

1. *Ivanov S.M., Lagunin A.A., Zakharov A.V., Filimonov D.A., Poroikov V.V.*

Computer search for molecular mechanisms of ulcerogenic action of nonsteroidal antiinflammatory drugs.

Peptic ulcers is the most frequent side effect of non-steroidal anti-inflammatory drugs (NSAIDs). Experimental data indicate that pathogenesis of peptic ulcers cannot be explained only by the inhibition of cyclooxygenases. The knowledge about other molecular mechanisms of action of drugs related with development of peptic ulcers could be useful for design of new safe NSAIDs. However, considerable time and material resources are needed for corresponding experimental research. For simplification of experimental search, we have developed an approach for in silico identification of probable molecular mechanisms of action of drugs related with its side effects. We have created the set of NSAIDs containing 85 substances with data about structures and side effects. The computer program PASS (Prediction of Activity Spectra for Substances) predicting more than 3000 molecular mechanisms of action based on structural formula of substances was used to estimate unknown molecular mechanisms of action for these set of NSAIDs. Statistically significant relationships between predicted molecular mechanisms of action and development of peptic ulcers have been established. We have discovered twenty-six molecular mechanisms of action (two known previously and twenty-four new) which probably related with development of peptic ulcers. By analyzing of Gene Ontology data, signal and metabolic pathways, publications in Medline, we formulated hypotheses about the role of ten molecular mechanisms of action in pathogenesis of peptic ulcer.

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2. *Gnedenko O.V., Ivanov A.S., Yablokov E.O., Usanov S.A., Mukha D.V., Sergeev G.V., Kuzikov A.V., Bulko T.V., Moskaleva N.E., Shumyantseva V.V., Archakov A.I.*

Protein-protein interactions of cytochromes P450 3A4 and 3A5 with their intermediate redox partners cytochromes.

Molecular interactions between proteins redox partners (cytochromes P450 3A4, 3A5 and cytochrome b) within the monooxygenase system, which is known to be involved in drug biotransformation, were investigated. Human cytochromes P450 3A4 and 3A5 (CYP3A4 and CYP3A5) form complexes with various cytochromes b: the microsomal (b5mc) and mitochondrial (b5om) forms of this protein, as well as with 2-hemichimeric proteins, b5(om-mc), b5(mc-om). Kinetic constants and equilibrium dissociation constants were determined by the SPR biosensor. Essential distinction between CYP3A4 and CYP3A5 was only observed upon their interactions with cytochrome b5om. Electroanalytical characteristics of electrodes with immobilized hemoproteins were obtained. The electrochemical analysis of CYP3A4, CYP3A5, b5mc, b5om, b5(om-mc), and b5(mc-om) immobilized on screen printed graphite electrodes modified with membranous matrix revealed that these proteins have very close reduction potentials -0.435- -0.350 V (vs. Ag/AgCl). Cytochrome b5mc was shown to be capable of stimulating the electrocatalytic activity of CYP3A4 to testosterone.

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3. *Ivanov Yu.D., Danichev V.V., Pleshakova T.O., Shumov I.D., Ziborov V.S., Krokhin N.V., Zagumenniy M.N., Ustinov V.S., Smirnov L.P., Shironin A.V., Archakov A.I.*

Irreversible chemical AFM-fishing for the detection of low-copied proteins.

The atomic-force microscopy-based method of irreversible chemical AFM-fishing (AFM-IF) has been developed for the detection of proteins at ultra-low concentrations in solution. Using this method, a very low concentration of horseradish peroxidase (HRP) protein (10⁻¹⁷ M) was detected in solution. A theoretical model that allows the description of obtained experimental data, is proposed. This model takes into consideration both the transport of the protein from the bulk solution onto the AFM-chip surface and its irreversible binding to the activated area.

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4. *Kolesanova E.F., Farafonova T.E., Aleshina E.Yu., Pyndyk N.V., Veremieva M.V., Novosylina O.V., Kovalenko M.I., Shalak V.F., Negrutskii B.S.*

Preparation of monospecific antibodies against isoform 2 of translation elongation factor 1A (eEF1A2).

Amino acid sequences of eukaryotic translation elongation factor isoform 1 (eEF1A1) and 2 (eEF1A2) were compared and two peptide fragments of eEF1A2 were chosen as linear antigenic determinants for generation of monospecific antipeptide antibodies. Selected peptides were synthesized, conjugated to bovine serum albumin (BSA) and used for mice immunizations. Antibodies, produced against the eEF1A2 fragment 330-343 conjugated to BSA, specifically recognized this isoform in the native and partially denatured states but did not interact with the eEF1A1 isoform. It was shown that these monospecific anti-eEF1A2 antibodies could be employed for eEF1A2 detection both by enzyme-linked immunosorbent assay and by immunoblotting.

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5. *Sirota T.V., Zakharchenko M.V., Kondrashova M.N.*

Cytoplasmic superoxide dismutase activity is a sensitive indicator of the antioxidant status of the rat liver and brain.

Several parameters of the cytoplasmic enzymatic antioxidant system of the liver and brain of the rat have been investigated under conditions of immobilization stress and of an antioxidant preparation in the diet of animals. These included superoxide dismutase (SOD) and glutathione reductase (GR) activities and nonspecific NADPH oxidation. Only changes in the activity of SOD both in the liver and brain were revealed. In the liver of animals that receive no preparation, a decrease in the activity of SOD after 30-min immobilization and its restoration after a 360-min immobilization were

observed. In the brain, the activity of SOD decreased only in preconditioned animals after 30 and 360 min of exposure to stress. In addition, the activity of SOD in the brain of preconditioned animals, both stressed and unstressed, was lower than in the corresponding groups of control animals. It is probable that, under the conditions of immobilization stress, the level of reactive oxygen species (ROS) and as a consequence the activity of SOD decrease. The intake of an antioxidant preparation under these conditions seems to be not correct.

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6. Aisina R.B., Mukhametova L.I., Ostryakova E.V., Seredavkina N.V., Patrushev L.I., Patrusheva N.L., Reshetnyak T.M., Gulin D.A., Gershkovich K.B., Nasonov E.L., Varfolomeyev S.D.

Polymorphism of the plasminogen activator inhibitor type 1 gene, plasminogen level and thrombosis in patients with antiphospholipid syndrome.

The frequency of venous and arterial thromboses and plasminogen level were investigated in 78 patients with antiphospholipid syndrome (APS), 35 of whom with systemic lupus erythematosus (SLE+APS) and 43 - with primary APS (PAPS). The levels and genotype of plasminogen activator inhibitor type 1 (PAI-1) were determined in 45 patients with APS, of whom 21 patients with SLE+APS and 24 patients with PAPS. A control group included 10 healthy individuals without autoimmune disease signs and thromboses on period of investigation and in past history. It was shown for the first time that for one third of 67 patients with APS and thromboses high positive levels of antiphospholipid antibodies (aPL) are associated with low plasminogen levels. The levels of PAI-1 antigen measured by ELIZA method, which detects active, latent and bound with plasminogen activator PAI-1, were opposed with frequency of thromboses in APS patients. Correlation between the high and increased levels of PAI-1 and high positive aPL levels was found for one third of 43 patients with APS and thrombosis. One of the possible mechanisms of this interconnection was considered. It was shown that arterial and, to a more extent, venous thromboses are associated with the 4G/5G polymorphism of PAI-1 gene and high plasma level of the inhibitor in 79% of APS patients. At the presence of the 4G allele patients with SLE+APS had higher PAI-1 levels than patients with PAPS. The obtained results show that measuring the levels of plasminogen and PAI-1 as well as the 4G/5G polymorphism of PAI-1 gene which is associated with thromboses may have the practical significance for identification of high risk of thrombosis in APS patients.

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7. Khaspekova S.G., Zyuryaev I.T., Yakushkin V.V., Naimushin Ya.A., Sirotkina O.V., Zaytseva N.O., Ruda M.Ya., Mazurov A.V.

Mean platelet volume: interactions with platelet aggregation activity and glycoprotein IIb-IIIa and Ib expression levels.

Increased mean platelet volume (MPV) is an independent risk factor of thrombotic events in patients with cardiovascular diseases. Interactions of MPV with platelet aggregation activity and contents of glycoprotein (GP) IIb-IIIa (allb/b3 integrin, fibrinogen receptor) and GP Ib (von Willebrand factor receptor) were investigated in this study. Investigation was performed in a group of healthy volunteers (n = 38) and in a group of patients with acute coronary syndrome (ACS). In patients blood was collected at days 1, 3-5 and 8-12 after ACS development. As an antiaggregant therapy all patients received acetylsalicylic acid (ASA, inhibitor of thromboxane A2 synthesis) and most of them – clopidogrel (ADP receptor antagonist) with the exception of part of the patients (n=44) at day 1 who had not taken clopidogrel before first blood collection. In volunteers platelet aggregation was stimulated by 1.25, 2.5, 5 and 20 M ADP, and in patients – by 5 and 20 M ADP. GP IIb-IIIa and GP Ib content on platelet surface was measured using 125I-labelled monoclonal antibodies. GP IIb-IIIa and GP Ib genetic polymorphisms were determined in ACS patients. In healthy donors significant correlations between MPV and aggregation levels were revealed at 1.25 and 2.5 M ADP (coefficients of correlation (r) - 0.396 and 0.373, p<0.05) and at 5 and 20 those interactions did not reach significant level (r - 0.279 and 0.205, p>0.05). Correlations between MPV and aggregation levels were observed at day 1 of ACS in a subgroup of patients who received ASA but had not started clopidogrel treatment (r - 0.526, p<0.01 and 0.368, p<0.05 for 5 and 20 M ADP respectively). Interactions between these parameters were not registered upon combined treatment with ASA and clopidogrel. Strong direct correlations between MPV and GP IIb-IIIa and GP Ib contents were detected in healthy donors and ACS patients (at all time points) – r from 0.439 to 0.647 (p<0.001 for all correlations). Genetic polymorphisms of GP IIb-IIIa (GP IIIa Leu33Pro) and GP Ib ((-5)T/C (Kozak) and Thr145Met) identified in ACS patients did not affect expression levels of corresponding glycoproteins. The data obtained indicated that increased MPV values correlate with increased platelet aggregation activity and enhanced GP IIb-IIIa and GP Ib expression.

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8. Ulanova T.S., Gileva O.V., Stenno E.V., Veikhman G.A.

Peculiarities of vanadium determination in whole blood by icp-ms.

The parameters of vanadium determination by ICP-MS in whole blood are presented. Conditions for blood sample preparation to reduce measure errors and to determine vanadium at the reference concentration level were optimized. The accuracy of the results is confirmed by analysis of standard blood samples Seronorm L1, L2 and L3. Vanadium mean in whole blood for the group of children from the town of Chusovoy (n=80) was $1.29 \pm 0.45 \text{ } \mu\text{g/L}$, and vanadium mean for grown-ups from the town of Chusovoy was $1.63 \pm 0.25 \text{ } \mu\text{g/L}$.

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9. Tsybulsky A.V., Popov A.M., Artyukov A.A., Krivoschapko O.N., Kozlovskaya E.P., Bogdanovich L.N., Krijanovsky S.P., Blinov Yu.G.

The effects of preparation "Gistochrom" in biochemical parameters of blood for patients with cardiopathologies.

The effects of the small doses of the preparation Gistochrom, containing natural polyhydroxynaphtoquinone echinochrom A from flat sea urchin *Scaphechinus mirabilis*, on blood biochemical parameters have been studied in patients with cardiovascular diseases. Gistochrom administration influenced the LPO-antioxidant protection system, indicating reinforcement of antioxidant protection mechanisms. Gistochrom modulated the immune status and the plasma cytokine profile. Thus, Gistochrom may be recommended as means of additional therapy for patients with cardiovascular diseases for correcting the metabolic, immunological and redox impairments.

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Advances of selenium supplementation in posttraumatic stress disorder risk group patients.

Posttraumatic stress disorder (PTSD) is a complex of symptoms developed in a patient after traumatic event. The basis of PTSD pathophysiology is hyper activation of neurones under stress factors influence, so-called excitotoxicity, followed by oxidative stress (OS) because of an accumulation of free radicals. Lipid peroxidation can lead to neurons damage. Neurons are especially susceptible to OS, changing signal transduction and information processing mechanisms. Clinically excitotoxicity preforms as different acute and/or chronic stress reactions and can cause PTSD. Selenium (Se) is involved on different stages of transport and metabolism of Glutamate. Research aim: to access PTSD incidence, OS parameters and their adjustment advances using organic Se in PTSD risk group patients. PTSD symptomatic severity (in PCL-M points) reduced for 5.85% to baseline, Prevalence Rate reduced for 46.03% to baseline in Se group patients. We can conclude that: 1) there is a statistically reliable correlations between the incidence of PTSD and OS parameters, between PTSD symptomatic severity and OS parameters; 2) the use of Se during the mission can reduce the OS parameters, minimize the incidence of PTSD and reduce the PTSD symptomatic severity.

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