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I/D polymorphism of the ACE1 gene in children with COVID-19 infection of varying severity

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Aim. The aim of this study was to investigate the association of genotypes and allelic variants of the I/D polymorphism of the ACE1 rs4946994 gene with the course of coronavirus disease COVID-19 in children from Ukraine. **Methods.** The material comprised blood samples from children aged 1 to 17 years (N = 234) with laboratory-confirmed COVID-19 of varying severity: mild, including asymptomatic, moderate, and severe, as determined by pediatricians during hospitalization. Additionally, there was a group of children (60) with a concomitant disease - juvenile idiopathic arthritis (JIA) in the presence of COVID-19. Methods included obtaining peripheral blood samples, DNA extraction, and purification using standard methods with a nucleic acid extraction kit, spectrophotometric analysis, and PCR reaction with real-time detection of melting products using Eva Green intercalating dye. Analysis of the melting curves of PCR products was conducted using the BIO-RAD iQ5 Optical System Software package, and statistical processing of results was performed using Fisher's criterion with Open Epi software. **Results.** Comparative analysis revealed no significant difference in the genotypes and alleles between children with COVID-19 and the control group. No differences in the genotypes and alleles were found in children with mild, moderate, and severe COVID-19. Allele I was more common in the patients with lung damage. Statistically significant differences were noted when comparing the proportion of carriers of allele I in the analyzed subgroups. The distribution of ACE I/D alleles in children with JIA and without concomitant diseases in COVID-19 showed a statistically significant difference. **Conclusions.** A statistically significant increase in the frequency of genotype I/I was found in the group of children with COVID-19 and pulmonary complications compared to the patients with COVID-19 without pulmonary complications. Additionally, a statistically significant increase in the frequency of genotype I/I was found in the group of children with a history of juvenile idiopathic arthritis and mild COVID-19. Thus, genotype I/I can be considered a risk factor for JIA.

Keywords: children; SARS-CoV-2 virus; COVID-19; *ACE1* gene; genetic polymorphism; disease severity.

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New continuous monitoring system integrated with electrochemical biosensor for the determination of heavy metal ions in water

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Aim. Heavy metal ions (HMI) pollution is a global problem, as HMI can cause irreversible harm to humans and other organisms [1]. Traditional methods for HMI detection are known for their high sensitivity and selectivity but have drawbacks such as high costs and complex, time-consuming processes. In contrast, biosensors present a viable alternative due to their affordability, ease of use, sensitivity, portability, and on-site monitoring capabilities [2]. Therefore, the aim of this work was to develop a continuous monitoring system that integrates an inhibition-based biosensor using enzyme to detect HMI in water samples. **Methods.** The system employs a peristaltic pump and active valves to control the supply of solutions, a flow cell with a biosensor, a measuring device, and a PC. Two pairs of planar thin-film interdigitated gold electrodes, deposited on a ceramic plate, were used as an electrochemical transducer. The enzyme urease was immobilized on the gold electrode surfaces through a glutaraldehyde crosslinking process. **Results.** During the research, the optimal flow rate for the system's efficient operation was determined to be 200 μL/min. The biosensor showed high sensitivity, stability, and reproducibility (RSD = 5.5 %). The biosensor's sensitivity to different HMI concentrations (0.01–10 μM) and reactivation with EDTA after inhibition for repeated analyses were explored. For most of the analyzed heavy metals, the detection limits were below foundational limits, indicating that the developed biosensor has potential for analyzing these heavy metals. **Conclusions.** A new continuous monitoring system integrated with an enzyme inhibition-based biosensor to detect HMI in water was developed, and its main analytical characteristics were investigated.
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Keywords: electrochemical biosensor, heavy metals, ecological monitoring

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S6K1 CoAlation as cellular response to oxidative stress

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Aim. The purpose of this work was to demonstrate that S6K1 undergoes CoAlation modification in HEK293/Pank1β cells as a cellular response to oxidative stress by using *in situ* proximity ligation assay (PLA) and immunoprecipitation (IP). **Methods.** The HEK293/Pank1β model cell line with stable overexpression of Pantothenate kinase 1β (Pank1β) was exposed to oxidative stress via a 30-minute treatment with 0.5 mM hydrogen peroxide for PLA and 0.5 mM diamide for IP. To detect CoAlation of endogenous S6K1 *in situ* PLA, using mouse anti-CoA monoclonal antibodies and rabbit anti-S6K1 polyclonal antibodies, was performed with further signal visualization using confocal microscopy. For additional verification of kinase CoAlation, IP of overexpressed p70S6K1 was conducted. HEK293/Pank1β cells were transiently transfected with pcDNA3.1/EE-p70S6K1 plasmid and precipitated onto anti-EE carrying beads with subsequent western blot analysis (WB). **Results.** In this study, we have shown an increased level of CoA covalent modification of S6K1 in oxidative stress-induced HEK293/Pank1β cells compared to the control. Specific recognition of S6K1-S-S-CoA was demonstrated using PLA and IP techniques. By measuring close proximity, PLA allows us to identify post-translational modifications that cannot be established by colocalization studies alone. The signal of CoAlated kinase in the assay appeared as a distinct dot and was visualized by fluorescent microscopy. HEK293/Pank1β cells treated with H2O2 showed a significantly higher level of CoAlated S6K1 compared to the control sample without treatment. To provide additional evidence of S6K1 modification by CoA in cellular immunoprecipitation of overexpressed p70S6K1 has been conducted from cells under normal or stress conditions caused by diamide. The overexpression was performed due to the difficulties of detecting an endogenously expressed CoAlated S6K1 in WB. The obtained results successfully demonstrated p70S6K1 to undergo CoAlation in response to the oxidizing agents. **Conclusions.** In previous studies, we have determine an increased level of CoAlated proteins in the cells undergoing oxidative stress, and such findings have been linked to the function of CoA as a major cellular antioxidant. To prove S6 kinase 1 is among CoAlated proteins during the cellular response to stress conditions, we have performed *in situ* proximity ligation assay and immunoprecipitation. Overall, here we have illustrated an increased level of CoAlated S6K1 in HEK293/Pank1β cells treated with different oxidizing agents. Thus, we can anticipate CoA molecules to play a protective role for S6K1 cysteine residues from overoxidation, preventing irreversible loss of protein function and subsequent degradation.

Keywords: CoA, S6K1, kinase, cell signaling, PTM, oxidative stress.

The effect of exogenous 24-epicastasteron application on phytohormone content in soybean

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Aim. Our study aimed to examine the effects of exogenous 24-epicastasterone (ECS) treatment on the levels of indole-3-acetic acid (IAA), abscisic, salicylic, and jasmonic acids in soybean leaves. ECS, a natural brassinosteroid phytohormone, is a direct biosynthetic precursor to epibrassinolide, which is widespread in various plants. This hormone plays a significant role in the plant growth regulation. By analyzing how ECS influences the levels of other hormones, this research provides deeper insights into the intricate network of interactions between brassinosteroids (BRs) and other plant hormones. **Methods.** Soybean seeds (*Glycine max L. cv. Terek*) were obtained from the experimental station of Poltava State Agricultural University (Ukraine). Plants were cultivated in an artificial climate chamber and sprayed twice with ECS solutions on the 21st and 28th days after planting. Changes in the endogenous content of phytohormones were quantified by LC/MS. **Results.** The research showed that the treatment with exogenous ECS under optimal conditions significantly increased the indole-3-acetic acid content in soybean leaves, while phenylacetic acid (PAA) and IAA ester with aspartate (IAA-Asp) levels remained unchanged. Exogenous 24-ECS treatment also led to decreased level of abscisic acid (ABA) and its glucose ester (ABA-GE) in soybean leaves, while the level of the inactive metabolite, dihydrophaseic acid (DPA), remained unchanged. These results highlight the role of brassinosteroids in modulating ABA levels in plants, indicating a potentially antagonistic relationship between these two hormone types in the regulation of plant growth and development. In the soybean plants treated with ECS, salicylic acid (SA) level decreased and benzothiadiazole (BzA) level significantly increased, with no notable difference between the ECS concentrations used, aligning with previous studies that suggest a role for BRs and SA in the plant adaptation to abiotic stresses. ECS treatment led to decreased levels of both JA and JA-Ile in soybean leaves, indicating a potential antagonistic interaction between brassinosteroids (BRs) and jasmonic acid (JA) signaling pathways, particularly in balancing plant growth and resistance under optimal conditions. **Conclusions.** Our study reveals that exogenous ECS treatment significantly influenced hormone balance in soybean leaves, notably increasing BzA and IAA while decreasing SA, JA, and JA-Ile levels without affecting DPA and PAA. These findings highlight the intricate crosstalk between brassinosteroids (BRs) and other plant hormones, suggesting that ECS plays an important role in regulating plant growth, stress responses, and hormonal content.

Keywords: brassinosteroids, epicastasterone, phytohormones, soybean.

Large-scale metagenomic data analysis using annotated de Bruijn graphs

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Aim. Metagenomics has transformed the study of microbial communities, enabling direct genomes. In turn, advances in computational power lead to the accumulation of vast datasets with many unannotated sequences. De Bruijn graph-based solutions aim to address these challenges. In this work, we explore the Counting de Bruijn graphs (Karasikov *et al.*, 2022) for sequence discovery in metagenomics, presenting an algorithm for read extraction. Evaluating on viral graphs, we extracted the SARS-CoV-2 spike protein gene sequence, enabling evolutionary studies. We envision that an efficient read extraction query could popularize Counting de Bruijn graphs for sequencing data storage and scalable sequence search. **Methods.** Modern short-read sequencing produces DNA segments of 20 to 500 base pairs long. To efficiently analyze this data, reads are split into k-mers, substrings of equal length. The de Bruijn graph concept encodes these k-mers in a directed graph, where nodes represent k-mers and edges connect consecutive k-mers. Counting de Bruijn graphs provide lossless indexing storing k-mer positions in a separate data structure. MetaGraph (Karasikov *et al.*, 2022) is a scalable and modular framework that employs the concept of Counting de Bruijn graphs. The proposed algorithm, implemented as a query within MetaGraph, involves iterative local graph traversal by visiting a fixed number of nodes at once. The implementation is available at: GitHub link. We used the read extraction query to retrieve reads covering the spike protein gene of SARS-CoV-2 from a graph storing 152,884 viral samples. The reads were assembled into gene sequences for each target sample using hifiasm, then aligned using MAFFT. The final multiple sequence alignment view provides an interactive way to investigate the evolution of the virus. **Results.** The developed read extraction algorithm enables the reconstruction of read sequences from Counting de Bruijn graphs. It involves local graph traversal, allowing for flexible adjustment of the traversal parameters to accommodate different datasets. The benchmarking results on different graphs, detailed in (Horyslavets D., Sequence-read extraction from Counting de Bruijn graphs, M.S. Thesis, 2023), highlight the need for further advances in processing graphs with a large number of samples. **Conclusions.** This work presents a read extraction algorithm from Counting de Bruijn graphs, providing a useful tool for identifying sequences within the constructed graph index. This method streamlines the storage and retrieval of sequencing data, offering an alternative to searching raw sequences in public databases. By enabling the reconstruction of full reads from the graph index, this solution enhances the practicality of these graphs in metagenomics research, paving the way for ongoing improvements in graph-based queries.

K e y w o r d s: metagenomics, k-mer sets, de Bruijn graphs, sequence search, graph traversal, MetaGraph

Study of the cytotoxic effect of nanocomposite complex AIMP1/p43 with HP-β-CD on the MDA-MB-231 cell line

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Aim. At the current stage of medical development, it is necessary to create new stable, non-toxic targeted drugs based on recombinant proteins that achieve target cells and inhibit pathological processes. AIMP1, aminoacyl-tRNA synthetase multifunctional protein 1, also known as p43 and proEMAP-II, is a non-catalytic component of the mammalian multi-tRNA synthetase complex (MSC). AIMP1/p43 has both tRNA-binding and cytokine activities. The AIMP1/p43 protein is an effective anti-cancer agent in mouse xenograft models containing the gastric cancer cells. This protein is a precursor of endothelial monocyte-activating polypeptide EMAP II, which promotes proinflammatory and anti-cancer processes. The antitumor activity of EMAP II was shown on prostate adenocarcinoma, glioma cells, and breast cancer cells. Protein aggregation is an important issue in the development and commercialization of biotechnology products. The AIMP1/p43 protein has an unstructured central region (71-146 a.a.), which leads to instability and a tendency to aggregate. To stabilize AIMP1/p43, we used 2-hydroxypropyl-β-cyclodextrin (HP-β-CD). These compound can form inclusion complexes with various molecules or their fragments, placing them in an internal hydrophobic cavity. Binding to HP-β-CD stabilises the AIMP1/p43 protein by increasing the fluorescence shift temperature of Trp271 from 43 °C to 50 °C, indicating a more stable protein structure (Kolomiiets *et al.*, 2021). **Methods.** We studied the cytotoxic effect of the AIMP1/p43 protein and its nanocomposite complex with HP-β-CD (in a ratio of 1:10) on the MDA-MB-231 cell culture. The protein was expressed in *Escherichia coli* BL21(DE3)pLysE cells and purified by metal-chelating chromatography. The MDA-MB-231 cell line is commonly used to model advanced breast cancer. The cells were trypsinized, washed, counted (10,000/well), transferred to a 96-well plate with complete DMEM-F-12 medium, and placed in a CO_2-incubator for 24 hours. The cells were washed with PBS buffer, and then DMEM-F-12 medium without fetal serum was added to the samples and incubated for 18 hours. Later, the cells were treated with MTT solution for 3 hours. The extinction was measured by a digital spectrophotometer (μQuant, BioTEK, USA) at 540 nm. **Results.** The results demonstrate a decrease in cell viability under the influence of AIMP1/p43 protein and the AIMP1/p43 complex with HP-β-CD. All the doses we studied inhibited the development of malignant cells. The strongest effect of the cytokine and its complex was observed at a dose of 0.04 μM. At this concentration, AIMP1/p43 cell survival was 38.07 % compared to the control. Under the influence of the complex, the cell survival was 31.73 %. The obtained results indicate the preservation of cytotoxic properties of AIMP1/p43 in the nanocomposite complex. **Conclusions.** The creation of the AIMP1/p43 complex with HP-β-CD promotes protein stabilization and does not interfere with the biological properties of the protein. The nanocomposite complex is a promising tool that requires additional research to determine its future use for the treatment of cancer.

Keywords: AIMP1/p43, 2-hydroxypropyl-β-cyclodextrin, nanocomposite complex, MDA-MB-231 cell line, antitumor activity.

Nociceptive signaling in lamina X neurons: impact of capsaicin and tetrodotoxin

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Aim. Understanding the mechanisms underlying the nociceptive processing in the spinal cord is crucial for developing the effective pain management strategies. Lamina X is yet another poorly studied center of the nociceptive information processing. Here, we investigated the impact of capsaicin and tetrodotoxin (TTX) on lamina X spinal neuron activity, focusing on changes in the miniature excitatory postsynaptic currents (mEPSCs). **Methods.** Electrophysiological recordings were conducted on P12 rats using an ex-vivo intact spinal cord preparation. Lamina X cells were visualized by oblique infrared LED illumination with a Sutter Instrument SOM microscope, LUMPlanFL N 40×/0.80 water-immersion objective conjugated with a CCD camera (BFLY-U3-23S6C-C, Flir Co.). Patch clamp recordings were performed on L4-L5 spinal segments, with all neurons probed with 3–5 MΩ borosilicate glass pipettes pulled by a P-87 puller (Sutter Instruments, USA) filled with intracellular solution containing (in mM): 10 CsCl, 130 Cs-Methanesulfonate, 4 MgATP, 0.4 NaGTP, 10 HEPES, 5 2Na-Phosphocreatine, 5 EGTA (pH 7.3). The spinal cord was continuously perfused with Krebs extracellular solution bubbled with 95/5 % O_2/CO_2. The signals were acquired and Bessel filtered at 2.6 kHz with a MultiClamp 700B amplifier under the control of pClamp 11.3 software (Molecular Devices, USA). For experiments involving the application of capsaicin and TTX, TTX was added to the perfusion solution to a final concentration of 0.5 μM, while capsaicin was added to a final concentration of 1 μM. **Results.** The changes in the amplitude and frequency of mEPSCs in lamina X spinal cord neurons were analyzed following the application of capsaicin in the presence of TTX (a blocker of voltage-gated Na+ channels). After baseline recordings (~10 min) and TTX addition, a decrease in the frequency and amplitude of mEPSCs was observed, indicating the suppression of spontaneous postsynaptic events, with only miniature postsynaptic events being observed. Subsequent addition of capsaicin, a TRPV1 receptor agonist, increased the frequency and amplitude of these miniature postsynaptic events. In 40 % of tested neurons, capsaicin application resulted in a significant increase in the amplitude and frequency of miniature events, while in the remaining 60 % of neurons, capsaicin affected mEPSCs to a lesser extent. After subsequent washout with a capsaicin-free solution, no significant differences were observed. **Conclusions.** The results of this study suggest that the activation of type 1 vanilloid receptors influences the activity of lamina X neurons, possibly through alterations in the neurotransmitter release from terminals of primary sensory neurons terminating on the neurons within this lamina. These results demonstrate that some lamina X neurons receive direct inputs from primary afferents and participate in the processing of nociceptive information.

Keywords: lamina X, spinal cord, nociception, primary afferents, miniature postsynaptic activity.

Analysis of ITSN-1 and SYNJ-1 proteins' interactions with components of the SUMOylation system

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Aim. Intersectin 1 (ITSN-1) is a scaffold protein implicated in several cellular mechanisms, including membrane trafficking and receptor-dependent signaling. One of the central partners of ITSN1 in the process of forming clathrin-coated vesicles is synaptojanin-1 (SYNJ-1), which simultaneously belongs to the family of proteins with phosphatase activity and RNA-binding proteins. Both proteins are closely involved in the development of neurodegenerative pathologies, such as early-onset hereditary Parkinson's disease, tauopathies, and Down syndrome. Additionally, the ITSN1 and SYNJ1 genes are located in the same cluster: the critical region of the Down syndrome (DSCR) on chromosome 21. Previously, ITSN1 demonstrated the ability to form conjugates with the small ubiquitin like modifier 3 protein (SUMO3), and ligases Ubc9 and PIAS2 *in vitro*. Considering the specific role SUMO proteins play in intracellular transport and their impact on pathological processes in neurons, it is important to investigate the interactions between components of the SUMOylation system, intersectins, and their protein partners. **Methods.** Co-immunoprecipitation of ITSN1 and SUMO proteins was conducted using mouse brain lysates and the neuroblastoma-derived SH-SY5Y cell line lysates. Detection of ITSN1 conjugates with SUMO proteins was performed using anti-SUMO1 and anti-SUMO2/3 antibodies. Mouse IgG was used as a negative control. Co-immunoprecipitation of SYNJ-1 was performed on 293 cells cotransfected with plasmids encoding Flag-tagged UBC9 and GFP-Flag-SYNJ-1 construct. Detection of SYNJ-1 conjugates with SUMO proteins was performed using anti-SUMO1 and anti-SUMO2/3 antibodies. **Results.** The results of precipitation demonstrated that ITSN-1 forms conjugates with SUMO2/3 proteins both *in vivo* and *in vitro*, which is important, as SUMO-1 and SUMO-2/3 modifiers usually have different substrate specificities. It is also worth noting that only SUMO-2/3 modifiers are capable of forming poly-chains through a linkage via the N-terminal lysine, a characteristic not inherent to SUMO-1. SYNJ-1, in turn, showed the ability to form conjugates with both SUMO-1 and SUMO-2/3 proteins. **Conclusions.** The findings of this study demonstrate that SUMO-2/3 is likely to be the primary SUMO-modifying protein partner for ITSN-1, both *in vivo* and *in vitro*. Additionally, SYNJ-1 is confirmed to undergo SUMOylation *in vitro*. While further *in vivo* studies are required to elucidate the exact role wich SUMOylation plays for SYNJ-1, particularly how it is connected to the protein's primary lipid phosphatase activity, there is a possibility of interplay between SUMOylation and the endocytic functions of both ITSN-1 and SYNJ-1, which could become a subject for further investigation.

Keywords: SUMOylation, ITSN1, SYNJ1, PTM, PPI.

Our experience in the use of autologous platelet concentrates in traumatology and orthopedics

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Aim. The goal of our work was the development of various types of autologous platelet concentrates and their classification. **Methods.** The following methods were used to isolate autologous platelet concentrates:
– Centrifugation (the main method for selecting platelet concentrates),
– A combination of the following modes was used, depending on the required number and ratio of cells to be obtained (1 — at 250 g for 10 min., 2 — at 2300 g for 5 min.),
– Mechanical methods,
– Cryopreparation.
Results. Our research demonstrates that PRP is not a single, stand-alone product. PRP is a group of autologous platelet concentrates unified by certain stages of manufacturing technology. In addition to the manufacturing technology, the final product is influenced by the personalized characteristics of the patient's condition at the time of biological material collection.
Conclusions. The classification of autologous platelet concentrates based on the number of platelets in their composition has been proposed:
– platelet-poor plasma (the number of platelets in the product is lower than the initial level in the patient's blood.);
– platelet-rich plasma of the plasma type (the number of platelets in the biotechnological product is higher than the initial concentration but less than 1 million per 1 μl);
– platelet-rich plasma (the number of platelets in biotechnological products is 3–4 times higher than the initial concentration and more than 1 million per 1 μl);
– concentrated platelets rich plasma (the number of platelets in the biotechnological product is 5–10 times higher than the initial concentration and is 3–10 million per 1 μl);
– high density platelet-rich plasma (the number of platelets in the biotechnological product exceeds the original by more than 10 times and is more than 10 million per 1 μl).

By cell ratio:
– leukocyte platelet-rich plasma (leukocyte concentration in platelet-enriched plasma is more than 2×10^9/l);
– poor-leukocyte platelet-rich plasma (leukocyte concentration in platelet-enriched plasma is less than 2×10^9/l);
– platelet cryolysate (a cell-free product based on platelet-enriched plasma).

A dosed autologous platelet concentrate was developed based on the cryolysate of platelets. Recommendations have been developed for the use of various types of autologous platelet concentrates in the pathology of the musculoskeletal system.

Keywords: platelet concentrates, composition, traumatology, orthopedics.

Development and probation of the amperometric biosensor for determining the AST activity level in blood serum samples

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Aim. The most common cause of death worldwide is cardiovascular disease, such as coronary heart disease, acute myocardial infarction, and stroke. These diseases are often chronic, requiring long-term monitoring of the patient's condition. One widely used biomarker of myocardial damage is the level of aspartate aminotransferase (AST) in the blood, which remains constant in a healthy person and significantly increases in case of myocardial damage. Currently, AST levels are primarily determined using a spectrophotometer, which hinders self-monitoring and rapid analysis. Thus, the development of biosensor methods for measuring AST levels is highly relevant, as the biosensors can address these limitations. **Methods.** The amperometric system was designed with a three-electrode measurement scheme using platinum disc working electrodes as electrochemical transducers. Bioselective element was created by covalent cross-linking immobilization of glutamate oxidase. An additional semipermeable membrane for increasing selectivity was formed by electropolymerizing of m-phenylenediamine. The AST detection was performed by dynamic measurement of the concentration of the AST reaction product (glutamate). The "calibration curve" and the "standard additions" methods were tested for AST detection in blood serum samples. Spectrophotometry was used as a reference method of analysis. **Results.** The method for forming a bioselective element was created and optimized. The working buffer parameters for AST functionality were optimized, specifically analyzing the influence of substrate (aspartate, α-ketoglutarate) and coenzyme (PLP) concentrations. The analytical characteristics of the developed biosensor, its stability, and its selectivity towards various interferents were investigated. Different methods for determining AST levels in blood samples with known concentrations (both normal and elevated) were compared, demonstrating that the "calibration curve" method provides more accurate results. The blood serum samples with unknown concentrations were analyzed using both the biosensor and spectrophotometric analysis, showing a high level of correlation between the two methods. **Conclusions.** A biosensor for determining AST activity in serum and a protocol for conducting research on real blood samples using the developed biosensor have been created. The high correlation between the biosensor results and the reference spectrophotometric method indicates the potential of this biosensor for use in clinical practice.

Keywords: amperometric, aspartate aminotransferase, biosensor, glutamate oxidase, liver test.

Investigation of intranasal administration of peptides to mice brain

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Introduction. Delivery of drugs to the brain is a complicated task due to the presence of the blood-brain barrier (BBB). Intranasal delivery allows bypassing the blood-brain barrier, has fewer systemic and side effects than oral and intravenous administration, and is non-invasive. For relatively large therapeutic agents that cannot cross the blood-brain barrier, such as peptides, this method is the most prospective choice. It is particularly important for the peptides targeting proteins causing neurodegenerative diseases such as Alzheimer's and Parkinson's. **Aim.** Our ultimate goal is intranasal delivery of α-synuclein-derived peptides to suppress Parkinson's disease progression. This work aims to investigate the effect of the amino acid sequence of peptides and their concentration on the efficiency of delivery by intranasal administration, the effect of additives on the delivery, as well as the pharmacodynamics of peptides. **Methods.** A set of 5 peptides of 14–25 amino acids was synthesized using standard solid-phase peptide synthesis (IOCB Prague) and covalently labeled with 5(6)-carboxyfluorescein at the N-terminus. C57Bl/6j mice were used. A 100-1000 µM solution of the peptide in a neutral buffer was injected into the nostrils of mice three times, 5 µl per nostril. After a specified time after injection, mice were decapitated, and brains were homogenized. The homogenate was centrifuged, and the concentration of peptides in the supernatant was measured based on the fluorescence intensity of fluorescein. The animals were kept, fed, and euthanized in accordance with the European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes (Strasbourg, 1986). **Results.** The best delivery was observed for the 14-amino-acid cationic (+4) peptide. The maximum concentration was reached 15–60 minutes after injection. The addition of β-cyclodextrin and microcrystalline cellulose improves the intranasal delivery of peptides by 60 % and 100 %, respectively. **Conclusions.** Small linear cationic peptides can be efficiently delivered to the brains of mice, reaching a final concentration in brain tissue of about 100–300 nM. We were able to achieve concentrations of the inhibitor peptide in the mouse brain in the range of 500 nM to 1 mM, which is correlated with its IC50 values *in vitro* experiments on inhibition of α-synuclein aggregation (about 500 nM). The percentage of delivered peptide decreases markedly with the concentration of the solution used for injection, which makes it impractical to use concentrations above 100 µM.

Keywords: blood-brain barrier (BBB), intranasal delivery, α-synuclein-derived peptides, neurodegenerative diseases, peptide synthesis, pharmacodynamics

The post-transfer editing of non-cognate amino acids by human cytoplasmic leucyl-tRNA synthetase

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Aim. Aminoacyl-tRNA synthetases can mischarge cognate tRNA with non-proteinogenic amino acids, which are toxic to cells. This study aims to investigate the post-transfer editing mechanism of human cytoplasmic leucyl-tRNA synthetase (LeuRS) against non-canonical amino acids. Using molecular modeling, molecular dynamic simulation, and quantum mechanical calculations, the involvement of Thr293, Thr390, and Asp399 in the hydrolysis of mis-acylated tRNA was discovered. Therefore, this research considers the deacylation of Ile-tRNALeuHS by editing-deficient mutant forms of hsLeuRS. **Methods.** 14[C]-Ile-tRNALeuHS was obtained under the following reaction conditions: 100 mM NA-HEPES 7.4, 15 mM KCl, 12 mM MgCl2, 2 mM DTT, 20 ug/ml BSA, 4 mM ATP, 10 uM tRNALeuHS, 75 uM 14[C]-Ile and 3 uM D399A. The aminoacylation reaction was performed at +37 °C for 20 min, and then stopped by adding 300 mM NaAc 5.02, followed by phenol purification and ethanol precipitation. The resulting 14[C]-Ile-tRNALeu was resuspended in 20 mM NaAc 5.02. The deacylation reaction was conducted under identical conditions (Na-HEPES, KCl, MgCl2, DTT, BSA, temperature) in the presence of 2.15 uM 14[C]-Ile-tRNALeu. The enzyme concentrations varied according to the aim of experiment. The reaction was quenched after specific intervals by spotting 10 μl onto trichloroacetic acid pre-soaked GF/C filters, followed by washing twice with 5 % TCA, drying, and quantifying by liquid scintillation counting. Each assay was conducted in triplicate, and results are presented as mean ± SD. The data analysis was performed using Origin 2017 software. **Results.** After 10 min incubation, the levels of remaining 14[C]-Ile-tRNALeu were 77 %, 31 %, 110 %, 36 %, 39 % in reactions without enzyme and in the presence of 25 nM WT, 250 nM D399A, 25 nM T390A and 100 nM T293A respectively. Kobs values were 0.276±0.05 s-1 for WT, 0.31±0.09 s-1 for T390A and 0.058±0.007 s-1 for T293A. Hence, the deacylation occurs 4.7 times slower for T293A LeuRS compared to WT, while T390A LeuRS hydrolyzes mischarged tRNA similarly to WT. **Conclusions.** These results indicate that the threonine residue at position 293 is not involved in post-transfer editing by human cytoplasmic LeuRS. On the other hand, even a 10-fold excess of D399A LeuRS does not cause hydrolysis of Ile-tRNALeu, suggesting that aspartic acid residue at position 399 plays a key role in the editing process.

Keywords: aminoacyl-tRNA synthetase, human leucyl-tRNA synthetase, deacylation, editing mechanism of aaRS.

Surface plasmon resonance detection of the sequence of Philadelphia chromosome oligonucleotides with nanoparticle-mediated signal enhancement

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Aim. The aim of this research was to demonstrate the fundamental possibility of enhancing the SPR signal using gold nanoparticles modified with probe oligonucleotides and surface blocking molecules for the detection of DNA sequences of the Philadelphia chromosome. **Methods.** One of the characteristic genetic markers of chronic myeloid leukemia is the hybrid gene BCR-ABL of the Philadelphia chromosome. To investigate the processes of immobilization and hybridization of its oligonucleotides, we used the two-channel SPR spectrometer "Plasmon SPR6" developed at the V.E.Lashkaryov Institute of Semiconductor Physics of the NAS of Ukraine. In accordance with the aim of the study, an 80 base pair long oligonucleotide from the e13a2 junction site of the Philadelphia chromosome was used as a target molecule; in this study, it is referred to as 80-mer BCR-ABL. The biosensor system employed for the detection of the target molecules consisted of two parts: the SPR sensor surface modified with probe oligonucleotides (mod-Ph) complementary to a 24 base pair region of the 80-mer BCR-ABL and gold nanoparticles (AuNPs) modified with a second DNA probe (SH-DP) complementary to an 18 nitrogenous base region of the 80-mer BCR-ABL target. For the hybridization of the target oligonucleotide with immobilized probes, the solution of the target sequence at various concentrations in the 2×SSC buffer was injected into the measuring flow cell and exposed for 10 minutes. **Results.** Compared to the biosensor without AuNPs, the use of the proposed hybridization biosensor system allowed the determination of the concentration of 80-mer BCR-ABL in the studied solution with much higher sensitivity and a much lower detection limit. The detection limit for measuring the concentration of 80-mer BCR-ABL with the proposed biosensor system was 100 pM, which is 500 times lower than that of the known biosensor [1]. The sensitivity of the system is 1201×10^{-6} ang. deg./nM, which is 6 times better than that of the known biosensor [1]. **Conclusions.** The linear operating range of the proposed system was shifted to low concentrations by two orders of magnitude, making it more promising for the analysis of real biological samples.

Keywords: Surface plasmon resonance, DNA hybridization biosensors, gold nanoparticles.

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The therapeutic effect of new non-nucleoside MGMT inhibitors during combined alkylating chemotherapy in vivo

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Aim. O6-methylguanine-DNA methyltransferase (MGMT) is an enzyme involved in repairing alkyl lesions in DNA, which occur during the alkylating chemotherapy in cancer cells. To enhance the efficacy of alkylating chemotherapy, MGMT inhibitors are administered in combination with the alkylating agents. The previous research has shown the promising attributes of novel non-nucleoside MGMT inhibitors, characterized by low cytotoxicity and high effectiveness *in vitro*. This study aims to assess the therapeutic potential of these inhibitors in the combination therapy using *in vivo* models. **Methods.** The aged female ICR mice that spontaneously developed tumors served as an *in vivo* model for investigating complex antitumor therapy. This included new MGMT inhibitors along with the alkylating agent N-methyl-N′-nitro-N-nitrosoguanidine (MNNG). The control groups consisted of mice treated solely with inhibitors, MNNG alone, and untreated mice. The tumor size changes were monitored dynamically, while Western blot analysis was employed to assess the post-treatment alterations in MGMT, and Caspases 3 and 9 protein levels. **Results.** In the MNNG control group, tumor growth decreased, whereas the MGMT inhibitors control group showed no change in the tumor size compared to untreated mice tumors. The combined treatment with 41 and 41B inhibitors resulted in a notable reduction in growth compared to the MNNG control group, while the 89 inhibitor treatment led to the complete tumor remission by day 17 of therapy. According to Western blot analysis, the combined therapy demonstrated a significant reduction in MGMT protein level compared with the MNNG control group, where alkylator treatment induced MGMT expression. Additionally, post-combination-treated tumors exhibited cleaved caspase 3, indicative of apoptotic pathway activation for tumor degradation, whereas control tumors only displayed inactive caspase 3. Levels of caspase 9 did not significantly differ between intact and treated tissues. **Conclusions.** The three newly analyzed non-nucleoside inhibitors enhance the therapeutic effect of the alkylating agent N-methyl-N′-nitro-N-nitrosoguanidine *in vivo*. The combined therapy resulted in the tumor growth reduction or remission. The obtained data indicate a decrease in the MGMT protein levels in tumor tissues and suggest an apoptotic mechanism for tumor degradation under the combined alkylating treatment.

Keywords: MGMT, inhibitors, alkylating chemotherapy, tumor reduction, ICR mice.

Descending control of dorsal horn neurons by rostral ventromedial medulla

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Aim. The rostral ventromedial medulla (RVM) is considered the most important relay station in the descending pain control pathway [1]. Located in the brainstem, RVM receives top-down projections from a number of encephalic regions and itself contains several classes of neurons projecting downward into the spinal cord, specifically into the grey matter of the dorsal horn [2]. The exact roles of the RVM projections to the dorsal horn in acute and chronic pain remain unclear. To study the mechanisms of this descending control pathway with the spinal cord neural network intact, an optogenetic approach was used. **Methods.** Adult mice were fixated in the stereotaxic frame, and AAV9-YFP-ChR2 vector was injected into the RVM. After 4–8 weeks, animals were sacrificed, and lamina I cells of the lumbar segments were studied using the whole-cell patch-clamp technique in the ex-vivo spinal cord preparation. Dorsal roots were stimulated at saturating current (150 uA, 1 ms) via a suction electrode [3]. Continuous 5 Hz train of 10 ms monochrome light (480 nm) was used to locally stimulate ChR2-expressing RVM axons through the microscope objective. After patch-clamp recordings, both spinal cords and brains were fixed and examined under a confocal microscope for post-factum verification of the correct injection site and resulting YFP expression. **Results.** Injection of AAV9-YFP-ChR2 vector into the RVM resulted in strong YFP expression in the axons within the dorsolateral funiculus and grey matter of the spinal cord, particularly in laminae I and X. In 4 out of 6 cells with excitatory post-synaptic currents (EPSCs) evoked by dorsal root stimulation, simultaneous train of 5Hz RVM axons stimulation resulted in a decrease of EPSCs integrals by 15–31 % ($p < 0.05$). In 2 of these cells, a decrease of monosynaptic EPSCs amplitudes was observed, indicating presynaptic inhibition as a possible mechanism of descending control. Primary-afferent-driven polysynaptic inhibitory currents were also decreased after optical stimulation (2 cells, $p<0.05$), similarly to cells with EPSCs. Additionally, one cell was also found to receive mono- and polysynaptic inhibitory inputs from RVM axons. **Conclusions.** This study reveals complex and diverse descending control of dorsal horn neurons by RVM that involves both pre- and postsynaptic mechanisms.

Keywords: presynaptic mechanisms, postsynaptic mechanisms, dorsal horn neurons.

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