction is necessary for myeloid progenitor cells survival (Wang et al, 2017). TNFα interacts with TNFR1 of osteoblast; this binding, via several intermediate steps results in NFkappaB signaling activation (either through classical p65/p50 or through alternative RelB/p52 mechanism) and RANKL (TNFSF11) is produced. When TNFα is recepted by osteoclasts the expression of cFms (CSF1R) is induced and the cell becomes susceptible to MCSF (CSF1). The CSF1CSF1R coplex on the osteoclast enables RANKL receptor synthesis – RANK. Thus, the osteoclast is able to perceive TNFα–osteoblast signals for further diffenrentiation: an increase in TRAP activity, alpha(v)beta(3) integrin, cathepsin K and MMP9 production (Yang et al, 2017). Still, the effects of TNFα on the osteoclast depend on the type of receptor involved – TNFR1 or TNFR2. Studies in mice deficient in TNFR1 and TNFR2 showed a stimulatory effect mediated by TNFR1, and an inhibitory one by TNFR2 (Kitaura et al, 2014).

Conclusions. Analysis of the available publications made it possible to clarify that in osteoclasts differentiation TNFα closes a kind of feedback loop, on the one hand indirectly by the induction of the synthesis of RANKL by osteoblasts, and on the other hand, directly interacting with the differentiating osteoclast, making it susceptible to that RANKL.

RESEARCH OF ALT-SENSITIVE AMPEROMETRIC BIOSENSOR CHARACTERISTICS

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Aim. Alanine aminotransferase is a specific liver enzyme. Its content in the blood is a constant value (up to 35 U/L). In acute or chronic liver damage, ALT activity in the blood increases, meaning it serves as a diagnostic marker of liver disease. Myocardial damage also causes a significant increase in serum ALT and AST activity. The characteristic dynamics of ALT and AST levels are different for types of lesions. To a lesser extent, a number of other diseases are accompanied by elevated ALT levels, such as diabetes, periodontitis, muscular dystrophy, lung cancer, etc., which allows the use of ALT to gain additional knowledge about the patient's condition. Accordingly, the development of a new amperometric biosensor for determining the content of ALT is very relevant.

Methods. In this work, a platinum disk electrode was used as an electrochemical transduser. Three-electrode scheme of amperometric enzymatic analysis (working platinum disk electrode, additional and Ag/AgCl electrodes) was used to detect ALT levels. The bioselective element on the working surface of the electrochemical transducer (pre-protected with poly-phenylene diamine membrane to increase selectivity) was formed by air-drying a mixture of glutaraldehyde with glutamate oxidase gel. Glutaric aldehyde forms crosslinks between the components of the enzyme gel, thereby forming a film.

Results. As the part of the biosensor development, the optimal concentrations of ALT substrates (alanine, αketoglutarate) and coenzyme (pyridoxal phosphate) were selected. The fundamental ability of this biosensor to measure AST activity was also demonstrated. The selectivity of the developed sensor for transaminases was tested: changes in the system under the presence of both enzymes were studied. In addition, the selectivity for a number of substances (amino acids, (non)electroactive molecules, ions) was tested. To predict the functioning of the sensor in biological samples, the amino acidic and ionic composition of blood serum was modeled and the response of the biosensor was analyzed. At the same time, the analytical and operational characteristics of the biosensor were studied (lower limit and range of determination, durations of signal and general analysis, operational and storage stability, and signal reproducibility).

Conclusions. A laboratory prototype of a new amperomeric biosensor based on GOD for selective determination of ALT has been developed. The obtained results allow us to proceed to the verification of the sensor's functioning in biological fluids and modeling of a two-enzyme system for the simultaneous determination of ALT and AST based on the developed biosensor.

HEMATOLOGICAL MARKERS OF LOW-GRADE SYSTEMIC INFLAMMATION IN RATS WITH DIFFERENT MODELS OF ALZHEIMER'S DISEASE

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Background. Results of numerous experimental studies and clinical observations suggest that low-grade systemic inflammation (LGSI) is inherent in Alzheimer's disease (AD), and can be considered both a disease-promoting factor and a consequence of disease progression. Persistent LGSI is marked by increased blood levels of circulating pro-inflammatory mediators, as well as by alterations in leukocyte differential count and other hematological indices. Deep insight into the LGSI phenomenon is required for developing anti-inflammatory strategies for AD treatment. This, in turn, necessitates the use of animal models, most correctly reproducing clinical features of LGSI in AD.

This study **aim**ed to compare low-grade inflammatory indicators in rats with different AD models.

Methods. AD was induced by intrahippocampal infusions of Aβ 140 and Aβ 2535. AD development was confirmed by the results of behavioral tests (Barnes maze test), as well as by the level of soluble beta-amyloid and tau protein in the hippocampus homogenates and by the level of death of dopaminergic neurons. Low-grade inflammatory indicators, such as systemic immune-inflammation index (SII), neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), and platelet-to-lymphocyte ratio (PLR), mean platelet volume-to-platelet ratio (MPV/PLT) was calculated by the result of hemogram analysis.

Results. The development of AD in animals with Aβ 140-induced AD was associated with the pronounced cognitive impairment, and death of dopaminergic neurons. However, hematological markers of LGSI were registered only in rats with Aβ 140-induced AD: NLR in lesioned animals was 6.5 times higher than in sham-operated rats, LMR was increased by more than 3 times as compared to intact and sham-operated animals, SII values was 10 times higher than those in control animals. In addition, hemoglobin level was increased in Aβ 140-lesioned animals by 1.6 times in comparison with animals from control groups. RBC count in these animals was slightly decreased as compared to control values. By contrast, animals from a group of Aβ 2535-induced AD were characterized by only slight increased NLR (0.83±0.11 vs 0.68±0.17) and SII (261.1±14.27 vs 175.7±11.08) values in comparison with intact and sham-operated rats. LMR in these animals was 3 times higher than that in control animals. Hemoglobin level and RBC count didn't differ significantly in comparison with values of animals from control groups.

Conclusions. Hematological indicators in rats with Aβ 140-induced AD indicate prominent LGSI. In addition, decreased RBC count along with increased hemoglobin levels in these animals specifies the development of anemia, which is also inherent in the clinical course of the disease. Thus, the Aβ 140-induced AD model reproduces one of the typical clinical features of AD pathophysiology – LGSI. However, the same cannot be said of Aβ 2535-induced AD model.

BIOTECHNOLOGICAL DERMAL COATINGS FOR THE REGENERATION OF TRAUMATIC SKIN DAMAGES

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Aim. Development of new dermal coatings or dermis equivalents with the included biological component of the cellular origin and pharmaceutical composite, indicated for the treatment of skin traumatic injuries.

Methods. The methods of cell biology (obtaining the cell culture or the medium conditioned by the cells *in vitro*), as well as various biotechnological methods, have been used.

Results. Regardless of the turbulent development of regenerative medicine, traumatic injuries of the skin are one of the most pressing problems worldwide. In Ukraine, there is a significant increase in the frequency of traumatic damages including burn wounds. This prompted us to develop new temporal replacements of the skin, which are used for the treatment of massive burn wounds, which cover more than 30% of the body surface. In order to create the new dermal coatings, the cells of the original line 4BL with properties of mesenchyme stem cells (MSCs) have been used. Living MSCs or their derivatives incorporated into the composition of various hydrogels have been applied as plastic paste-like coatings, or they have been plotted on the membranes of natural and synthetic materials, and then - on the surface of experimental burn wounds of model animals for the regeneration of their dermis. The achievement of new dermis equivalents is that the living MSCs in the structure of the product have been replaced on the conditioned by the cells media containing a complex of biologically active compounds. And, additionally, a pharmaceutical composite with immune-modulatory and antiviral properties has been included. It has been shown that the new biotechnological product is highly effective in the treatment of burn wounds of the model animals. Analysis of the toxicological characteristics leads us to the conclusion that new dermis equivalents are safe in general and could be given to a specialized toxicological laboratory for certification, and if allowed - for further clinical trials at the medical institutions of Ukraine.

Conclusions. A new biotechnological product, dermis equivalent, which is highly effective and safe in the treatment of experimental burn wounds in preclinical studies *in vivo*, has been created. In comparison with other foreign analogs of this product, it could cost about 10 times cheaper.

THE THERAPEUTIC EFFECT OF MESENCHYMAL STEM CELLS DERIVED FROM THE HUMAN UMBILICAL CORD, USING DIFFERENT METHODS OF DELIVERY TO AN ANIMAL MODEL OF LIVER CIRRHOSIS

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Mesenchymal stem cells (MSCs) are among the most frequently used cell type for regenerative medicine due to their unique properties. In particular, it is the possibility to use MSCs for the treatment of liver damage.

Aim. This study aimed to compare the efficiency of human umbilical cord MSCs transplantation in the conditions of intravenous injection of MSCs as cell suspension and intraperitoneal injection of MSCs encapsulated in alginate microcapsules for the treatment of rat liver cirrhosis.

Methods. MSCs were isolated from the human umbilical cord using the explants method. After culturing *in vitro* MSCs meet the minimum criteria accepted by The International Society for Cellular Therapy. Wistar rat's liver cirrhosis was caused by intraperitoneal injections of CCL4 solution in olive oil (1:1) according to a particular scheme. Methods of histology and molecular biology determined pathological changes in the liver of rats. After 13 weeks, the histological study showed the presence of changes in the structure of liver parenchyma typical for cirrhosis. At the same time, the level of *EGF* (epidermal growth factor) and *αSMA* (α-Smooth muscle actin) gene expression has sharply increased. At this stage, one group of animals (n = 6) was injected with human umbilical cord MSCs (6-7x10^6 cells/kg of rat weight) of the second passage into the tail vein, another group was intraperitoneally injected with the same amount of MSCs encapsulated in alginate microcapsules. Control animals received either intravenously normal saline or intraperitoneally "empty" alginate microcapsules.

Results. A study of *EGF* and *αSMA* expression in the liver of experimental animals showed that it begins to fall in both transplantation cases. After 3 weeks, a decrease in the level of EGF expression was observed 3 times faster with the introduction of encapsulated MSCs than in the form of a cell suspension. In particular, 6 weeks after transplantation of both encapsulated and cell suspension MSCs in the liver of rats, EGF expression was observed as normal. Transplantation of the cell suspension into the tail vein results in the disappearance of the expression of EGF after 12-13 weeks. In control animals, the liver recovery process is very slow and after 13 weeks there are clear signs of cirrhosis in the liver of those animals. Also, 3 weeks after the introduction of encapsulated MSCs, the decrease in the rate of collagen accumulation occurred faster in comparison with MSCs introduced in the form of a cell suspension. Other morphological parameters of the liver parenchyma also recovered faster with the introduction of MSCs encapsulated in alginate microcapsules.

Conclusions. Thus, liver recovery occurs faster after intraperitoneal administration of encapsulated MSCs compared with intravenous administration of MSC suspension.

THE MCU COMPLEX IN HEALTH AND DISEASE

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The exciting discovery of MCU as the calcium channel allowing rapid Ca^{2+} uptake in mitochondria upon cell stimulation was followed in the following years by the elucidation of a complex molecular machinery, including two pore subunits (MCU and MCUb), three regulatory elements (MICU1, MICU2 and MICU3) and an assembly factor (EMRE), endowed with different functional properties. It is well accepted thus that the molecular composition of the MCU complex accounts for major flexibility in Ca^{2+} uptake capacity in different tissues and physiological and pathological conditions. To unravel the functional significance of this complexity, molecular intervention in physiologically significant model systems must be carried out. In this presentation, I will report data in a muscle regeneration model. I will show that MCUb, the dominant-negative subunit of the mitochondrial calcium uniporter (MCU) complex, which is highly expressed in immune cells, promotes muscle regeneration by controlling macrophage responses. Macrophages lacking MCUb lose the ability to efficiently acquire the anti-inflammatory profile and mice with MCUb-deficient macrophages show exhaustion of the satellite cell pool and delayed regeneration. MCUb ablation alters macrophage metabolism and is accompanied by the stabilization of HIF-1α, the master transcriptional regulator of the macrophage pro-inflammatory program. Together, these data demonstrate that the high expression of the MCUb subunit in macrophages is critical for enabling satellite cell functional differentiation and recovery of tissue homeostasis after damage.

NANOPORE SEQUENCING AS AN EFFICIENT METHOD TO STUDY STRUCTURAL VARIATION IN ARABIDOPSIS THALIANA POPULATION

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Background. Intraspecies structural variation (SV) describes the genomic polymorphisms that occur between different individuals of the same species. SV has spectacular meaning for plant populations, in which it often overlaps large genomic fragments.

Aim. To utilize the modern sequencing technology – nanopore sequencing, that generates long sequences (reads), based on the example of one non-reference *Arabidopsis thaliana* accession (Mitterberg 2185). To optimize all the steps of bioinformatic sequence analysis to assemble the entire genome *de novo*. To compare the assembled chromosomes with the reference, to identify new accession-specific variants.

Methods. Genomic high-molecular weight DNA was isolated from plant material. Sequencing library was prepared by DNA purification and ligation of sequencing adapters. Sequencing was performed using the Oxford Nanopore MinION sequencer. The resulting data was preprocessed and formatted to FASTA format. The *de novo* genome assembly was performed using the overlap-layout-consensus algorithm. The draft sequences were then arranged to consensus sequences equivalent to *Arabidopsis* chromosomes.

Results. We have assembled the genome of Mitterberg 2185 into five sequences. Considering the fact that nanopore sequencing produces sequences with lower per-base accuracy than other technologies, the final assembled sequences demonstrated good quality and high accuracy. Whole-genome comparison with the reference showed strong synteny, as well as numerous SV events (duplications, deletions, and inversions).

Conclusions. Nanopore sequencing is a reliable method to study SV at the sequence level. The access to well-quality genome sequence allowed us to resolve the structure of rearranged regions in the genome. This study will contribute to our understanding of the genetic base of plant adaptation and evolution.

FEATURES OF PROTEIN SYNTHESIS IN THE NUCLEI OF RAT HEPATOCYTES AFTER GENERAL COOLING AND REHABILITATION

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Aim. The response of the nuclear apparatus of cells to general cooling of animals and at the stage of subsequent rehabilitation is a poorly studied problem because of the existing a priori opinion on the stability of nuclear structures under the effects of cell cooling and freezing. Although, there is a possible reprogramming of the genome in a response to stress.

Methods. The synthesis and concentration of nuclear proteins (histones and non-histone proteins) in rat's liver cells after acute cooling of animals (immersion into water with a temperature of +4°C for 10 min to reduce the rectal temperature to 2022°C), as well as in the recovery period (1 day at +20°C) were investigated. Labeled amino acids (14Cprotein hydrolyzate) were administered at a dose of 50 μCi per 100 g of animal weight. The synthesis and concentration of proteins were determined after isolation of the nuclei from the liver tissue by ultracentrifugation through a 1M layer of sucrose and using a Triton X100 detergent followed by chemical fractionation using the modified Schmidt-Tonhauser method. The protein concentration was photometrically determined at A260 wavelength.

Results. It was found that in the control group of animals the histone concentration was 285γ/1000γ chromatin (the same units hereinafter), after an acute cooling it was 120 and after a day of rehabilitation that was 110. The specific radioactivity was 3, 53 and 142 ppm, respectively per γ of histones. A similar trend is observed when studying the non-histone proteins (NHP). In the control, their concentration is 225γ/1000γg of chromatin, after cooling that was 70 and after 1 day it made 90. The specific radioactivity of the NHP in the control is 4.0 ppm per γ of NHP, after cooling that was 1.9 and after a day it reached the control level, that was 4.1.

Conclusions. Thus, the synthesis of histones in liver cells during the rehabilitation period significantly increased in comparison with the synthesis of NHP, i.e. in 24 hrs after the animals' staying under the normothermy conditions, the chromatin was still in a condensed state and, probably, with low transcriptional activity.

THE FATTY ACID SPECTRUM OF HEPATIC LIPIDS OF RATS UNDER THE CONDITIONS OF ACETAMINOPHEN-INDUCED TOXIC DAMAGE IN THE BACKGROUND OF ALIMENTARY PROTEIN DEFICIENCY

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Background. An important component of lipid homeostasis is fatty acids (FA). An impaired synthesis and utilization of free fatty acids are one of the causes of liver disease: from the triacylglycerol (steatosis) deposition to inflammation and necrosis of hepatocytes – steatohepatitis. The liver is the main organ involved in lipid metabolism in the body and at receipt of chylomicrons from food, lipid transport from hepatocytes to other organs occurs through hepatocytes.

The **aim** of the study was the evaluate the fatty acid content of rat hepatic tissue in an experimental model of acute acetaminophen-induced injury in the background of dietary protein deficiency.

Methods. The model of the experiment provided for the division of animals into groups: C – intact animals (control); LPD – rats kept on a low-protein diet; TI – animals simulated with acetaminophen toxicity (APAP); LPD/TI – animals that simulated APAP-induced injury in the background of alimentary protein deprivation. The determination of FA content was performed by gas-liquid chromatography with a chromatograph HRGC 5300 (Italy). FA was identified using a standard mixture of their methyl esters from Sigma (Germany).

Results. According to the results of studies in the liver of the control group of rats, 28 FA were identified, including 43.7% saturated, 14.56% monounsaturated and 41.72% polyunsaturated; in the LPD group – 35 FA (42.9% saturated, 16.15% monounsaturated, 39.98% polyunsaturated, 0.02% unidentified), TI – 37 FA (38.3% saturated, 19.9% monounsaturated, 41.7% polyunsaturated, 0.02% unidentified), LPD/TI – 29 FA (25.5% saturated, 34.7% monounsaturated, 39.6% polyunsaturated). It should be noted that palmitic (C16:0), stearic (C18:0), linoleic (C18:2), oleic (C18:1), arachidonic (C20:4), docosahexaenoic (C22:6) dominate in the liver tissue of the rats' control group. Compared with the control in the liver of rats LPD group, an increase in the percentage of palmitic (C16:0), oleic (C18:1) acids and a significant decrease in docosahexaenoic acid (C22:6) was detected. A feature of the TI group compared to the control indicators is the decrease in the content of stearic (C18:0) with the simultaneous accumulation of margaric (C17:0) and arachidonic acid (C20:0). In the liver of rats LPD/TI group, compared with controls, a decrease in the content of palmitic (C16:0), stearic (C18:0) and arachidonic (C20:4) acids and a significant increase in the amount of palmitoleic acid (C16:1) was observed.

Conclusions. The fatty acid spectrum of rats' liver lipids under the experimental conditions is characterized by an imbalance in the ratio of the amount of saturated FA, unsaturated FA, and the amount of PUFA. The revealed probable decrease in saturated FA in the background of increasing the amount of monounsaturated FA in the liver of rats with an acetaminophen-induced injury can be considered as a prerequisite for impaired lipid metabolism due to increased activation of their peroxidation.

LEPTIN INDUCED CHANGES IN GENE EXPRESSION, BARRIER INTEGRITY AND CELL MORPHOLOGY IN HUMAN AIRWAY EPITHELIAL CELLS

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Introduction. Obesity is a major risk factor for asthma, and metabolic stress was found to influence changes in different cell types, including nasal and bronchial epithelial cells. However, precise factors which influence the change are not fully understood.

Aim. The purpose of this study was to determine the response of airway epithelial cells to conditions of metabolic stress that characterize obesity.

Methods. We investigated the effects of factors associated with obesity and metabolic stress on gene expression related to the extracellular matrix (ECM) remodeling. Human bronchial epithelial cell line BEAS-2b and primary human nasal epithelial cells were grown on transwell plates in medium with normal, physiological concentrations of 6mM glucose and 0.9 μM insulin. Confluent cells were treated with increasing concentration of glucose, insulin and leptin to mimic conditions of metabolic stress common in obesity, with medium changed every 48 hours. Expression of ECM markers, Trans-epithelial electrical resistance (TEER), and changes in cell morphology were measured after the treatment.

Results. The expression of markers of remodeling in airway epithelial cells changed in response to conditions of metabolic stress. Five days exposure (20 ng/ml) to leptin resulted in increased expression of HAS1 (hyaluronan synthase I), ITGAL (integrin subunit alpha 1), NCAM1 (neural cell adhesion molecule I), and MMP15 (matrix metallopeptidase 15) in both types of the epithelial cells. Prolonged (14 days) simultaneous treatment with leptin, insulin and glucose resulted in significant decreases in E-cadherin, smooth muscle actin (SMA), collagen 1A1 and MMP-9 (matrix metallopeptidase 9) expression. No significant changes were found in a number of pro-inflammatory cytokines after high glucose, insulin or leptin treatment. We found that leptin treatment caused a 2-10% increase in TEER values and was accompanied by the cell shape change in human nasal epithelial cells.

Conclusions. Changes in airway epithelium contribute to psychophysiology in asthma. Data from the current study suggest that conditions of metabolic stress characteristic of obesity, specifically higher levels of leptin lead to changes in markers of extracellular matrix remodeling, and barrier integrity. Leptin could possibly induce proliferation and permeability of the airway epithelial cells.

USE OF 5S RDNA IGS POLYMORPHISM TO RECONSTRUCT POSTGLACIAL MIGRATION ROUTES OF UKRAINIAN POPULATIONS OF QUERCUS

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Aim. Migration is one of the central processes in the dynamics and evolution of plants. The study of patterns of plant migration is important for predicting the dynamics of ecosystems during the global climate change. This is especially true for common groups of woody, forest-forming plants, including, in particular, representatives of «white oaks» group (sect. *Quercus*). In recent years, molecular markers were used for studying of ecological and evolutionary success of species of the genus *Quercus*. A promising molecular marker that is just beginning to be used for biogeographic research is the intergenic spacer (IGS) of 5S rDNA. In previous studies, we were able to identify 9 major IGS haplotypes characteristic for three European species: *Q. robur L.*, *Q. petrea (Matt.) Liebl.*, *Q. pubescens Willd*. In this work, we sequenced 5S rDNA IGS of oaks from three western regions of Ukraine and added them to the general analysis.

Methods. Oaks specimens were collected in the vicinity of the settlements of Vynohradiv (Transcarpathian region), Khomets (Lviv region) and Shatsk (Volyn region). DNA samples were isolated from herbarized plant material using a modified CTAB method. 5S14a and 5S15 primers complementary to the coding region were used for PCR amplification of 5S rDNA. The obtained PCR products were cloned into the plasmid vector pJET 1.2. After screening for recombinant plasmids, one to three clones for each sample were sequenced.

Results. Alignment of sequences obtained in this work with the consensus sequences of nine IGS haplotypes identified previously revealed that the new sequences belong to haplotypes H1 (Vynogradiv: clones QuVyn3, QuVyn4, QuVyn5; Shatsk: QuSha2; Khomets: QuKho9) and H3 (Khomets: QuKho1). H1 is characterized by the transition T→C at position 204 of the IGS. The sequence of the H3 haplotype is identical to the general consensus of all nine haplotypes. Therefore, we consider H3 to be the original haplotype for European white oaks.

Conclusions. In general, our results confirm the previous hypothesis that one of the main postglacial colonization routes of European white oaks passed from the Transcaucasian refugium through the territory of the Eastern Black Sea coast. For this migration routes, the predominance of oaks possessing the haplotype H1 is further confirmed by our new data.

SPA–B-LACTAMASE PROTEIN AS A SECONDARY IMMUNOREAGENT

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Background. *Staphylococcus* protein A (SPA) is a cell wall component that can bind the constant regions of antibodies. SPA has an affinity for IgG subclasses 1, 2, and 4 and a lower affinity for IgG3, IgM, and IgA. If the primary antibody in ELISA or blot analysis is of the IgG isotype, SPA fusion can be used for detection.

Aim. Our research **aim**ed to apply the fusion protein SPAβLac in immunoassays as a secondary immunoreagent.

Methods. Recombinant DNA technology assures the elaboration of genetic constructions without disadvantages of chemical conjugation, such as high heterogeneity of the final product or the necessity of separating full-size conjugates from nonconjugated components. βLactamase was selected as a fused component due to its advantages, such as stability and the possibility of obtaining high concentrations in bacterial systems of expression in soluble active form. The DNA sequence of *E. coli* βlactamase was subcloned into plasmid vector pET24-SPA. *E. coli* BL21(DE3) cells were transformed by obtained plasmid vector. SPAβLac expression was induced by adding IPTG and by the autoinduction protocol. The iodometric method for the detection of βlactamase activity was applied.

Results. The protein of interest was accumulated in the cytoplasmic fraction of *E. coli*. The possibility of long-term storage of the protein at –20°C without loss of its functional activity was shown. The functional activity of SPAβLac was confirmed with ELISA.

Conclusions. Application of SPAβLac as a universal secondary immunoreagent allows extending the range of primary antibodies for antigen detection in ELISA or blot analysis (SPA detects Fc fragments of IgG of different animal species and human IgG).

PROTECTIVE EFFECT OF PIOGLITAZONE, THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA LIGAND (PPARΓ), IN A RAT MODEL OF HYPERGASTRINAEMIA

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Aim. Hypergastrinaemia, resulted from hypochlorhydria, is known to occur during atrophic gastritis, to cause mucosal proliferation and dysplasia and further develop to gastric cancer. Recently, peroxisome proliferator-activated receptor γ (PPARγ), a member of the nuclear hormone receptor superfamily, has been recognized as a regulator of overall anti-cancer responses in various cell types, possibly due to its anti-proliferative, pro-differentiation and pro-apoptotic activity. This study was aimed to determine effects of pioglitazone, a specific PPARγ ligand, in the gastric mucosa of hypergastrinaemic rats.

Methods. Long-term hypergastrinaemia in adult male Wistar rats was induced by daily i.p. injections of omeprazole (14 mg/kg bw) during 28 days, with gastrin levels regularly checked using radio-immuno assay (RIA). Pioglitazone (10 mg/kg bw) was given i.p. to omeprazol-treated rats also daily during 28 days. Gastric antral mucosa response was evaluated using histological examination and morphometry.

Results. Chronic administration of omeprazole was found to gradually increase blood gastrin during the experiment, up to 4fold at the 28th day of administration, as well as to cause acute inflammation and dysplasia in the antral mucosa, including lymphocytic infiltration, glandular atrophy and appearance of undifferentiated cells, local thickenings or damages of the epithelial layer, overall presuming early stages of tumorigenesis in the mucosa. Pioglitazone was shown injections to omeprazol-treated rats were found to result in significant thinning of epithelial layer and normalization of histological and morphometrical parameters of the mucosa. As omeprazole-induced gastrin was still detected high under the pioglitazone treatment, this ligand was presumed not to affect development of hypergastrinaemia in rats, but to reduce inflammation and cell proliferation in the antral gastric mucosa.

Conclusions. Pioglitazone was shown to significantly inhibit the effects of omeprazole-induced hypergastrinaemia on inflammation and cell proliferation in rat gastric antral mucosa, and thus may be considered a promising pharmacological agent in the search of anti-cancer drugs.

NON-NUCLEOSIDE MGMT INHIBITORS MODULATES ANTI-TUMOR EFFECT OF ALKYLATING DRUG

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Background. Alkylating chemotherapy is applied in the treatment of oncological diseases with a severe prognosis, such as brain gliomas. The effectiveness of treating is significantly reduced due to the presence of an active O6-methylguanine-DNA methyltransferase (MGMT) repair enzyme, which removes alkyl radicals from the most harmful adduct, O6-methylguanine. So MGMT repair enzyme activity is a negative factor influencing clinical effect of alkylating drugs in tumor cells. And the main strategy for resolving this problem is the complex therapeutic strategy including nucleoside-derived MGMT inhibitors, such as O6-benzylguanine (O6-BG) and its analogs. But these known inhibitors are quite toxic, especially for bone marrow MSCs. Therefore, the search for new low-toxic MGMT inhibitors is still ongoing actual.

Aim. Studying possible therapeutic action of MGMT repair enzyme inhibitors as possible cancer chemotherapy drugs in model systems.

Methods. *In vitro* estimating the cytotoxicity of the studied compounds in cell cultures, *in vivo* acute and subacute toxicity testing, *in vivo* tumor growth dynamics assay.

Results. In this work we studied two novel non-nucleoside MGMT inhibitors (41, 41B) which are analogs of one another. Both inhibitors have been shown to be less cytotoxic than O6-BG with using MTT-test and clonogenic test *in vitro*. Relatively low own toxicity of the studied inhibitors has been confirmed on the level of an organism. At testing acute toxicity, the death of the model animals has been absent and at testing subacute toxicity, definite morphological changes of animals internal organs has been shown after the introduction of an inhibitor in a concentration 20 mg/kg, which exceeded the therapeutic doses by a factor of tens of times. Studied inhibitors sufficiently increased the cytotoxic effect of alkylating agent N-methyl-N'-nitro-nitrosoguanydine (NG) in tumor-derived cell cultures, what gives evidence of their activity. When studied at *in vivo* rodent tumor model (spontaneous mammary gland tumor), both inhibitors were shown to increase anti-tumor effect of alkylating compounds. At the combined action of compounds 41 and NG diminishing in the size of the tumors by 1.5-2 times in the studied animals during the first week of therapy has been observed. On the second week of therapy, the growth of tumors has been much slower than in the group of animals, which has been treated by alkylating agent only. The inhibitor 41B in the case of mutual treatment with NG significantly decreased the growth of tumors of the studied animals compared with those ones which were treated with alkylating compound.

Conclusions. Based on the results of studies with using *in vitro* and *in vivo* models both novel non-nucleoside MGMT inhibitors increased anti-tumor activity of alkylating compound, so their further studies as possible drugs for complex cancer chemotherapy could be advisable.

CARBOXYLESTERASE FROM PIG LIVER CYTOSOL

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Background. Carboxylesterases (CES) (EC 3.1.1.1) are the most studied enzymes, catalyzing the enantioselective hydrolysis of an exceptional range of acyclic, carbocyclic, and heterocyclic compounds. These enzymes are localized in the endoplasmic reticulum and cytosol. Microsomal carboxylesterase is the extensively studied isoenzyme involved in xenobiotic metabolism. There are several works devoted to porcine cytosolic carboxylesterase.

The **aim** of this work is to isolate carboxylesterase from the cytosol of pig liver, study its biochemical properties and oligomeric structure.

Methods. Isolation of cytosolic carboxylesterase was performed from the cytosolic fraction, obtained after microsomal fraction preparation. Then fractional precipitation of proteins by ammonium sulfate was carried out. The most active fraction was loaded onto a Sephadex G-200 column and then to a DEAE-Sepharose column. Protein concentration was determined according to modified Lowry method. Esterase activity was determined by hydrolysis of 1-naphthyl acetate. Purified esterase was analyzed by SDS-PAGE and Native-PAGE. According to Michaelis equation, the activity assays were measured in different concentrations of 1-naphthyl acetates (90-1250 μM) and 2-naphthyl acetates (30-250 μM) in order to determine Michaelis-Menten constant (K_m) and maximum reaction rate (V_{max}).

Results. Present study describes a simple carboxylesterase isolation method from pig liver cytosol. The special activity of purified enzyme reached 730 μmol/(mg protein per min) by 1-naphthyl acetate, which was 243-fold of the crude homogenate. SDS-PAGE and Native-PAGE of final purified enzyme were studied. A single protein band of both methods established the homogeneity of the enzyme. The molecular weight of purified carboxylesterase determined by SDS-PAGE was found to be 61.8 kDa. Obtained by Native-PAGE molecular weight of carboxylesterase was estimated about 175 kDa, which can indicate the trimeric structure of isolated pig liver cytosolic carboxylesterase. The K_m and V_{max} values of carboxylesterase were determined from Hanes-Woolf plot. V_{max} values were 0.78±0.016 and 1.63±0.095 mmol/(min×mg protein), respectively for 1- and 2-naphthyl acetate. K_m values of studied enzyme were 0.51±0.025 and 0.18±0.019 mmol/L, respectively. The analysis of pig liver cytosolic carboxylesterase K_m showed more affinity of 2-naphthyl acetate than to 1-naphthyl acetate.

Conclusions. Carboxylesterase was obtained from pig liver cytosol in an electrophoretically homogeneous form. Its molecular weight, oligomeric structure, biochemical and kinetic characteristics were studied.

BLOOD-BRAIN BARRIER DISFUNCTION AND DEVELOPMENT OF EPILEPTIC SEIZURES

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Background. Blood-brain barrier dysfunction (BBB) is an important factor to the development of epilepsy and its behavioral comorbidities. Disruption of its integrity is accompanied by the ingress of blood components, including thrombin, into the cerebrospinal fluid. The effect of thrombin is mediated mainly through its major receptor, protease-activated receptors 1 (PAR1). Our recent studies have shown that the concentration of serine protease, thrombin, increases significantly in brain tissue after experimentally induced status epilepticus (SE). Moreover, thrombin contributes to neuronal damage and epileptogenesis caused by SE.

Aim. Since thrombin and PAR1 are involved in synaptic plasticity and memory formation, the aim of this study was to determine the role of PAR1 in synaptic plasticity and behavioral deficits after SE.

Methods. Using lithium-pilocarpine model of seizures, the methods of behavior testing and field potential electrophysiology technique we studied the effect of PAR1 inhibition on such behavior characteristics as anxiety, emotion excitability, conditioning, and sociability; and such electrophysiological parameters as synaptic efficacy, short-term and long-term potentiation in CA1 region of the hippocampus.

Results. We show that downregulation of PAR1 activity reduces anxiety and aggressive behavior in epileptic rats. Also we demonstrate that inhibition of PAR1 rescues SE-induced synaptic plasticity deficits in CA1 region of hippocampus. Although treatment with PAR1 antagonist does not ameliorate spatial learning deficits, it attenuates anxiolytic-like behavior in experimental rats after SE. Taken together; our data suggest an important role of PAR1 in SE-induced synaptic and behavioral alterations and provide a new insight into cellular mechanisms underlying behavioral impairments associated with epilepsy.

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