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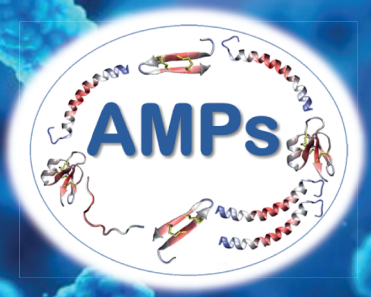
“Antimicrobial peptides as prototypes of novel antibiotics”



21 July 2022
Saint Petersburg
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ALMAZOV National Medical Research Centre

World-Class Research Centre for Personalized Medicine

INSTITUTE OF EXPERIMENTAL MEDICINE 1890 IEM

IInd International Seminar "Antimicrobial peptides as prototypes of novel antibiotics"

21 July 2022

World-Class Research Centre for Personalized Medicine Institute of Experimental Medicine, Saint Petersburg

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The Seminar is holding in the frame of realization of the project "Infection diseases and antimicrobial therapy" at the World-Class Research Centre for Personalized Medicine (with the financial support of the Ministry of Science and Higher Education of the Russian Federation, Agreement №075-15-2020-902).

The Seminar is devoted to the prospects of developing novel antibiotics on the base of antimicrobial peptides (AMPs) as tools for combating multidrug resistant bacteria causing severe hospital infections including those arising during or after COVID-19. Varied structural classes of AMPs are considering as templates for creation of such potent drugs (alpha-helical, beta-hairpin, proline-rich peptides), the pros and cons of these substances will be discussed. An application of AMPs in combination with conventional antibiotics as well as with metal nanoparticles also will be under consideration. Other types of the biological activity of AMPs will be discussed: immunomodulatory activity, interaction of AMPs (mostly beta-hairpin) with the complement system.

The main aim of the seminar is finding new ways for scientific collaboration.

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ABSTRACTS

SYNTHETIC ANALOGUES OF ANIMALS' ANTIMICROBIAL PEPTIDES ARE PROMISING ANTI-INFECTIVE TOOLS AGAINST DRUG-RESISTANT BACTERIA

Shamova O. V., Zharkova M. S., Sukchareva M. S., Vladimirova E. V., Komlev A. S., Protasov E. A., Khaydukova M. M., Filatenkova T. A., Goncharov A. E., Chernov A. N., Klimov N. A., Korneva E. A., Krenev I. A., Berlov M. N., Orlov D. S.

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Introduction. Bacterial resistance for conventional antibiotics is rapidly growing pointing to the urgent need for creation of alternative antimicrobials with principally new mode of antibacterial action. Natural antimicrobial peptides (AMPs) of the innate immune system and their synthetic analogous are considering as such novel antibiotics due to their unique multi-targeted way for killing bacteria. In contrast to the common antibiotics, AMPs in addition to their antimicrobial activity, possess an array of varied defensive features.

The main **purpose** of our study is developing the prototypes of new antibiotics based on synthetic analogues of natural AMPs for the treatment of severe infections caused by antibiotic-resistant bacteria.

Methods. An array of synthetic peptides has been chemically synthesized and their antimicrobial activity has been explored using broth microdilution assay and other approaches; cytotoxicity for the host cell also was examined.

Results. It was shown that the synthetic analogues of natural AMPs (proline-rich bactenecin 3.4, cathelicidin buCATHL4D, beta-hairpin peptide arenicin 1 and protegrin 1) exert potent antimicrobial activity against multi-drug resistant (including those resistant to carbapenem) strains of *Klebsiella pneumoniae* and *Acinetobacter baumannii* isolated from flushes obtained after bronchoalveolar lavage of patients hospitalized for COVID-19 treatment. Minimal inhibitory concentrations (MICs) were in a range of 0.1-2 microM). These peptides also demonstrated a high antimicrobial activity against clinical isolates of bacteria from infected wounds of patients (*K. pneumoniae, A. baumannii, Stenotrophomonas maltophilia*), MICs varied from 0.1 to 16 microM. An ability of AMPs to prevent the bacterial biofilm formation has been studied, we have shown that some

peptides in sub-microbicidal concentrations effectively prevented forming of biofilms by multi-drug resistant *P. aeruginosa* MDR 522/17 and *A. baumannii* 7226/16. Beta-hairpin AMPs manifested the most significant antibacterial activity. But when the cytotoxic activity of AMPs towards host cells (human erythrocytes, PBMC, cultured cells normal human fibroblasts, EA.hy926, A549, K-562) was examined *in vitro*, it turned out that some beta-hairpin peptides are toxic to the studied cells. At the same time, certain bactenecin analogues exhibited insufficient cytotoxic activity against human cells *in vitro*. Considering that these AMPs demonstrated marked antibacterial activity we selected two ChBac3.4' variants with optimal properties for the further investigation in *in vivo* models.

Conclusion. The obtained data confirm the prospects of developing new effective antibiotics based on synthetic analogues of AMPs of the innate immunity for combating severe hospital infections caused by antibiotic-resistant bacteria. Several promising structural variants of natural AMPs have been selected for the further comprehensive study directed to creation such prototypes of novel antibiotics. The project is funded by the Ministry of Education and Science of the Russian Federation, Agreement № 075-15-2022-302 (20.04.2022).

THYMALIN PEPTIDE DRUG: PERSPECTIVES OF APPLICATION IN COVID-19

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Introduction. Thymalin is a medicinal drug, a complex of peptides with a molecular weight of up to 10 kDa, isolated from the thymus of calves. Thymalin regulates the number of T- and B-lymphocytes, stimulates cellular immune responses, and normalizes blood clotting, including cases of COVID-19 in older patients. The active components of Thymalin are short peptides Thymogen (drug, dipeptide EW), Vilon (dipeptide KE), Crystagen (tripeptide EDP).

Purpose. To valuate the effect of Thymalin and its constituent short peptides on gene expression and cytokine synthesis by human blood mononuclear cells in an inflammatory response model.

Methods. Isolation of human blood mononuclear cells was performed by sedimentation in a density gradient. Bacterial lipopolysaccharide was added to stimulate cytokine synthesis (an inflammatory response model). Thymalin or one of the short peptides was added to the experimental groups of cells at concentrations of 0.001, 0.01, 1 mg/ml. The expression of cytokine genes (IL-1β, IL-2, IL-4, IL-6, IL-10, TNFα) was assessed by RT-PCR, and protein synthesis was assessed by enzyme immunoassay. Data analysis was performed according to the Mann-Whitney U-test.

Results. Crystagen and Vilon (1 mg/ml) increased the expression of IL-2 gene by 25 % and IL-1β gene by 3.2 times in mononuclear cells. Vilon, Thymogen, Thymalin (1 mg/ml) increased IL-4 synthesis in mononuclear cells by 16.4–23.8 %. Vilon, Thymogen and Thymalin in all studied concentrations increased IL-10 synthesis in mononuclear cells by 53.1—170.2 %. Thymalin (1 mg/ml) increased the synthesis of IL-1β in mononuclear cells by 37.5 %. Vilon, Thymogen, Thymalin in all studied concentrations increased IL-2 synthesis in mononuclear cells by 20.5 — 53.6 %. Vilon (1 mg/ml) increased the synthesis of IL-6 in mononuclear cells by 39.4 %. Thymogen (0.01 mg/ml) increased IL-6 synthesis in blood mononuclear cells by 56.6 %. Vilon, Thymogen, Thymalin (1 mg/ml) increased TNFα synthesis in mononuclear cells by 16.8–44.9 %.

Conclusion. The therapeutic effect of Thymalin and its constituent short peptides in COVID-19 is based on the gene expression and cytokine synthesis regulation by blood mononuclear cells. Further study of the immunomodulatory effects of Thymalin and its constituent short peptides is promising for identifying the mechanisms of its effect on the immune system cells' functions and clarifying the optimal use of Thymalin in COVID-19 to correct the disorders caused by the "cytokine storm".

RECURRENT RESPIRATORY INFECTIONS IN CHILDREN DURING THE CORONAVIRUS PANDEMIC

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Introduction. According to WHO, every third inhabitant of the planet suffers from acute respiratory infections every year. Globalization increases the risk of the spread of these diseases, contributes to the emergence of new infections that are rapidly transmitted and poorly amenable to traditional therapy.

Purpose. To study the structure and etiology of respiratory diseases in children during a pandemic.

Methods. 280 sick children aged from 1 to 17 years were examined. The control group consisted of 30 healthy children. General laboratory, biochemical, microbiological and immunological research methods were carried out.

Results. Recurrent respiratory infections (RRI) predominate in the morbidity structure, more often (58 %) occur at the age of 1-2 years, as well as acute obstructive bronchitis — 54 % of cases at the age of 1 to 6 years. There is an increase in cases of community-acquired pneumonia in children from 1 to 6 years of age, which in the structure is 42 % of cases. Boys predominated (63 %), 88 % attended preschool educational institutions. 38 % of children had monthly episodes of acute respiratory infections, 35 % had 6 to 10 episodes with a "light interval" all year round. Among frequently ill children (FIC), elevated ASLO titers were determined in 21 % of patients. In a diagnostically significant amount, S. aureus was detected more often than others in 44 % of cases in the group from 3 to 6 years, in 40 % in the group from 1 to 2 years, and 27 % among schoolchildren. In 23 % of sick children, there was an association of two or more bacterial agents. Respiratory mycoplasmosis was diagnosed in 17 %, and chlamydia in 5 % in the FIC group from 3 to 6 years. RRI in children aged 1 to 6 years in 65 % of cases is associated with active herpesvirus infection, in children aged 3 to 6 years in 12 % of cases with streptococcal, in 11 % with mycoplasma and in 4 % with chlamydial infections. Giardiasis was diagnosed in 2 % of FIC, ascariasis in 0.8 %. A three-fold increase in the level of TNF-α and a synchronous increase in IL-1β and IL-6 in the blood of sick children were established both in acute and RRI with broncho-obstructive syndrome (BOS). And also in FBI, there is a decrease in the concentration of IFN-γ and phagocytosis compared to the control group.

Conclusion. The results obtained indicate the independence of dynamic changes in the concentration of the studied cytokines from the frequency of RRI and BOS episodes. Etiological deciphering at early stages of infectious diseases in frequently and long-term ill children and optimization of therapy can reduce both the frequency of respiratory diseases and the frequency of chronic somatic pathology in this category of patients.

ANTIMICROBIAL PEPTIDES FROM BLOOD SERUM

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Introduction. The growing resistance of pathogenic microbes to conventional antibiotics is a serious challenge for modern medicine. Antimicrobial peptides (AMPs) could be a promising source for next-generation antibiotics. However, many AMPs reduce their activity under physiological conditions. In this regard, it seems somewhat paradoxical that natural AMPs from blood serum have not yet been comprehensively investigated. The microbicidal activity of blood serum has been known since the 19th century. While the activity against Gram-negative bacteria is mainly provided by the lytic action of complement membrane attack complex, the exact components responsible for the activity against Gram-positive bacteria are still unknown. These components, referred to in the early literature by the obsolete term β-lysin, were characterized as a fraction of cationic AMPs mainly derived from platelets. Generally, investigations in this field were carried out on rabbit serum.

Purpose. To purify and characterize cationic AMPs from human and rabbit blood sera retaining their activity against Gram-positive bacteria under physiological conditions.

Methods. Blood serum depleted of cationic factors was obtained by its incubation with a suspension of CM-cellulose. The components bound to CM-cellulose were purified by solid phase extraction, gel filtration at Bio-Gel P-10 and reverse-phase HPLC at C18. The samples were analyzed by Tricine-SDS PAGE. Antimicrobial activity against *Listeria monocytogenes* EGD and *Bacillus subtilis* 534 was evaluated by colony counting assay.

Results. Although native human and rabbit sera are microbicidal against Gram-positive bacteria, the sera depleted of cationic polypeptides lose this activity. Several serum AMPs were isolated. In particular, a 3.7 kDa peptide was purified from human serum and its activity against Gram-positive bacteria at 10 μg/ml at physiological ionic strength was demonstrated. Two AMPs with Mw in the range of 8—9 kDa were isolated from rabbit serum. They exhibited antimicrobial activity at 12.5 μg/ml and revealed ability to restore the microbicidal activity of depleted serum.

Conclusion. The microbicidal activity of blood serum against Gram-positive bacteria depends on the cationic polypeptide fraction. The spectra of AMPs contained in mammalian sera are species-specific. Serum AMPs are able to act against Gram-positive bacteria under physiological conditions.

β-STRUCTURAL ANTIMICROBIAL PEPTIDES AS COMPLEMENT SYSTEM MODULATORS

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Introduction. Complement system plays a pivotal role in immune defense but is involved in many unfavorable processes. Search for novel complement inhibitors is still an important task. On one hand, we consider antimicrobial peptides as potential complement modulators. On the other hand, action on complement may be considered as a possible underestimated adverse effect of alternative peptide-based antibiotics.

Purpose. To characterize the action of β-structural antimicrobial peptides on human complement system activation *in vitro*.

Methods. Arenicin-1[V8R], arenicin-2, AA139, tachyplesins I and III, gomesin, capitellacin, androctonin, antimicrobial peptide from *Echinostoma caproni*, thanatin were expressed as recombinant peptides; arenicin-1, arenicin-1[C/A] and protegrin-1 were obtained by solid-phase synthesis; defensin from *Calliphora vicina* was purified from larvae; human α-defensins were purified from leukocytes. Human normal serum was used as the source of complement. The models of the classical (CP) and the alternative pathways (AP) of complement contained antibody-sensitized sheep erythrocytes or rabbit erythrocytes, respectively. Complement activity was measured by hemolysis as well as by ELISA for assessment of C3a and C5a anaphylatoxins accumulation.

Results. Most of the studied β-hairpin peptides showed bidirectional effect on complement activation. Tachyplesins, ALP-1 and capitellacin possessed the most pronounced activating capacity, especially in the CP model. The potentiating effect was reduced at high concentrations (of about 80—160 μg/mL) up to complement-inhibiting effect. Arenicin-1[V8R], arenicin-2 and protegrin-1 appeared to be the most potent inhibitors. Arenicin-1[V8R] was a "pure" inhibitor in the CP model (IC_{50} = 15.4 μM, 0.75 μM and 3.16 μM for inhibition of C3a, C5a accumulation and hemolysis, respectively) while arenicin-2 — in the AP model (IC_{50} = 26.9 μM, 8.9 μM and 18.1 μM). Human α-defensins exerted inhibitory action, especially in the CP model. Defensin from *C.vicina* was a weak activator in the CP model.

Conclusion. Screening of 16 natural and artificial β-structural antimicrobial peptides revealed different patterns of sequence-depending action on human complement. Three promising β-hairpin peptides were selected for further development of complement inhibitors — arenicin-1[V8R], arenicin-2 and protegrin-1. A prospective arenicin analogue was suggested.

THE INFLUENCE OF HYPERHOMOCYSTEINEMIA ON THE INFLAMMATORY PROCESS

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Introduction. In modern medicine, the problem of hyperhomocysteinemia is relevant and subject of many studies. Homocysteine (HC) is a natural sulfur-containing, non-proteinogenic amino acid that is a product of methionine metabolism. Normally, its blood level does not exceed 15–20 μM/l, but in case of impaired HC metabolism a condition of hyperhomocysteinemia is observed, which is characterized by increase in its blood level to 100–500 μM/l and above. At the same time, an increase in HC concentration in blood plasma can be both cause and consequence of pathological conditions, and can be hereditary or acquired. It is also known that hyperhomocysteinemia can lead to depletion of the functional activity of nervous, cardiovascular and immune systems of body.

Purpose. The purpose of this work is to study the influence of hyperhomocysteinemia on activation of neutrophils and production of α-defensins.

Methods. Blood of 76 patients with β0-thalassemia (thalassemia major) was examined, while the control group consisted of 20 healthy donors. The patients were divided into 2 groups before and after splenectomy (group I – 41 patients and group II – 35 patients, respectively). From biochemical parameters, the levels of ferritin, hemoglobin, serum iron, bilirubin and its fractions were determined. Levels of biochemical parameters (hemoglobin, free and bound bilirubin and serum iron) were determined using the "Diasys" kits. The concentration of α-defensin was determined by ELISA method using a commercial Hycult Biotech kit. The level of homocysteine was identified with Immulite 2000 System. Study was conducted with informed consent of patients in line with ethical principles of Helsinki Declaration (2013). Statistical analysis was performed with Mann-Whitney test. Statistical significance was determined at $p < 0.05$.

Results. Study of biochemical parameters revealed noticeable increase in the levels of direct and indirect bilirubin by average 2,5 times in both groups compared to control. In addition, against the background of decrease in hemoglobin concentration, there was a sharp increase in levels of serum iron and ferritin in group of patients after splenectomy ($p < 0.001$), which indicates a violation of liver function. In liver, HC is remethylated with the participation of 2 enzymes: betaine-homocysteine methyltransferase – in this case, betaine (trimethylglycine) and methionine synthetase act as methyl group donor. The cofactor is pyridoxal-5-phosphate, a derivative of vitamin B_6. In case of impaired liver function, observed in group

II patients due to excess iron, accumulation of HC may occur. The concentration of homocysteine was 22 μM/l in group I and 70 μM/l in group II, compared to the control values at 17 μM/l. In group of patients before splenectomy, the mean value of α-defensins was 524,0 ± 7,07 ng/ml. In group II patients, this indicator rose to 1582 ± 142,2 ng/ml, which is 15,5 times higher than in control group (102 ± 0,7 ng/ml). Our data revealed that the level of α-defensin also increased depending on the state of liver and neutrophil activation and correlated with the values of homocysteine.

Conclusion. Therefore, it is possible to assume that hyperhomocysteinemia may be the cause of the activation of cells of immune system, which can lead to a number of negative manifestations in the body.

ANTIBIOTIC ACTIVITY OF PEPTIDES FROM INNATE IMMUNE SYSTEM AND NATURAL DETERGENTS

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Introduction. Antimicrobial peptides (AMPs) of the innate immune system are promising candidates for a role of novel antibiotics. However, some cytotoxicity of AMPs toward host cells limits their active implementation in medicine and forces attempts to design numerous ways of usage of AMPs with optimized properties. Modern strategies include: creation of a wide array of synthetic structural analogs for selecting the peptides with the best features and/or application of antimicrobial peptides conjugated with certain nanoparticles. An alternative route: search of synergistic antibacterial effects of AMPs in combination with natural substances of different chemical nature.

Purpose. We supposed that *in vivo* antimicrobial activity of host defense peptides could be modulated by other biologically active substances, in particular natural detergents: bile acids and others. The aim of our experimental work was to study the antibiotic activity of AMPs in the presence of bile acids (BA).

Methods. AMPs were produced via the Fmoc solid phase synthetic approach on a synthesizer Symphony X (Protein Technologies, USA). The purity and molecular masses of AMPs have been characterized by RP-HPLC and MALDI-TOF mass spectrometry. For antibacterial assays we used the following bacterial strains: *Escherichia coli* ML-35p, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* SG511. Minimal inhibitory concentrations (MICs) were determined using broth microdilution assay. Combined antimicrobial activity was examined by Checkerboard titration assay for calculation the Fractional Inhibitory Concentration Indices (FICI). The effects of peptides on the permeability of *E. coli* ML-35p inner and outer membranes were assessed spectrophotometrically, using reporter molecules.

Results. As was expected, the structurally different AMPs of varied origins demonstrated a high level of activity, taken individually. MICs of human defensin hBD-3 were the following: 30μM against *E. coli* ML-35p, 0,05 μM for *P. aeruginosa* ATCC 27853, 0,8 μM for *St. aureus* SG-511.

MICs of porcine PG-1 were: 3.1 μM against *E. coli* ML-35p, 0,125 μM — *P. aeruginosa* ATCC 27853, 0,03 μM — *St. aureus* SG-511. MICs of caprine ChBac3.4: *E. coli* ML-35p — 3.1 μM, *P. aeruginosa* ATCC 27853 — 1.6 μM, *St. aureus* SG-511 — 3,1 μM. BA — lithcholic, tauroholic, glycolic, desoxycholic, chenodesoxycholic exerted a negligible antimicrobial activity with an average MIC >> 2500 μM. But we have found a synergy of antimicrobial activity when applied BA in combination with the peptides — using AMPs in concentration 1/3 MIC, bile acids — 1/8 MIC. Porcine PG-1 + chenodesoxycholic — synergy against *E. coli* ML-35p (FICI = 0.5), *P. aeruginosa* ATCC 27853 (FIC = 0.38), *St. aureus* SG-511(FIC = 0.5), porcine PG-1 + deoxycholic — synergy against *P. aeruginosa* ATCC 27853 (FIC = 0.5). Goat ChBac 3.4+ chenodesoxycholic –synergy: *P. aeruginosa* ATCC 27853 (FIC = 0.5), *St. aureus* SG-511 (FIC = 0.38). ChBac3.4 + deoxycholic — synergy: *St. aureus* SG-511 (FIC = 0.38). Human HBD-3 + chenodesoxycholic synergy — *St. aureus* SG-511 FIC = 0.5. Thus chenodeoxycholic acid was the most effective taken together with AMPs. We suppose that mechanism of activity of BA could be related with action on bacterial membranes. Using the permeabilization assay we found that in presence of bile acids AMPs obtain a significantly higher capability to disrupt membranes of microorganisms.

Conclusion. BA demonstrated very low level of antimicrobial activity. But in combination with other membranoactive substances — AMPs they acquire a remarkable antibiotic action. These results may support an idea stating an importance of interactions between AMPs and bile acids in gut homeostasis. Combination of AMPs and natural detergents — bile acids could be a useful finding for developing new anti-infective drugs.

ROLE OF HUMAN SALIVARY CATIONIC PROLINE-RICH PEPTIDES IN THE IMPLEMENTATION OF PROTECTIVE REACTIONS OF THE ORAL CAVITY

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Introduction. Saliva plays an important role in the implementation of anti-infective functions of the oral cavity. It contains antimicrobial cationic peptides, but their concentration in the salivary fluid is relatively low, as well as proline-rich proteins and peptides, the functions of which are currently poorly understood and not elucidated. With the emergence of new infections, in particular, coronavirus infection, the study of the protective factors of various barrier organs and tissues is important. Thus, deciphering the molecular and cellular basis for the implementation of the protective functions of proline-rich peptides PRPs is an urgent task of medicine and biology.

Purpose. Elucidation of the role of cationic proline-rich peptides of saliva in the implementation of protective reactions in the oral cavity.

Methods. The following methods were used in the work:

1. Method of serial dilutions in a liquid nutrient medium, containing microorganisms.
2. ELISA.
3. Flow cytometry.
4. Wound healing in a model of a full-thickness skin wound in mice, the wound surface of which was treated with the analyzed peptides.

Results.

1. Chemically synthesized fragments of proline-rich human saliva proteins: P-F (43-61), P-H (37-51), IB6 (98-116), p1932 have low antimicrobial activity or practically do not show it against gram-negative and gram-positive bacteria.
2. It has been established that PRPs of saliva can increase the antibacterial activity of peptides of the innate immunity system present in saliva.
3. PRPs of human saliva (P-H (37–51), P-F (43–61), IB6 (98–116), and p1932) at concentrations of 5 and 10 μM suppress the respiratory burst reaction of human blood phagocytes *in vitro* stimulated by the introduction of *Escherichia coli*.
4. Preliminary data have been obtained, which show that when peptides P-H (37-51), P-F (43-61), IB6 (98-116), p1932 are added to the culture medium, a significant inhibition of the release of pro-inflammatory

cytokines IL-1β, TNFα and, to a much lesser extent, the anti-inflammatory cytokine IL-10 is observed.

5. The use of proline-rich human saliva peptide IB6 (98-116) has a positive effect on the dynamics of wound healing in experimental animals.

Conclusion. The results of the assessment of various types of biological activity of the studied cationic proline-rich peptides make it possible to assume that such peptides play a significant role in ensuring antimicrobial protection of the oral cavity, modulating the activity of the peptides of congenital immunity, and may also be involved in the regulation of the process of inflammation and reparation processes. The project is funded by the Ministry of Education and Science of the Russian Federation, Agreement № 075-15-2022-302 (20.04.2022).

COMBINED ACTION OF AMPs AND SILVER NANOPARTICLES ON HUMAN CELLS AND ANTIBIOTIC-RESISTANT BACTERIA

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Introduction. Currently, much attention is paid to the study of antimicrobial peptides (AMPs) of innate immunity as candidate molecules for the role of a new class of antibiotics. Protegrin-1 (PG-1) is an AMP which has antimicrobial activity against a wide range of microorganisms, but some of its toxicity to human cells is an obstacle to its use in medicine. The solution to this problem can be the combined use of PG-1 with silver nanoparticles, which are also considered as promising antibiotics, in order to identify synergistic effects. This will reduce the effective concentrations of substances and, accordingly, toxicity.

Purpose. To evaluate the combined antimicrobial activity and hemolytic activity of PG-1 and silver nanoparticles.

Methods. To investigate the joint antimicrobial action of the studied substances serial dilutions according to the "chessboard" method were used; a hemolytic test was used to assess toxicity. PG-1 was kindly provided by Prof. R. Lehrer (UCLA, USA). Nanomaterials made on the basis of natural silicates, containing silver nanoparticles, were provided by the staff of the scientific group, Doctor of Chemistry. O. Yu. Golubeva (Grebenshchikov Institute of Silicate Chemistry).

Results. The study revealed that PG-1 exhibits synergistic antibacterial effects with samples of silver nanoparticles against antibiotic-resistant bacteria *Acinetobacter baumannii* 7226/16, *Escherichia coli* ML-35p, *Pseudomonas aeruginosa* 522/17. It is important to note that no cases of antagonism were identified. Under the combined action of PG-1 and samples containing silver nanoparticles, a decrease in the hemolytic activity of the peptide was observed. At a concentration of 25 μM, the hemolytic activity of PG-1 in combination with nanoparticle samples was approximately 2-fold lower than the individual activity of the peptide. When using combinations at concentrations close to the MIC, the hemolytic properties of PG-1 were practically not manifested. In general, the hemolytic activity of the combined preparations is significantly lower than the activity of individual PG-1, which suggests that the combined use of the peptide and samples of silver nanoparticles is an effective method for reducing their hemolytic activity and, consequently, toxicity.

Conclusion. The combined use of PG-1 and samples of silver nanoparticles is an effective way to increase their antibacterial activity and reduce their toxicity and can be considered as a way to obtain effective antibiotic drugs. The project is funded by the Ministry of Education and Science of the Russian Federation, Agreement № 075-15-2022-302 (20.04.2022).

MACHINE LEARNING TECHNIQUE FOR PREDICTION OF THE AMP'S MINIMUM INHIBITORY CONCENTRATION

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Introduction. Peptides with antimicrobial activity are considered as potential alternatives to antibiotic-based therapies. At the moment most of active antimicrobial peptides (AMPs) are natural substances or were inspired by them. Activity of the AMP can be characterized by minimum inhibitory concentrations (MICs). MIC is the lowest concentration of AMP that inhibits the visible growth of a microorganism after overnight incubation. This parameter can be estimated when a peptide already physically exists in a laboratory. Therefore MIC assessment of the absolutely random sequences rarely can be done. However, such information might be useful in a context of the peptide development. For example, an approach which gives an opportunity for a quantitative prediction of MICs is based on the amino acid (AA) sequences.

Purpose. Proof of the concept that information from database and machine learning technique can be used for MICs prediction.

Methods. Data for modeling were downloaded from DRAMP database. From these data we assembled subsets based on the peptides' antimicrobial activity against: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*. Partial least squares regression (PLS) was applied to relate the sequences (recoded in numeric array) with MICs (in μM). PLS decomposes the matrix with sequences into latent variable space and seeks for the linear combination of variables that would have maximum correlation with MICs. Full cross-validation procedure was applied to yield an estimate of modeling precision, and root mean square errors of prediction (RMSECV) were calculated.

Results. Three PLS models were built for the following cases: 1) the peptide with a length of 18 AA and MIC against *P. aeruginosa*; 2) 14 AA and MIC against *K. pneumoniae*; 3) 20 AA and MIC against *A. baumannii*. All models have coefficients of determination (R^2) exceed 0,95 in calibration and relatively low value of RMSECV.

Conclusion. An application of information from databases and PLS for quantitative prediction of the a peptide's MIC was studied. This approach provides an opportunity to predict MICs even for random sequences. However, this computation is not universal and has to be validated individually.

ANTIMICROBIAL ACTIVITY OF COMPLEMENT PEPTIDE C3f UNDER VARIOUS CONDITIONS

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Introduction. The complement system plays a key role in protection against pathogens. The C3f peptide is a cleavage product of the complement component C3b. Previously, the antimicrobial activity of C3f was described. The C3f peptide contains 2 histidine residues, which can be protonated depending on pH and bind metal ions.

Purpose. To study the antimicrobial activity of the synthetic peptide C3f against *Listeria monocytogenes* EGD and *Micrococcus luteus* A270 under various conditions.

Methods. C3f peptide was obtained by solid-phase synthesis. The antimicrobial activity was assessed by colony counting. The effect of pH was studied on *L. monocytogenes* and *M. luteus*, using 0.01 M PBS with pH values of 5.0; 6.0; 7.5; 8.0; final C3f concentrations are 16-64 μM and HNPs — 4–32 μM. The effects of Zn^{2+}, Cu^{2+}, Fe^{3+} and 0.15 M NaCl were studied on *L. monocytogenes* using 0.01 M Tris-HCl buffer pH 7.4 and final C3f concentrations are 1–32 μM. The concentrations of $CuSO_4$, $FeCl_3$ exceeded the concentration of C3f by 1.2 times and $ZnCl_2$ exceeded the C3f by 1.2; 4 or 8 times.

Results. The antimicrobial activity of C3f was lost in the presence of 0.15 M NaCl. Zn^{2+}, Cu^{2+}, Fe^{3+} had no antimicrobial activity against *L. monocytogenes*. In the presence of Zn^{2+} and the peptide, antimicrobial activity increased. Viability was 44 % of control ($p < 0.01$ compared to C3f alone) at 4 μM of C3f, 26 % ($p < 0.001$) at 8 μM, 16 μM 4 % ($p < 0.05$) at 16 μM. Cu^{2+} and Fe^{3+} did not increase the antimicrobial activity of C3f. The pH dependence of the antimicrobial activity of C3f was different for the 2 bacterial species. Against *L. monocytogenes* the C3f had the highest antimicrobial activity at pH 7.5: viability was 4 % of control, pH 5.0 was 100 % ($p < 0.001$ compared to pH 7.5), pH 6.0 was 10 %, pH 8.0 was 15 %. Similarly: HNPs had the highest antimicrobial activity at pH 7.5 and were inactive at pH 6.0. Against *M. luteus* C3f had the highest antimicrobial activity at pH 6.0: at 16 μM viability was 20 % of control ($p < 0.001$). At pH 7.5, viability increased. At pH 8.0: at 16 μM viability was 86 %, 32 μM was 69 % ($p < 0.001$), 64 μM was 49 % ($p < 0.001$).

Conclusions. An increase in ionic strength decreases the antimicrobial activity of C3f. Zn^{2+} in contrast to Cu^{2+}, Fe^{3+} increases the antimicrobial activity of C3f. Against *L. monocytogenes* C3f and HNPs have the highest antimicrobial activity at pH 7.5, but do not work at pH 5.0 or pH 6.0. C3f enhances antimicrobial activity against *M. luteus* at lower pH.

NATURAL MILK PROTEIN COMPLEXES AS A METHOD OF DELIVERY AND PRESERVATION OF BIOLOGICALLY ACTIVE PROTEINS AND PEPTIDES FOR NEWBORNS

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Introduction. Milk is not only a source of nutrients for mammals, but also contains a wide range of proteins, peptides, and other components necessary for the growth and development of newborns, as well as protection from viral and bacterial infections. A number of biochemical processes are carried out by biologically active proteins and peptides as part of protein complexes capable of preserving and transporting functionally significant components in the aggressive environment of the gastrointestinal tract. The characterization and identification of milk proteins, including protein complexes, is important for understanding the biological role of milk.

Purpose. The purpose of this work was to obtain comparative characteristics of the protein profile and to identify protein complexes of human, goat and cow milk whey.

Methods. Preparations of milk and whey, as well as fractions of human, cow and goat milk whey after ultrafiltration on Vivaspin with a pore diameter cutting off molecular weights were used 1000, 300, 100, 50, 30, 10 and 3 kDa. The analyzed samples were characterized by electrophoresis, RP HPLC, MALDI MS and immunochemical methods.

Results. The results showed similarities and differences in the protein profile of human, cow and goat milk preparations. The main difference between milk, whey and human milk whey fractions after ultrafiltration from the corresponding cow and goat milk preparations is the absence of β-lactoglobulin, the presence of lysozyme, a less pronounced casein fraction and a more pronounced fraction of high-molecular proteins, including lactoferrin. The presence of low-molecular proteins in the composition of high-molecular fractions of human, cow and goat milk whey after ultrafiltration has been shown. Individual antimicrobial proteins and peptides (HNP 1-3 and HNP4, α-lactalbumin, lysozyme, lactoferrin, lactoperoxidase and myeloperoxidase) were identified in human milk and whey preparations, as well as whey fractions after ultrafiltration of more than 1000 kDa and 100−300 kDa and fractions of HPLC. Low-molecular proteins α-lactalbumin and β-lactoglobulin were found in the composition of high-molecular complexes of goat milk whey in fractions 30-50, 50-100, 100-

300 and more than 1000 kDa and cow in fractions – 50-100, 100-300, 300-1000 and more than 1000 kDa.

Conclusion. In this way, antimicrobial peptides of human milk whey HNP 1-4 are found in high-molecular complexes with such antimicrobial proteins as α-lactalbumin, lysozyme, lactoferrin, lactoperoxidase and myeloperoxidase.

CYTOTOXIC ACTIVITY OF PROTEGRIN-1 AGAINST TUMOR CELL CULTURES OF THE BRAIN AND NORMAL CELLS

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Introduction. Despite recent advances in medicine, many cancer drugs usually do not have much specificity for transformed cells, they also kill healthy cells, proliferate rapidly, with toxic side effects. Another disease of chemotherapy is the development of resistance. Thus, a more promising alternative may be other classes of drugs that have properties that specifically target cancer cells without being toxic to normal cells with a stable developmental trend. Antimicrobial peptides (AMPs) may be a possible solution of this problem. At this point, the presence of signs and mechanisms of the antitumor effect of innate immunity peptides in the nervous system has probably been established.

Purpose. The aim of the study is to establish the antitumor activity of the protegrin-1 (PG-1) peptide against brain tumor cells.

Materials. Four cell cultures were identified in the study: C6 and U-251 human rat glioma and, respectively, K562 — human erythromyelomas (taken as a tumor culture control), Eahy 926 — human hybrid endotheliocytes (taken as a «normal» control). Four dilution series of 4, 8, 16, and 32 μM were selected as final concentrations.

Methods.

1. Counting cells in Goryaev's body using trypan blue vital dye.

2. Evaluation of the cytotoxic effect of drugs on cells using the MTT test. Two types of controls were used in the experiments:

1) Positive control (100 % susceptible cells, nutrient medium with cells).

2) Negative control (0 % susceptible cells, culture medium without cells).

3. Measurement of the optical sum and processing of results. Measurement of optical concentration in the wells of a plate produced at a wave frequency of 540 nm using a plate spectrophotometer POLARstar Omega (Labtech, Italy).

4. Statistical calculations are performed using the Prism 4.5 package (GraphPad Software, USA).

5. To verify the results of the photometric MTT test with real manifestations of cell diseases in severe cell diseases on a number of samples of parallel derived cell counts in Goryaev's body.

Results. The results of the MTT sensor test that PG-1 suppresses normal cells of human endothelial cells more strongly, while tumor cells are more resistant to its cytotoxic effect. However, with the appearance of tumor lines of rat and human glioma, it can be seen that the more stable con-

trol line K562. IC_{50} values were also calculated for all cell lines; according to the results of the experiment IC_{50} K562 to the literature data, the values for C6 and Eahy 926 are quite close to each other, while for human glioma a value close to 22.4 μM was determined.

Conclusion. When collecting the MTT test, the results are higher than when collecting trypan blue. What can be confirmed is that PG-1 stimulates metabolic activity at low concentrations. Based on the received data better understanding of PG-1 and/or combination with other drugs and peptides of innate immunity will shed further light on the optimal approaches to the use of these AMPs for suppression of cancer cells.

ANALYSIS OF ANTICANCER IMPACT OF CATELICIDIN LL-37, PROTEGRIN PG-1, AND NERVE GROWTH FACTOR NGF ON HUMAN BRAIN TUMOR CELLS

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Introduction. Brain tumors are the most malignant among other cancer types, and their surgical resection is extremely difficult. The majority of frequently malignant brain tumors belong to glioblastoma (GBM), anaplastic astrocytoma (AA) in adult and medulloblastoma (MB) in children. The basic reason of the low efficacy of therapy of brain tumors is a heterogeneity of cellular content of tumors and selection of neoplastic clones resulted in fast development of drug resistance to chemotherapy. Therefore, there is a keenly necessity for the search of new and more efficient therapeutic drugs and studying of their proliferation and migration effects on tumor cells. It is well known that some biologically active molecules such as growth factors, in particular nerve growth factor (NGF), and peptides of the innate immunity can regulate a variety of biochemical, physiological and immunological processes. To date it is found than some antimicrobial peptides (AMP) have an antitumor effect. We investigated the anticancer mechanisms of two peptides of cathelicidin family with different structures: α-helical cathelicidin (LL-37) of azurophilic granules of human neutrophils and β-hairpin protegrin-1 (PG-1) from pig neutrophils.

Purpose. An exploration of the effects of NGF, LL-37, PG-1 and chemotherapeutic drugs on human anaplastic astrocytoma (AA), glioblastoma (GBM), medulloblastoma (MB), primitive neuroectodermal tumor (PNET) tumor cells.

Methods. The specimens of tumors of AA, GBM, MB, PNET were taken from 4 patients after surgical operations in Pediatric and Adult Neurosurgical Departments of Polenov Neurosurgical Institute of Almazov Medical Research Center (Saint Petersburg). To assess the cytotoxic antitumor efficacy of NGF, PG-1, LL-37, and chemotherapeutic agents on human tumor cell cultures, we determined concentrations at which a 50 %

inhibition of cell viability was reached (IC_{50}) using the MTT assay. The descriptive statistics were performed using the GraphPad Prism software (version 6.01, 09.21.2012, company, San Diego, CA, USA).

Results. We have studied the cytotoxic effects of NGF, PG-1, and LL-37 towards human MB, PNET, GBM, and AA cells in comparison with chemotherapeutic drugs (Table). According to the guidance for preclinical drug testing, a new class of compounds is considered to be cytotoxically active at $IC_{50} \leqslant 10^{-4}$M, and an analog of a known anticancer drug is rated as cytotoxic if its IC_{50} is less than or equal to the IC_{50} of the reference drug.

Table. *The IC_{50} of chemotherapeutic agents, NGF, LL-37, and PG-1 on medulloblastoma cells from patients, according to MTT*

Peptides, chemotherapeutic agents	Patient 1	Patient 2	Patient 3	Patient 4
	Medulloblastoma, IC_{50}, μM	Primitive neuroectodermal tumor, IC_{50}, μM	Anaplastic astrocytoma, IC_{50}, μM	Glioblastoma, IC_{50}, μM
Doxorubicin	1036.0	1135.8	1277.7	3350.3
Carboplatin	1534.7	32177.0	416.9	39792.9
Temozolomide	1718.0	28656.0	22296.3	43539.3
Cisplatin	494.9	1925.7	253.0	11919.7
Etoposide	25.0	49.5	93.1	86.5
NGF	0.0038	23.1	0.0383	0.029
LL-37	13.3	24.9	52.3	32.2
PG-1	35.0	145.8	44.9	123.6

Data in the Table demonstrate that IC_{50} values of NGF, LL-37, and PG-1 are significantly less than 10^{-4} M. Consequently, NGF, PG-1, and LL-37 exert the cytotoxic effects towards MB, PNET, AA and GBM cells from patients 1, 2, 3 and 4 in comparison with the effects of chemotherapeutic drugs that showed moderate or weak cytotoxicity in regard to the cells of PNET, AA and GBM from patients 2, 3, and 4. These data indicate that GBM, AA, and PNET cells possess multidrug resistance to chemotherapy. Our study revealed that MB, PNET, AA, and GBM cells exhibit *in vitro* individual sensitivity inherent to these tumors in the level of organism.

Conclusions. NGF, PG-1, and LL-37 show strong cytotoxic action towards clinical isolates of MB, PNET, AA and GBM. Further experimental and clinical studies will facilitate the use of NGF, PG-1, and LL-37 for the treatment of brain tumors. The project is funded by the Ministry of Education and Science of the Russian Federation, Agreement № 075-15-2022-302 (20.04.2022).

SYNTHETICALLY MODIFIED ANTIMICROBIAL PEPTIDES

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Introduction. Nowadays, searching for new effective antimicrobials is an important goal of biomedical research. Cationic peptides of animal origin are promising candidates for a role of novel effective antibiotics. Many peptides have already been discovered and have been tested *in vivo* and *in vitro*. However, most of these peptides have a low stability and short half-life in biological systems.

Purpose. The aim of this study was to modify recently discovered antimicrobial peptides in order to improve their properties, e.g. biocompatibility and stability.

Methods. The selected peptides were obtained by solid-phase synthesis and covalently modified on resin, then characterized and identified by reverse-phase high performance liquid chromatography (RP-HPLC) and MALDI-TOF MS.

Results and discussion. Two recently discovered beta hairpin peptides — RC and MC, were selected for the modification through lipidation at the C-terminus and PEGylation at the N-terminus of the peptide's molecules. Fatty acid was introduced by formation of amide bond with amino group of lysine, specially added at C-terminus. Lauric acid was chosen as the fatty acid. Polyethylenglycol (PEG) was covalently attached to the N-terminus via amide bond as well. Introducing a fatty acid presumably will enhance an ability of a peptide to interact with lipid membranes of target microorganisms. The inclusion of a PEG residue into the peptide molecule, according to the literature data, is a very useful tool for improving the peptide's characteristics, important for the practical usage: on the one hand this modification should reduce the solubility, which can be decreased after an addition of a fatty acid to the C-terminus of a peptide. On the other hand, it should lead to reduction of biodegradation of the abovementioned peptides *in vivo*.

Conclusion. Modifications of antimicrobial peptides have been successfully obtained by solid-phase synthesis. Further studies of the antimicrobial activity and stability of these peptides will be carried out at the next step of our research.

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CYTOKINE STATUS OF PREGNANT WOMEN WITH COVID-19 OUT OF PREGNANCY

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There is a possibility of a negative impact of the transferred coronavirus infection on human reproductive function, in particular on female fertility. With COVID-19, especially in moderate and severe cases, hyperthermia and an inflammatory reaction, accompanied by systemic oxidative stress, can have a negative effect, which can have a damaging effect on the cells and tissues of the female reproductive system. And also, the impact of COVID-19 on the reproductive system of a woman can be mediated by the toxic effects of the drugs used and the decompensation of concomitant chronic diseases. It is currently unknown what long-term effects on women's reproductive health may be associated with past COVID-19. In this regard, women who have had COVID-19, especially in a severe form, should be assigned to a high risk group for the development of complications and be subjected to more thorough dispensary observation.

The aim of the study was to study the features of cytokine synthesis in pregnant women with a gestational age of up to 13 weeks.

In 2020—2021, the Obstetric Department of the Fergana City Medical Association (Fergana region, Uzbekistan) received obstetric complaints from 36 pregnant women up to 13 weeks of gestation who had COVID-19 in the preconception period. 18 women with a physiological pregnancy at the same gestational age made up the control group. Immunological studies were carried out by determining the content of cytokines IL-1β, IL-4, IL-6 and IFNγ in blood serum by ELISA (test system of "Vector Best", Russian Federation). The data obtained were processed by the method of variation statistics with the determination of the reliability $p < 0.05$.

When analyzing the anamnestic data, it was found that 66.7 % of pregnant women in the preconception period suffered a mild course of coronavirus infection. 25.0 % of women experienced moderate to severe COVID-19. The coronavirus infection proceeded in a severe form in 2 women. An analysis of changes in the level of pro- and anti-inflammatory cytokines in women who recovered from COVID-19 showed that in the first trimester of pregnancy, the content of IL-6 increased by 1.2 times compared with similar indicators during a physiological pregnancy ($p < 0.05$). The level of IL-1β also increased by 1.37 times in the first trimester, and the concentration of the anti-inflammatory cytokine IL-4 decreased by 1.3 times ($p < 0.05$). The concentration of IFNγ in the first trimester in wom-

en of the main group exceeded that in the comparison group by 1.5 times ($p < 0.01$). An analysis of the outcome of pregnancy showed that of the women examined, 12.5 % had a non-developing pregnancy, and 25 % had a spontaneous abortion.

Thus, increased production of interferon at the beginning of the gestational period in pregnant women who recovered from COVID-19 indicates the activation of maternal Th1 cells, which mediate the most dangerous cellular immune response for the fetus. Our results indicate that in the early stages of gestation, a number of immune disorders occur in the mother's body, which can be the direct cause of the developed complications.

SOME PARAMETERS OF THE IMMUNE SYSTEM OF PREGNANT WOMEN WITH COVID-19 IN THE THIRD TRIMESTER OF GESTATION

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It has been shown that coronavirus infection has a negative impact on pregnant women and their newborns. Publications on the impact of COVID-19 on gestation, fetus and newborn note that pregnant women are less tolerant of respiratory pathogens and therefore suggest that they are more susceptible to COVID-19 as well. High susceptibility to respiratory infections and severe pneumonia in pregnant women has been associated with immunosuppression and other physiological changes during pregnancy. In most cases, pneumonia develops during infection in the third trimester — during the period of maximum change in cell-mediated immunity, while there is a rapid development of respiratory failure and secondary bacterial complications.

The aim of the study was to study the cellular and humoral parameters of the immune system in women with COVID-19 who were in the third trimester of pregnancy.

We studied the clinical and immunological features of the course of the gestational process in 57 women with coronavirus infection, which consisted of 2 groups depending on the severity: group 1 — 45 women with a moderate degree of coronavirus infection and group 2 — 12 women with severe severity.

Immunological studies were carried out by quantitative study of the number of lymphocytes with the phenotype CD3, CD4, CD8, CD20 in peripheral blood using monoclonal antibodies of the LT series (test system "Sorbent", Moscow, RF), determining the level of IgG, IgA, IgM in blood serum by ELISA using test systems of LLC "Vector Best" (RF), according to the attached instructions. The control group consisted of 20 women with physiological pregnancy. Statistical data processing was performed using the Student's t-test using the standard Windows 2000 statistical software package.

Analysis of the results of the studies showed that the SARS-CoV-2 virus leads to pronounced disorders in the coagulation link of hemostasis. Despite the use of both antiplatelet agents and injectable anticoagulants, 7 (10.4 %) pregnant women developed thrombophilic complications. Immunological studies have shown that pregnant women with coronavirus

infection are characterized by suppression of the cellular link of immunity. The suppression of the immune system is manifested by general lymphopenia.

At the same time, the level of B-lymphocytes was significantly increased ($p < 0.05$), and the maximum value was recorded in women with a severe course of coronavirus infection ($p < 0.05$). In the humoral link, class G dysimmunoglobulinemia was observed, while the levels of IgA and IgM were significantly elevated ($p < 0.05$), and these changes are deeper in severe coronavirus infection.

In conclusion, during physiological pregnancy, the inclusion of fetoprotective mechanisms is observed that activate the immune system in the direction of the Th2-type immune response and, probably, have a modulating effect on the Th1-dependent immune response. With coronavirus infection in pregnant women, this inclusion is not observed, which leads to a breakdown in tolerance and the occurrence of a number of pregnancy complications. In pregnant women with a gestational age of more than 32 weeks, coronavirus infection has a damaging effect on immune system parameters, depending on the severity of the disease. The more severe the disease is, the stronger the changes in the state of the immune system are observed.

CLINICAL AND IMMUNOLOGICAL PREDICTORS OF RHINOGENIC COMPLICATIONS DUE TO COVID-19

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The SARS-CoV2 coronavirus causes COVID-19, a pandemic that threatens millions of people. Because protective immunity does not exist in humans and the virus is able to evade innate immune responses, it can replicate unhindered in initially infected tissues. Subsequent cell death leads to the release of viral particles and intracellular components into the extracellular space, which leads to the recruitment of immune cells, the formation of immune complexes, the synthesis of a wide range of cytokines and associated damage.

The aim of the study was to study the parameters of innate immunity in patients with ENT diseases against the background of moderate and severe severity of COVID-19.

We examined 57 patients with a confirmed diagnosis of coronavirus infection, which was established on the basis of the detection of SARS-CoV-2 RNA by PCR in swabs from the nasopharynx and oropharynx. 38 patients (66.7 %) were moderate, 33.3 % were severe COVID-19. Most of the patients were men (73 %).

The levels of IFNα and IFNγ, antibodies to IFNα, C3 and C5 complement components in blood plasma were studied by ELISA using test systems from "Vector Best" (RF).

The main manifestations of ENT diseases in the moderate form were acute rhinosinusitis, unilateral hemisinusitis, sinusitis or acute rhinopharyngitis. Patients with severe COVID-19 had more severe fever, headache, and general weakness. Manifestations of acute rhinosinusitis were expressed in the form of hemisinusitis or pansinusitis, or bilateral sinusitis with lesions mainly of the maxillary and ethmoid sinuses. 2 patients with a severe form of COVID-19 were hospitalized with a diagnosis of cavernous sinus thrombosis.

An analysis of the results of studying the level of interferons showed that in patients with COVID-19 with moderate severity, the level of IFNα was 2.8 times higher than in the control group, and in patients with severe severity, this cytokine was increased by 3.5 times ($p < 0.001$). The concentration of IL-8 in patients with moderate severity was increased by 3.2 times ($p < 0.001$), and in severe patients 5.4 times higher than the values

of the control group. The level of IFNγ, regardless of the severity, was increased in patients with COVID-19 by 4.3 times ($p < 0.001$).

To conclude, ENT diseases in moderate and severe forms of COVID-19 have pronounced manifestations of the hematopoietic system. The data obtained indicate that the likelihood of an unfavorable course increases with an increase in the serum level of inflammatory process indicators, which should be used as prognostic criteria for the development of complications of COVID-19.

CYTOKINE PROFILE OF PATIENTS WITH JUVENILE RHEUMATOID ARTHRITIS

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The pathogenesis of juvenile rheumatoid arthritis (JRA) is still one of the most complex and intensively studied problems. Considering that JRA is a potentially disabling disease, mainly due to the state of the musculoskeletal system, further study of the factors affecting the processes of bone remodeling is an urgent task of pediatric rheumatology. The causes of bone metabolism disorders in JRA are numerous and have not been fully established. The development of the inflammatory process in this pathology is based on mechanisms associated with an imbalance in the formation of inflammatory and anti-inflammatory cytokines, which may be the starting point in the development of bone tissue pathology.

The purpose of the study is to study the state of the cytokine status in patients with juvenile rheumatoid arthritis living in Karakalpakstan.

We examined 46 patients with JRA, aged 7 to 17 years. Determination of the quantitative concentration in the blood serum of indicators of cytokine status (TNF-α, IL-1, IL-6, IL-4, IL-10) was carried out by ELISA using test systems "Vector Best" (Russia). The control group consisted of 20 practically healthy peers. Statistical data processing was performed using the Student's t-test using the standard Windows 2000 statistical software package.

Analysis of anamnestic data showed that the articular-visceral form of the disease was diagnosed in 15.2 % of children, predominantly articular — in 71.7 % of patients. For the period of the survey, 15.2 % of patients had a moderate degree of process activity, 47.8 % had a minimal degree, and 21.7 % of children were in remission. According to the duration of the disease, the patients were distributed as follows: in 18 children, the duration of the disease was from 1 to 3 years, in 12 — from 3 to 6 years, and in 16 — more than 6 years.

Our study showed that in patients with JRA, the values of both pro-inflammatory (TNF-α, IL-1β, IL-6) and anti-inflammatory (IL-4, IL-10) cytokines significantly ($p < 0.01$) exceed the values control group. The level of the main pro-inflammatory cytokine TNF-α was 68.54 ± 2.7 pg/ml (against 21.36 ± 0.94 pg/ml in control), the level of IL-1β was 42.34 ± 2.13 pg/ml (versus 18.24 ± 1.72 pg/ml in control), and the level of IL-6 was 19.81 ± 2.52 pg/ml (against 5.84±1.27 pg/ml in control). The content of anti-inflammatory cytokines was also significantly higher than in healthy peers.

Thus, the level of IL-10 was 45.73 ± 2.37 pg/ml (against 17.24 ± 2.15 pg/ml in control), and the content of IL-4 was 48.21 ± 3.4 pg/ml (versus 5.96 ± 1.13 pg/ml in control). It is known that TNF-α significantly increases bone resorption, weakens osteoclastogenesis and bone formation, regulates the interaction of osteoblastic and osteoclastic cells, and IL-1β accelerates the maturation of osteoclasts. IL-1β activates the inflammatory process through various mechanisms, including by increasing the synthesis of IL-6. Interleukin-6 has a pleiotropic effect and triggers systemic inflammation (stimulates the synthesis of proteins in the acute phase of inflammation in the liver), affecting hematopoiesis (leads to the development of thrombocytosis and anemia) and many types of cells of the immune system. Thus, our study showed that in juvenile rheumatoid arthritis, there is a violation of the processes of bone tissue remodeling associated with immune changes underlying the pathogenesis of the disease.

CYTOKINE LEVEL IN PERSONS PROFESSIONALLY EXPOSED TO EXHAUST GASES

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Air pollution by oil products and vehicle exhaust gases cause significant harm to both the environment and our planet, as well as human health. It is the products of oxidation and combustion of both gasoline and diesel fuel that make up a significant part of the carcinogens in the atmosphere. Air pollution with exhaust gases can cause the development of inflammatory and allergic reactions and cause diseases of the respiratory and immune systems.

The aim of the study was to study the level of pro- and anti-inflammatory cytokines in car service workers in Tashkent. 24 employees of car service center No.5 in Tashkent were examined. The age of the surveyed persons ranged from 20 to 45 years, with work experience from 1 to 20 years in the automotive service.

Depending on the work experience, 2 groups were compiled: the 1st group — work experience up to 5 years and the 2nd group — more than 5 years. 15 practically healthy young people made up the control group. The level of TNFα, IL-1β, and IL-4 was determined in blood serum by ELISA ("Vector Best", Russia). Anamnestic data showed that 54 % of the examined persons were men under the age of 30 years. 75 % of the surveyed men were married. Of these, 3 (16.7 %) did not have children, were married for more than 3 years. An analysis of somatic diseases among the examined men revealed the following: allergic rhinitis occurred in 25 %, 12.5 % complained of allergic dermatitis, 16.7 % had diseases of the gastrointestinal tract, 20.8 % of workers had bronchopulmonary pathology, arterial hypertension was in 2 men (8.3 %), urological diseases were observed in 4 men (16.7 %).

So according to the anamnestic data, among the examined men there was not a single one who would not complain about anything. The conducted studies on the study of the level of cytokines showed that the concentration of IL-1β in the examined persons with work experience up to 5 years was 1.65 times higher than the values of the control group ($p < 0.01$). And in people with a higher work experience, the level of this cytokine was 1.15 times lower than the values of the control group ($p < 0.05$), which indicates the presence of chronic diseases, in particular, of the respiratory system. Analysis of the data on the study of the level of TNF-alpha showed an increased level in all examined individuals, and its level was

higher in group 2 ($p < 0.01$). It was in the second group that there were more workers with diseases of the cardiovascular system. The results of the study of the level of anti-inflammatory cytokine — IL-4, showed that in 42.8 % of the examined patients, an increase in the concentration of this cytokine was observed. Consequently, among the surveyed contingent there were quite a few people suffering from various allergic diseases.

In conclusion, the results of the studies have shown that exhaust gases are the main cause of the development of a number of diseases. An increase in the concentration of carbon dioxide in itself is an extremely unfavorable factor in the development of hypercapnia (one of the forms of hypoxia) and disruption of the respiratory system. Often, near major urban roads, the level of carbon dioxide is tens, and in extreme cases, hundreds of times higher than the permissible limits. But not only it is dangerous to human health. Exhaust gases are one of the main sources of lead pollution. Lead (a component of fuel additives that increase the octane number) is extremely toxic to humans, including the nervous, cardiovascular and respiratory systems, and it also has a powerful teratogenic effect.

PROSPECTS FOR USE OF SHORT PEPTIDES AS SYSTEMIC REGULATORS OF NEURODEGENERATIVE PROCESSES IN ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a neurodegenerative disease and the most common cause of dementia. Memory loss and psychophysiological disorders are observed in AD. The wide range of molecular and cellular AD-associated processes, such as neuroinflammation, oxidative stress, mitochondrial dysfunction, calcium imbalance, impaired neurogenesis, blood-brain barrier (BBB) breakdown lead to the development of the above clinical symptoms. Modern drugs approved by the Food and Drug Administration (FDA) for the treatment of AD do not significantly slow down the progression of the disease. In addition, these drugs are known to have side effects.

Short peptides (2–7 amino acid) are promising neuroprotectors with a wide range of biological effects. It was shown that EDR and KED peptides prevented the elimination of mushroom spines in the 5xFAD mouse model of AD. The EDR peptide increased the activation of signaling mitogen-activated ERK1/2 kinase, extremely essential in the survival of neurons and synaptic plasticity. The EDR peptide has binding sites in the promoter region of the superoxide dismutase SOD2 gene, the levels of which decreased in the human postmortem frontal cortex and correlated with cognitive impairment in AD patients. The AEDG peptide regulates the synthesis of IL-1β in thymic epithelial cells. At the same time, the pro-inflammatory cytokine IL-1β is activated in the early stages of AD development. The AEDG peptide has been shown to regulate the neurogenic differentiation of human periodontal ligament stem cells by increasing mRNA expression and synthesis of Nestin, GAP43, β Tubulin III, and Doublecortin neurogenesis proteins. The KED peptide modulated the state of capillary walls, increasing their resistance and permeability, and it also improved cerebral circulation, contributed to the restoration of VEGF expression in aortic endotheliocyte culture obtained from patients with atherosclerosis that indicates the expediency of further study of the KED peptide as a vasoactive component.

Thus, the action of short peptides is associated with the regulation of key pathophysiological processes contributed to the disruption of the functioning of neural networks with the subsequent development of cognitive dysfunction and memory loss in AD. In this regard, short peptides are the prospect therapeutic agents in AD treatment.

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