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ХІТОЗАН: ПРОТИМІКРОБНА АКТИВНІСТЬ ТА ПЕРСПЕКТИВИ БІОМЕДИЧНОГО ВИКОРИСТАННЯ

Суходуб Л.Б.

CHITOSAN: ANTIBACTERIAL ACTIVITY AND PERSPECTIVES OF THE BIOMEDICAL APPLICATION
Sukhodub L.B.

In the last decades, serious attention is attracted by the use of natural antimicrobial drugs instead of the usual ones because of pathogens resistance to antibiotics. Chitosan (CS) is widely used as an antimicrobial agent owing to its high biodegradability, nontoxicity and antimicrobial properties. CS is a cationic polysaccharide obtained by partial deacetylation of chitin, the major component of crustacean shells. In last time cultivation of fungi provides an alternative source of the CS obtaining: Chitin makes up 45 % of the *A. niger* and *M. rouxii* cell wall content and up to 20 % of the *P. notatum* cell wall content. In contrast to other polymers, chitosan is a hydrophilic polymer with positive charge and has three types of functional groups: amino group at position C-2 in each deacetylated structural unit, as well as primary and secondary hydroxyl groups at C-6 and C-3 positions respectively. This causes its ability to form new hydrophilic medicals on the basis of known drugs, as well as the formation of drug release systems. CS is unique adsorbent and it is possible to combine it with another drugs. The natural ability of CS for gelation is used in the preparation of the hemostatic agent "Celox", that is effective for preventing fatalities when arterial bleeding occurs on the battlefield. The clotting of "Celox" occurs much faster than other hemostatic agents. Antimicrobial activity of chitosan against many Gram-positive and Gram-negative bacteria, filamentous fungi and yeasts has been widely demonstrated in the scientific literature. There are some reported mechanisms for antibacterial activity: positively charged due to NH_3^+ groups Chitosan interact with negatively charged functional groups at the cell surface and compromise the cell wall or outer membrane. In the case of Gram-positive bacteria, lipoteichoic acid could provide a molecular linkage for chitosan at the cell surface, allowing it to disturb membrane functions. Lipopolysaccharides in the Gram-negative bacteria outer membrane are held together by electrostatic interactions with divalent metals. These cations may compete with CS, that also disturb the cell functions. Some authors reported that CS binds to DNA and inhibits RNA synthesis.

Significant role in antibacterial activity belongs to the physical and chemical properties of Chitosan, including its cationic structure, molecular weight, degree of the deacetylation, concentration. Owing to the high content of amino and carboxyl groups, Chitosan can form chelate complexes with metals. Silver (Ag) ion antimicrobial activity against Gram-negative and Gram-positive bacteria is well known. Complexes Chitosan-Silver are used in medicine for example as part of the protective coatings on metal implants in dentistry and orthopedics in order to reduce the risk of postoperative infection. The antibacterial activity of the Silver-Chitosan-doped hydroxyapatite (HA) coating was examined using spectrophotometry by measuring the optical density of the culture medium *E. coli* ATCC 25922 containing the experimental samples. After 48 hours immersion of the substrate in medium, concentration of microbial cells (C, CFU/ml) was decreased from log 7 to log 4,8, what is evidence of the coating antibacterial activity. It was studied the ability of the biomaterials based on HA with Chitosan and Silver content to influence the adhesive properties of *E. coli* ATCC 25922 and *S. aureus* ATCC 25923. It was proved that under Ag^+ ions action, added to the coating material, the adhesive index for *E. coli* decreases in relation to formalinized ram erythrocytes on 17 % as compared to control sample (pure HA) and the adhesive index for *S. aureus* – on 13 %. Also was found that chitosan as a component of bioactive coating decreases the adhesive index *E. coli* on 29 %, and those for *S. aureus* on 22 %. Thus, from this short overview follows the conclusion that CS can be used in medicine as a very perspective antimicrobial agent. Also, application CS in combination with HA-Ag coatings on medical metal implants, using biomimetic technology should be taken to the attention. CS has a great potential for its using as a component of the composite biomaterial with all necessary properties (porosity, biodegradation, nontoxicity) in nanomedicine, particular for bone regeneration and stomatology. Once more direction is connected with a property of CS to bind with DNA, RNA and that open the possibility to create novel materials for gene therapy. But for more effective using all CS and its derivatives properties in practical medicine it's necessary to perform further deeper investigations.

Key words: Chitosan, Gram-negative, Gram-positive microorganisms, antimicrobial activity.

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ЕКСПЕРИМЕНТАЛЬНІ РОБОТИ (EXPERIMENTAL STUDY)

ФАРМАЦІЯ (PHARMACY)

ПОРІВНЯННЯ ІМУНОГЕННОСТІ І БІЛКОВОГО СКЛАДУ ЕКСПЕРИМЕНТАЛЬНОЇ ТА ОФІЦІНАЛЬНОЇ ВІРОСОМАЛЬНИХ ПРОТИГРИПОЗНИХ ВАКЦИН

Волянський А.Ю., Погоріла М.С., Романова О.А., Давидова Т.В., Мартинов

А.В., Ігумнова Н.І., Сидоренко Т.А., Юхименко В.І., Перемот С.Д., Смілянська

М.В., Кашпур Н.В.

COMPARED OF IMMUNOGENESITY AND PROTEIN COMPOSITION OF EXPERIMENTAL AND OFFICIAL
VYROSOMAL INFLUENZA VIRUS VACCINE

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Peremot SD, Smilianska MV, Kashpur NV

Introduction Due to the high mutagenicity that distinguishes influenza A virus, there are viruses of this type of new antigenic properties, the prevention of which the immune system has no immunological memory. It is therefore important to find new ways to create and study patterns of immunological impact of influenza vaccines. The success of liposomal vaccines and the growing interest in their improvement can be attributed to a number of key advantages over other systems of delivery of antigens and enhance the immune response. The main advantage of liposomes is their safety and good tolerance, as demonstrated by the use of approved based on liposomes of anticancer and anti-infective drugs.

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Material and methods As part of the search for new ways to create, study functional characteristics and patterns of immunological impact of influenza vaccine immunogenicity was investigated newly formed liposomal vaccine "Lipos 2" compared to the trivalent vaccine officinal virosomal vaccine Infleksal and analyzed their protein composition using bioanalyzer «Agilent 2100." Immunogenicity of vaccines studied by hemagglutination inhibition test (HIT) with a specific antigen (influenza virus). The composition of proteins containing vaccine was studied using bioanalyzer «Agilent-2100» («Agilent Technologies», USA) using the method of SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis). Analysis on «Agilent-2100" conducted by using chips to analyze 12 samples simultaneously studied vaccines. The protein composition of the vaccine was determined by electrophoregram receiving data on molecular weight protein fragments, their concentration and percentage of study medication. Also, determine the average number of proteins in vaccine strain.

Results and discussion Considerable experimental vaccine immunogenicity, which caused a persistent increase in the level of specific antibodies to all three viral antigens in most experimental animals compared with the vaccine, which was in control. Based electrophoregram defined the quantitative composition of proteins, their total weight and percentage of trivalent subunit vaccine Influvak and trivalent virosomal vaccine Infleksal, that the number of components of different protein components by molecular weight.

Keywords: virosomal trivalent influenza vaccine, protein composition, immunogenicity..

БІОФАРМАЦЕВТИЧНЕ ОБГРУНТУВАННЯ РОЗЧИННИКА У СКЛАДІ ІМУНОБІОЛОГІЧНОГО ПРЕПАРАТУ ДЛЯ ПОПЕРЕДЖЕННЯ ТА ЛІКУВАННЯ КАНДИДАМІКОЗІВ

Рибалкін М. В., Стрілець О. П., Стрельников Л. С., Калюжна О. С.

BIOPHARMACEUTICAL SUBSTANTIATION OF THE SOLVENT IN THE COMPOSITION OF THE IMMUNOBIOLOGICAL DRUG SOLUTION FOR PREVENTION AND TREATMENT OF CANDIDAL INFECTION

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Today diseases caused by potentially pathogenic microorganisms become increasingly important. This phenomenon is connected with increase of power of influence of the environment: chemical pollution, radiation, irrational use of antibiotics and hormone therapy; it leads to decrease of the immune response and human nonspecific resistance. For the last years one of the indicators of failure of the human body immune protection is chronic and local candidiasis caused by potentially pathogenic fungi of *Candida* genus. Prevalence and risk of candidal infections determine the need for searching new medicines with a high efficiency and safety for human. Development of a vaccine for prevention and treatment of candidal infection is being actively conducted in many countries of the world. It should be noted that currently no domestic vaccine is produced in Ukraine and no candidiasis vaccines have been registered. Therefore, development of such vaccine is the topical issue of modern pharmacy and medicine.

In our previous studies it was found that the immunobiological drug based on the antigens of fungi of *C. albicans* with the protein concentration of 3 mg/ml and *C. tropicalis* with the protein concentration of 5 mg/ml in the ratio of 1:1 possesses the protective and therapeutic effect. At the current stage of research it is necessary to substantiate the solvent in the composition of the immunobiological drug. The aim of this work is the experimental substantiation of the solvent in the composition of the immunobiological drug based on the antigens of *C. albicans* and *C. tropicalis* fungi.

Materials and Methods. The immunobiological drug with the protein concentration of 4 mg/ml was investigated using various solvents. The following solvents was studied: water for injections, 0.9 % isotonic saline solution, phosphate buffer solution. To determine the protective and therapeutic activity of the immunobiological drug based on the antigens of *C. albicans* and *C. tropicalis* fungi the research was conducted in healthy two-month white mice with the body weight of 18-22 g. There were 6 animals in the control and test groups. Mice were intramuscularly injected 0.2 ml of the immunobiological drug twice with an interval of 14 days. To determine the protective activity of the immunobiological drug the animals were infected intraperitoneally in 3 months after the second introduction. For this purpose the suspension of *Candida albicans* fungi of CCM 335-867 strain in the amount of 20 mln. of cells and *Candida tropicalis* of ATTC 20336 strain in the amount of 60 mln. of cells in the volume of 1 ml was used; they were introduced with the interval of 1 hour. After that in 14 days the animals were examined and the results were determined. To determine therapeutic activity of the immunobiological drug the animals were infected according to the scheme described, in 5 days a double injection of the drug was introduced to mice. After that in 14 days the animals were examined and the results were determined. As a **result of the research** conducted it was found that the immunobiological drug based on the antigens of *C. albicans* and *C. tropicalis* fungi with all solvents studied protected 100 % of animals from infection in 1 and 3 months. The therapeutic effect of the immunobiological drug based on the antigens of *C. albicans* and *C. tropicalis* fungi with all solvents was 100 %. The therapeutic effect started to exhibit in 8 - 14 days after the first introduction of the vaccine, and in 8 - 14 days after the repeated introduction of the vaccine the full recovery of animals occurred. In animals of the control group the signs of infection corresponding to the moderate form of the disease and the advanced form of the disease were registered. Taking into account the fact that the immunobiological drug with all solvents has shown the similar results the use of phosphate buffer solution is more expedient for further study because it preserves the constant value of the pH medium for a long period of time. Thus, it provides the drug stability since an insignificant change in pH can greatly affect the activity of antigens, and it will have an influence on the drug activity.

Keywords: candidiasis; antigen; vaccine; immunity; solvent

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ВЕРИФІКАЦІЯ ВЕРХ МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ АМЛОДИПІНУ В ТАБЛЕТКАХ

Ханін В. А., Комарицький І. Л., Бевз Н. Ю., Георгіянц В. А.

VERIFICATION HPLC METHOD OF QUANTITATIVE DETERMINATION OF AMLODIPINE IN TABLETS

Khanin V. A., Komarytskyy I. L., Bevz N. Yu., Georgiyants V. A.

Introduction. Amlodipine ((±)-2-[(2-aminoetoksi)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridine dicarboxylic acid 3-ethyl 5-methyl ester) as besylate and small tally belongs to the group of selective long-acting calcium channel blockers, dihydropyridine derivatives. In clinical practice, as antianginal and antihypertensive agent for the treatment of cardiovascular diseases. It is produced in powder form, substance and finished dosage forms (tablets of 2.5, 5 and 10 mg). The scientific literature describes methods of quantitative determination of the drug by spectrophotometry – by his own light absorption and by reaction product with aloksan, chromatography techniques, kinetic-spectrophotometric method in substances and preparations and methods chromatomass spectrometry and stripping voltammetry. For the quantitative determination of amlodipine besylate British Pharmacopoeia and European Pharmacopoeia recommend the use of liquid chromatography method. In connection with the establishment of the second edition of SPH and when it is comprised of articles on the finished product, we set out to analyze the characteristics of the validation

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of chromatographic quantitative determination of amlodipine besylate tablets and to verify the analytical procedure.

Material & methods. In conducting research using substance amlodipine besylate series number AB0401013. Analysis subject pill "Amlodipine" series number 20113 manufacturer of "Pharmaceutical company "Zdorovye". Analytical equipment used is: 2695 chromatograph with diode array detector 2996 firms Waters Corp. USA using column Nova-Pak C18 300 x 3,9 mm with a particle size of 4 μm , weight ER-182 company AND Japan, measuring vessel class A. Preparation of the test solution. To accurately sample powder tablets equivalent to 50 mg amlodipine, add 30 ml of methanol, shake for 30 minutes, dilute the solution to 50.0 ml with methanol and filtered. 5 ml of methanol solution adjusted to a volume of 100.0 ml. Preparation of the working standard solution sample amlodipine besylate. 50.0 mg of amlodipine RCC dissolved in methanol and dilute with the same solvent to 50.0 ml. 5.0 ml of this solution argue with methanol to volume 100.0 ml. Before the major controlled trials validated the existence of documents certifying the suitability vykorystovanoho equipment, raw materials and chemicals. Validation of the methodology was carried out in accordance with the requirements of SPhU.

Results & discussion. Linearity methods defined within 80-120% of nominal concentrations. The linearity of the methods supported by the entire range of concentrations studied ($b=0.9845$, $S_b=0,01473$, $a=1.5282$, $S_a=1.4956$, $S_r=0.5486$, $r=0.9992$). It is proved that the validated method characterized by sufficient convergence and accuracy over the entire range of concentrations ($\Delta Z=1.03$, $\delta\%=0.09$).

Conclusion. During verification methods of quantitative determination of amlodipine besylate tablets were studied characteristics validated HPLC method: accuracy, linearity, precision, specificity, and internal laboratory precision. Validation technique characteristics do not exceed the critical value of error (1.6%) and characterized by qualitative analytical indicators. This technique can be correctly reproduced in the laboratory conditions, and is independent of the excipients.

Keywords: amlodipine, HPLC, method validation

АНТИМІКРОБНА АКТИВНІСТЬ СТЕРЕОІЗОМЕРІВ ПОХІДНИХ ХОЛАНОВОЇ КИСЛОТИ В ПОРІВНЯННІ З ХОЛЕВОЮ КИСЛОТОЮ

Барсук Д. О., Савченко Д. С., Криський О. С., Коваленко С. М.

ANTIMICROBIAL ACTIVITY OF STEREOISOMERS OF CHOLANIC ACIDS' DERIVATIVES COMPARED TO CHOLIC ACID.

Barsuk D.O., Savchenko D.S., Kryskiv O.S., Kovalenko S.M.

A series of membrane-active cationic derivatives of cholic acid with antimicrobial activity may have common aspects of the action mechanism with previously found cationic-peptide antibiotics. Bactericidal action of some substances was found for a wide range of gram-negative and gram-positive microorganisms, other derivatives cholic acid were weakly active against gram-negative microorganisms, but effectively penetrated through the outer membrane and increased the sensitivity of bacteria to hydrophobic antibiotics. The negative feature of membrane-active antimicrobial agents is a frequent expression of hemolytic properties that may be an obstacle to their regular use. Level haemolytic of bile acid derivatives ranged from very weakly hemolytic to high hemolytic. The aim of this work was to explore new stereomeric compounds: derivatives of bile acids with weak hemolytic activity and potentially high antimicrobial effect. According to previously conducted research on optical structure and pharmacological action of stereoisomers stereoisomers α / β -amino, it was found that bile acids change their pharmacological properties depending on the stereo-configuration therefore to continue the analysis of bile acid derivatives it were taken already studied stereoisomers amino functionalized cholic acid. In 3D-models of 3β -amino-7 α , 12 α -dihydroxy-5 β -cholanolic acid has a more unfavorable position of the amino group at the 3rd carbon atoms and requires a large amount of activation energy and reaction conditions are more stringent than 3α -isomer need, so therefore bacterial metabolism blocking is possible through this mechanism. Thus reactions involving 3β -amino group are complicated because of its shielding by voluminous substituent at 10th position steranic fragment (CH_3 group) in contrast to 3α -amino which is directed in the opposite direction.

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Materials and methods. The antimicrobial activity was measured by the method of serial dilutions in a solid nutrient medium. To compare the effects of spatial configuration on the antimicrobial activity of substances we used 8 compounds (4 α -stereoisomers and 4 β -stereoisomers) with the same substituents, which were obtained by synthesis of corresponding α/β -amino cholanolic acid with acylchloride.

Result and discussions. The results of antimicrobial activity show that all studied drugs can be characterized by a wide spectrum of antibacterial properties against strains of aerobic bacteria and fungi and they have higher rate of growth delay compared to their $3\alpha/\beta$ predecessors. $\alpha\text{CyclBut}$ showed a greater influence on growth delay of *Candida albicans*, *Escherichia coli* and *Pseudomonas aeruginosa*. Almost all of α isomers showed weaker antimicrobial activity against *Proteus vulgaris*, *Bacillus subtilis*, *Staphylococcus aureus* compared with their β analogues and in absolute values.

Conclusions. All compounds have showed a wide range of antimicrobial activity and might be used as potential antibiotics. $\alpha\text{CyclBut}$, $\beta\text{CyclBut}$, $\beta\text{3Me-Ph}$ caused the greatest growth delay of microorganisms.

Keywords: bile acid, 3-acylated α - stereoisomers and β 3-amino-7 α , 12 α -dihydroxy-5 β -cholanolic acid antimicrobial activity.

МЕДИЦИНА (MEDICINE)

EFFECT OF CYTOKINE CEREBROPROTECTIN ON THE STATE OF ANTIOXIDANT THIOL-DISULFIDE SYSTEM IN THE BRAIN TISSUE OF RATS WITH EXPERIMENTAL DIABETES MELLITUS

Suprun E.V., But N.A., Suprun A.S.

Agents, providing both for damaging effect and cell viability system in the ischemia/hypoxia area, include cytokines – intercellular communication transmitters in health and disease, which establish communication signal network between cells of the immune system and cells of other organs and tissues. According to present-day ideas, nature of immune response and peculiarities of development of the pathophysiological changes in ischemic/hypoxic tissue disorders depends on preemptive activation of the T-lymphocyte subpopulations, their synthesis of cytokines of various types and formation of "cytokine cascade", i.e. relation between pro-inflammatory and anti-inflammatory cytokines. Therefore, application of cytokine preparations may become effective perspective link in the complex therapy of post-ischemic complications in DM. In case of ischemic damage to the brain tissue in DM model, TDS balance shifts due to decrease in its reduced intermediates on the background of oxidized forms, with considerable lowering of reduced glutathione level and GR and GP activity. Similar pathological biochemical changes cause significant functional changes in cells and are often irreversible. Changes on TDS activity and oxidation of thiol groups of a cystein-dependent protein region of the mitochondrial internal membranes cause depolarization and destabilization of the mitochondrial internal membranes, with so called non-selective PT-pore (permeability transition pore – PTP) being formed. Opening of such channel in the internal membrane results in establishing ion balance in the matrix and mitochondrial intermembranous space, distributes hydrogen ion gradient (H^+) to the internal membrane and breaks respiratory chain. Also, this causes volume dysregulation of mitochondria due to the matrix

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hyperosmolality, results in the increased matrix volume, breaks of the external membrane and growing destabilization of mitochondria and enzyme system, leads to development of persistent mitochondrial dysfunction, and as a result, to mitochondrial death – mitoptosis. Moreover, IL-1, produced in response to hypoxia, expresses inducible NOS (iNOS) in the glial cells, which results in NO hyperproduction and toxic effects due to its excessive amount. Excessive amount and its highly toxic derivatives nitrosylate protein-clinging enzymes of the respiratory chain of mitochondria and Krebs cycle, and inhibit them. Dysfunction of mitochondrial enzyme complexes (MEC) is formed, which causes qualitative changes of iron-sulfur centers in the mitochondrial enzymes and their functions, as well as suppression of a main (NAD-dependent) pathway for the substrate oxidation in respiratory chain. Aerobic energy synthesis is suppressed, thus bioenergetic (tissue) hypoxia is developed. Under conditions of the impaired generation of cell energy, caused by mitochondrial dysfunction, loss of NAD and ATP results in death of cells by necrosis or apoptosis. These pathophysiological changes form the basis of occurrence of early or late post-ischemic DM complications, resulting in disturbance of the usual lifestyle and lowering of life quality, persistent loss of occupational capacity and rapid progression of heavy neurological consequences up to lethal outcome. To gain maximum protective effect in the DM therapy, it is necessary to achieve interruption of pathogenetic ischemic/hypoxic cascade at earlier stages, which includes stage of thiol-disulfide imbalance establishing. Normalization of TDS state allows prevention of depolarization and destabilization of the mitochondrial internal membrane followed by development of mitochondrial dysfunction, energy imbalance and other post-ischemic consequences.

Keywords: interleukin-1, IL-1ra, experimental diabetes mellitus, thiol-disulfide system

ОСОБЛИВОСТІ ВПЛИВУ БАБЕЗІЙНОЇ ІНФЕКЦІЇ НА БУДОВУ ШЛУНКА НЕЛІНІЙНИХ МИШЕЙ (експериментально-морфологічне дослідження)

Похил С.І., Торяник І.І., Тимченко О.М., Чигиринська Н.А., Костиця І.А., Болецка Т.А.

PECULIARITIES OF THE BABESIOSIS INFECTION INFLUENCE TO NONLINER MICE STOMACH STRUCTURE (experimental and morphological investigation)

Pokhil S.I., Torianik I.I., Tymchenko O.M., Chigirinska N.A., Kostyria I.A., Boletch'ka T.O.

The babesias are one of the most ubiquitous and widespread blood parasites in the world based on numbers and distribution of species in animals, second only to the trypanosomes. They generally have two classes of hosts, an invertebrate and a vertebrate host. The maintenance of *Babesia* spp. is dependent on both hosts; the specific tick vector must feed on a vertebrate reservoir that is competent in maintaining the *Babesia* organisms in an infectious state. Therefore, *B. microti* presents itself as an emerging zoonosis only in areas where there is a primary competent reservoir. The first documented case of babesiosis in humans was in 1957. A splenectomized farmer in Yugoslavia was diagnosed with a *B. bovis* infection. But now most cases of babesial infections in humans have been acquired in temperate regions of the USA and Europe. That a tick-transmitted protozoan parasite, one of the causative agents of piroplasmiasis (babesiosis). The disease is characterized by signs of malaise, inappetence, fever, hemolytic anemia and hemoglobinuria. The parasite has a wide distribution and occurs on all continents, except Australia. The life cycle of the parasite in animals organism (equine hosts, traveling dogs, cats) comprises two intracellular stages: sporozoites inoculated by infected ticks develop into schizonts within lymphocytes where they multiply and subsequently transform into microzoites, which then invade erythrocytes. **Purpose** of the experiment's to study the influence of the babesiosis infection to nonliner female mice stomach structure.

Materials and methods. The examinational material of this investigations are the female nonliner control intact mice of the 4-6-week-old (n=15) and such patterns, which were with the babesiosis infection (n=45). For all of examinational animals groups were used macroscopic and histological methods. Macroscopic analysis was included organoleptic, biomechanical, microtopographical methods of investigations (external status, volum, size, syn-, holo-, skeletoty). Microscopic examination was carried out in a traditional way. Bits of the material were removed, washed, fixed in 12 % formaldehyde, subjected to postfixation and dehydrated (providing have been worked in standart algorithm's). Sections were contrasted by hematoxylin and eosin, azur and eosin, Brashé, sudan III- sudan IV, analysed under a microscope LOMU (LOMO, Russia): x 300; x400; 1350 and photographed with a digital camera "Canon EOS-3000".

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Results. Clinically disease manifestations of babesiosis are caused by the asexual reproductive stage of the organism in digestive system organs, erythrocytes of the host and the subsequent lysis of same host cells. Consequently, there is a very broad clinical spectrum which is probably directly reflective of the level of parasitemia in the blood. Morphologically: gastrical tunics (mucosal, muscular, serosal) of the control groupe animals are very visible and norm. The changes of abdominal microtopography it was not found. Megalia, oedema, hyper-, atrophia, inflammatory processus are absent. It was found out that a pronounced structural-functional changes of stomach, submucosal lymphoid tissues and endothelium of gastrical microvessels took place during first days of the observation of animals with the babesiosis. The above regression was attributed to development of thrombosis, stasis, trophic changes, development of destruction and necrosis. Characteristic morphological signs consisted of changes in the nucleus/cytoplasm ratio of cells, development of lympho-leucocellular, lympho-histiocellular infiltration, proliferation, vacuolization of cytoplasm, caryorexis, transformation of chromatin, appearance of megakaryocytes in the bloodstream and their markedly increased count.

Conclusions: macro- and microscopic changes in the stomach of 4-6-week-old nonliner female mice (with the babesiosis) were with the phase character, have been depended upon terms of the début of babesiosis and consisted in destruction of stomach structural components, gastric microvessels roofs.

Key words: babesiosis, macroscopic, microscopic changes, stomach, nonliner female mice.

ХАРАКТЕРИСТИКА СИСТЕМНОГО ТА МІСЦЕВОГО ІМУНІТЕТУ ХВОРИХ НА ХРОНІЧНИЙ ТОНЗИЛІТ

Коляда Т.І., Вдовіченко Н.І., Тупотілов О.В., Коляда О.М.

CHARACTERISTICS OF SYSTEMIC AND LOCAL IMMUNITY IN PATIENTS WITH CHRONIC TONSILLITIS

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Palatine tonsils are the most significant cluster of lymphadenoid tissue of throat. Performing a protective function they are very prone to acute and chronic inflammation and thus they are a source of chronic infection in the body. Therefore the problem of chronic tonsillitis (CT) requires a serious attention. Rheumatoid arthritis (RA) is one of the important factors that could significantly complicate the course of chronic tonsillitis. RA is a chronic immune inflammatory disease that progressively affects connective tissue mostly of the peripheral joints and it has a wide range of extra-articular manifestations. The aim of our study was to explore the dynamics of immunologic indicators during the active disease and convalescence in patients with various severity of chronic tonsillitis, including tonsillitis complicated with RA, and to define indicators of local and systemic immunity for further improvement of tactics of immune correction in the complex treatment.

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Material and methods. 41 patients with various forms of chronic tonsillitis in active period of disease observed during the study.

Patients were divided into the following groups: 19 persons with the compensate form of CT, 15 persons with the decompensate form of CT, 9 persons with the decompensate form of CT complicated with RA in remission stage. The control group consisted of 15 apparently healthy persons. Average age of the observed persons was 34.4 ± 0.8 years. Concentrations of serum immunoglobulins sIgA, IgA, IgM, IgG were determined by the method of radial immunodiffusion by Mancini. Levels of Ig E, IL-4 and IFN - γ in the blood serum of patients were evaluated using ELISA test systems of "Vector-best". The detection of circulating immune complexes (CIC) was performed by the method based on a selective precipitation of antigen complexes in 3.5% solution of polyethylene glycol (PEG) with subsequent photometric determination of density of the precipitate. In peripheral blood the relative level of CD₃₊, CD₄₊, CD₈₊, CD₁₆₊, CD₁₉₊-cells was determined with the help of monoclonal antibodies to the differential antigens of lymphocytes by indirect immunofluorescence method.

Results and discussion. It is noted that there are marked changes in the immune system from both humoral and cellular components under the influence of chronic infection persisting in palatine tonsils. The immunity indicators in different groups of patients with chronic tonsillitis were compared after undertaken conservative treatment. The fastest positive changes for the normalization of indicators occurred in the group CT (the compensate form), except IFN - γ . Indicators sIg A, Ig A were significantly reduced in group CT+RA, indicators IgG, IgE, CIC, IFN - γ remained significantly high relatively to control group. The decompensate form of CT was characterized by the deficit of sIg A, IgA and IgG in the context of increased levels of IFN - γ and IL-4. Thus, the studied changes in the levels of all fractions of immunoglobulins, IFN - γ and IL-4 indicate that the undertaken conservative treatment was insufficient and it requires further improvement with the immune correction usage in the complex therapy in all patient groups except of the group with the compensate form of CT. Decrease of level of sIg A relatively to control group, a significant increase of level of IFN - γ was observed in patients with the compensate form of CT before starting treatment. After treatment the normalization of all analyzed indicators was observed except of IFN - γ which level was decreasing but remaining above the control value. Thus, the existence of adequate immunologic reactivity makes application of immunomodulators by simple form of CT inappropriate. Deficit of sIg A, IgA and IgG in context of increased levels of IFN - γ and IL-4, a reliable increase of CD₁₉₊ content and increase of level of lymphocytotoxic autoantibodies were observed in patients with the decompensate form of CT. After treatment in patients with the decompensate form of CT the indicators of subpopulations CD₈₊, CD₁₆₊ content were decreased, deficit of sIg A, IgA and IgG, increased levels of IFN - γ and IL-4 was observed, also level of lymphocytotoxic autoantibodies were increased. The obtained data shows that conservative treatment of the decompensate form of CT is insufficient and application of immunomodulating remedies of systemic action is appropriate. A significant number of CD₄₊ cells with simultaneously low number of CD₁₆₊ is a peculiarity of phenotype of white blood cells of peripheral blood in patients with the decompensate form of CT that develops in the context of RA. Also the hyper production of immunoglobulins IgM, IgG and IgE in context of decreased levels of sIg A, IgA, the increase of level of the CIC, IFN - γ and also lymphocytotoxic autoantibodies was noted. After the conservative treatment of CT most of these indicators does not reach the control values, an imbalance of T-cell link remains and that points to the expediency of application of immunomodulating remedies.

Keywords: immunity, chronic tonsillitis, a subpopulation of lymphocytes, immunoglobulins, cytokines

ОСОБЕННОСТИ ИММУНОЛОГИЧЕСКИХ ПОКАЗАТЕЛЕЙ ПРИ ХРОНИЧЕСКОМ ТОНЗИЛЛИТЕ, АССОЦИИРОВАННОМ С ВИРУСОМ ЭПШТЕЙНА-БАРР У ВЗРОСЛЫХ

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FEATURES IMMUNOLOGIC INDICATORS OF CHRONIC TONSILLITIS ASSOCIATED WITH EPSTEIN-BARR VIRUS IN ADULTS

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Chronic tonsillitis are the most common diseases of the upper respiratory tract. One of the causes of tonsillitis, with severe clinical manifestations or erased is the Epstein - Barr virus (EBV). According to the literature, more than 90% of the adult population infected with EBV and are lifelong carriers of the virus. After primary infection replication of the virus in asymptomatic or in the case of a weakened immune system may develop infectious mononucleosis. Primary EBV infection in adolescence and adults is much greater than in children and often causes the formation of chronic forms. The main entrance gate is EBV oropharyngeal epithelium. In epithelial cells undergoing complete EBV replication with lysis of cells and the formation of a large number of virions. EBV infects B lymphocytes through the interaction of the surface gp320 virus with CD21 (receptor for complement component C3d). In EBV-infected B lymphocytes are two possible kinds of replication: lytic and latent process. During replication of EBV lytic expressed approximately 100 proteins are immunogenic but are 4 types of proteins, which have specific antibodies: early antigen - EA; viral capsid antigen - VCA; Epstein-Barr nuclear antigen - EBNA; latent membrane protein - LMP. LMP-1 induced bcl-2 (a blocker of apoptosis in B-cells) and promotes proliferation and migration of B-lymphocytes. Thus, EBV infection is characterized by widespread, reactivation of infection from infected parts that most often manifests itself with recurrent infection with symptoms of chronic tonsillitis. Objective: what features of general and local immunological parameters inherent in chronic tonsillitis in the acute stage, caused by reactivation of EBV infection. 50-55

Materials and methods. The study included 311 patients with chronic tonsillitis subcompensated in the acute stage. Microbiological testing of samples produced from the throat, determined EBV DNA in saliva, EVB-VCA-IgM, EVB-EA-IgG and EVB-NA-IgG in the serum; CBC was performed, the determination of CD3, CD4, CD8, CD16, CD19, IgM, IgG, IgA, IgE, CEC serum cytokines and sIgA in the oropharyngeal fluid.

Results and discussions. Group of patients who are not identified in the throat *S. pyogenes* and *S. aureus* revealed EBV DNA and identified EVB-EA-IgG and EVB-VCA-IgM was 20 people (chronic tonsillitis associated with EBV infection). A group of patients in whom there was no EBV DNA in saliva and throat was isolated *S. pyogenes*, *S. aureus* was 60 people (chronic tonsillitis associated with bacterial infection). Control group consisted of relatively healthy people - 20 people. Patients with EBV infection was observed leukocytosis, lymphocytosis; increasing the number of B lymphocytes (CD19), IgM, IgA and IgE, increasing the CEC, INF- α , L-1, IL-4, IL-6, IL-10, IL-17 and sIg A. Patients mentioned bacterial tonsillitis increased Ig G, IgA, and IgE, and a significant increase in CEC; increase INF- γ , IL-1, IL-4, IL-6, IL-17. INF- α , IL-10 levels were not significantly different from the control. Number sIg A was reduced.

Conclusions: 1. Relationship between exacerbation of tonsillitis and EBV infection was confirmed by the presence of fluid in the oropharyngeal EBV DNA and antibodies to EBV early antigens 2. For acute EBV infection is characterized by leukocytosis, lymphocytosis; possibly in the presence of abnormal blood mononuclear cells in a small amount 3. For acute EBV infection is characterized by increase in the number of B-lymphocytes and levels of IgM, IgA and IgE in serum, as well as increasing the CEC. 4. In the local cytokine status for acute EBV infection, in contrast to bacterial tonsillitis characterized by increased levels INF- α and IL-10. 5. The treatment of acute exacerbations of chronic tonsillitis often empirically prescribers ampicillin and amoxicillin. In the case of EBV infection activation function of these antibiotics is contraindicated and may cause allergic reactions, up to anaphylactic shock.

Key words: chronic tonsillitis, VEB, subpopulation of lymphocytes, immunoglobulin, cytokines

РАСПРОСТРАНЕННОСТЬ ИНФИЦИРОВАНИЯ ГЕРПЕТИЧЕСКИМИ ВИРУСАМИ СРЕДИ ЧАСТО БОЛЕЮЩИХ ДЕТЕЙ

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THE PREVALENCE OF INFECTION WITH HERPES VIRUSES AMONG FREQUENTLY ILL CHILDREN

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Frequently ill children (FIC) - a group of dispensary an inclusion criterion which is the frequency of episodes of colds are over 4-6 throughout the year depending on age. In children population among all diseases marked the absolute predominance (90%) of acute respiratory infections (ARI). The maximum incidence of acute respiratory infections among children there is between the ages of 1 to 3 years, and then gradually decreases. Among primary school children the incidence is 2-5 cases a year, among teens - no more than 2-4 diseases throughout the year. Opinions of scholars and practitioners pediatricians as to the legality of frequent classification of cases of children to pathologic conditions of the immune system are different and often diametrically opposed.

Objective: To determine the prevalence of infection with herpes viruses of frequently ill children.

Materials and methods. The analysis consists the results of clinical and laboratory examination of 170 frequently ill children. The criterion for selection of children for the study is the frequency of episodes of colds according to the classification of A.A.Baranov V.Yu.Albitskiy. Analysis of clinical and anamnesis data revealed that in the observed group of children there is a high rate of recurrent respiratory diseases. Thus, in the observed group of children the average incidence of ARI was $7,42 \pm 0,92$ episodes a year. The average duration of an episode of disease was $9,12 \pm 2,75$ days. The complicated course occurred in 32% of cases, the average duration of a complicated episode grew to $12,37 \pm 3,91$ days.

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This study led to the following **conclusions:**

1. To 3 years of age, about 85% frequently ill children are infected with at least one virus of the family Herpesviridae. By 6 years of age the number grows to 95%, to 11 years - to 98%. 2. Infectiousness 3 or more herpes viruses among children up to 3 years is more than 30%, among children 3-6 years is 48%, in the age group 6-11 years more than 67%. 3. Most often children of all age groups infected with herpes simplex virus (HSV) and cytomegalovirus (CMV). 4. With age the rate of infection with varicella-zoster virus (VZV), Epstein-Barr virus (EBV), human herpesvirus 6 (HHV6) higher than those for the HSV and CMV, and to 11 years in the frequency of detection of antibodies to any of the 5 studied types of herpes viruses among frequently ill children exceeds 50%.

Key words: herpetic viruses, antibodies, seroconversion, frequently ill children

Історія медицини

АЛЕРГИЯ І ПРОТИТУБЕРКУЛЬОЗНИЙ ІМУНІТЕТ В ПРАЦЯХ М. М. ЦЕХНОВИЩЕРА

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THE QUESTIONS OF ALLERGY AND ANTI-TUBERCULOSIS IMMUNITY IN THE WORKS OF M.M. TSEHNOVITSER

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The mechanism of anti-tuberculosis immunity drew the attention of scientists since the established of the infectious nature of tuberculosis. The famous ukrainian microbiologist and immunologist M.M. Tsehnovitser in period from 1921 to 1940 years spent a lot of original experiments for elucidation of the role of allergy in the anti-tuberculosis immunity. M.M. Tsehnovitser believed that a common cause of infectious allergy is tuberculosis granuloma, which even at rest eliminated weakened microbes and their products in general lymphatic and blood stream of the body. In his experiments M.M. Tsehnovitser discovered: 1 When the body comes in contact with *M.tuberculosisi* formed tuberculosis centre. Infection meets local tissue reaction and in incubation period formed sensitization. In this state the body manifested as a natural susceptibility and resistance to infection. During this period organism going through the initial stage of allergy. 2. Meanwhile, the infectious process goes on and the *M.tuberculosisi* giving rise. The body reacts to this change in the formula blood - leukocytosis, monocytosis, eosinophilia. Tuberculosis focus represents a formed granuloma. This phase of tuberculosis infection accompanied by severe allergy. 3. Then there are two versions of the process. In the first case happened the generalization of tuberculosis infection. The blood reacts are leukopenia, monocytosis, eosinophilia and lymphocytosis due to toxic processes. In the second case *M.tuberculosisi* multiplied only local in the granuloma and is not generalization of tuberculosis process. In this case, natural immunity is raised. There are allergy and positive anergy in later. 4. It is exclusively unique phenomenon for tuberculosis process is the regression of the fire with his sterilization. This type of tuberculous process is in BCG-infection. In the source of infection observed complete resolution of pathological tissue, blood initially reacts slightly, but quickly comes back to normal. There is a natural infectious immunity and allergic states. M.M. Tsehnovitser made the following conclusions: 1. Tuberculosis allergy arise in infected organism as index and indicator of infection, accompanied by acquired immunity. 2. Receptive cells at the time of exposure to tuberculosis antigens produced toxic complex (such as histamine) that caused local tissue sensitization (by action on the walls of capillaries and nerve endings) and total allergic reactions. 3. Tuberculosis allergy stimulates innate immunity. 4. Receptive tissue and blood cells at the time of contact with tuberculosis allergens intensified sharp phagocytic reaction which retards the spread in the body of the allergen. 5. Tuberculosis allergy plays a role in the body's fight against tuberculosis.

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Key words: M.M. Tsehnovitser, tuberculosis, allergy, sensitization.