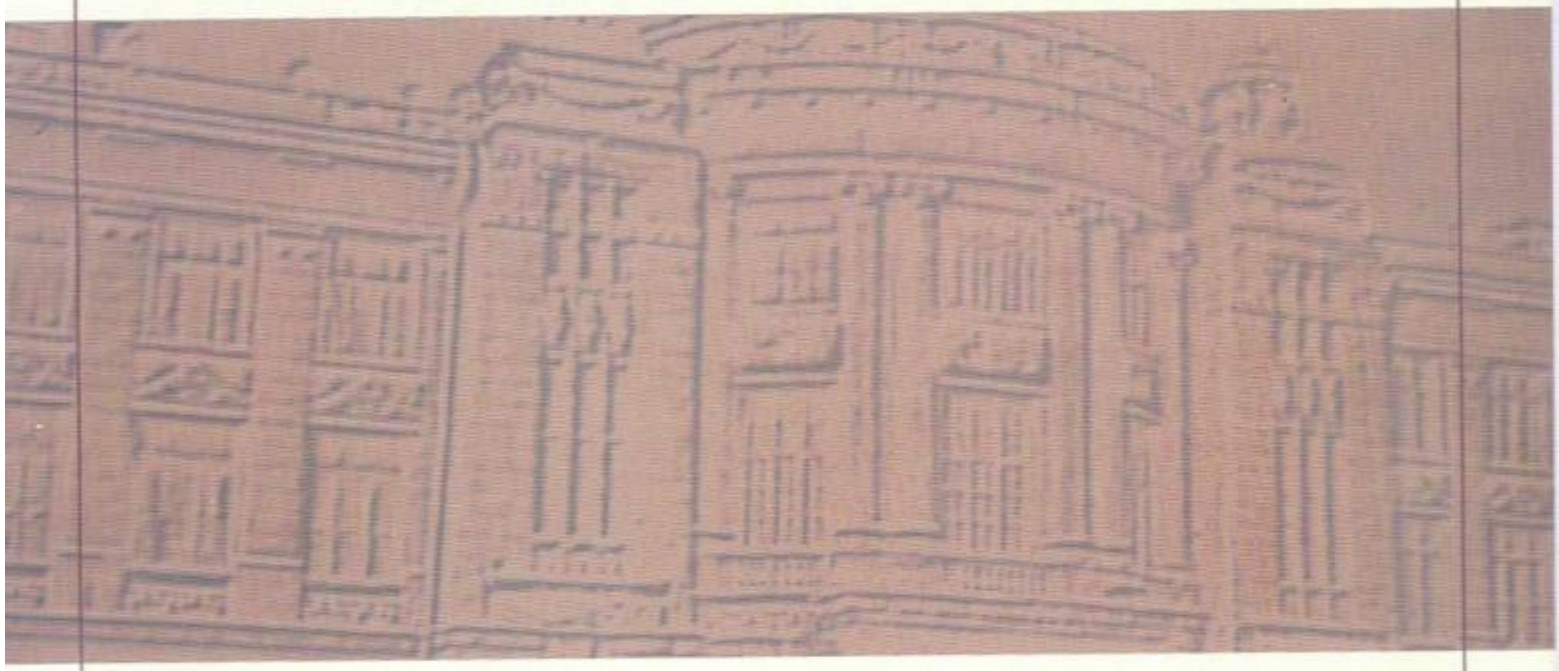
АНАЛИ МЕЧНИКІВСЬКОГО ІНСТИТУТУ



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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 elektrophoregram 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 elektroforegram defined the quantitative composition of proteins, their total weight and percentage of trivalent subunit vaccine Inflyuvak 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

Rybalkin M. V., Strilets O. P., Strelnikov L. S., Kalyuzhna O. S.

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 candidiases 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.
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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 doulble 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**

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 SPhU 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, Sb=0,01473, a=1.5282, Sa=1.4956, S_0=0.5486, r=0.9992). It is proved that the validated method characterized by sufficient convergence and accuracy over the entire range of concentrations (ΔZ=1.03, δ%=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., Криськів 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 expressionof hemolytic properties that may be an obstacle to their regular use. Level haemolyticity of bile acids 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-7α, 12α-dihydroxy-5β-cholanic 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. **28-33**
Materials and methods. The antimibicrobial activity was meseared 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 cholanic 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 3α/β predecessors. αCyclBut showed a greater influence on growth delay of *Candida albicans, Escherichia coli and Pseudomonas aeroginosa.* 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.
Conclutions. All compounds have showed a wide range of antimicrobial activity and might be used as potential antibiotics. αCyclBut, βCyclBut, β3Me-Ph caused the greatest growth delay of microorganisms.
Keywords: **bile acid, 3-acylated α- stereoisomers and β 3-amino-7α, 12α-dyhydroxy-5β-cholanic acid antimicrobial activity.**

МЕДИЦИНА (MEDICINE)

EFFECT OF CYTOKINE CEREBROPROTECITON 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 port-ischemic complications in DM. In case of ischemic dmaage 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 disregulation of mitochondria due to the matrix **34-39**

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 die 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., Костиря І.А., Болецька Т.А.

PECULIARITIES OF THE BABESIOUS 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 worldbased 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 competante 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 asquired in temperate regions of the USA and Europe. That a tick-transmitted protozoan parasite, one of the causative agents of piroplasmosis (babesiosis). The disease is characterized by sings 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 innoculated by infected ticks develop into schizonts within lymphocytes where they maltiply and subsequently transform into microzoites, which then invide erythrocytes. **Purpose** of the experiment's to study the influence of the babesious infection to nonliner female mice stomach structure.

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

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 microthopography 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-hystiocellular 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

Kolyada T.I., Vdovichenko N.I., Tupotilov A.V., Kolyada O.N.

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.

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 Manchini. 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 CD_3+, CD_4+, CD_8+, CD_{16}+, CD_{19}+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 indcators occured 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 та 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 contest of increased levels of IFN – γ and IL-4, a reliable increase of CD_{19}+ 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 CD_8+, CD_{16}+ 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 CD_4+ cells with simultaneously low number of CD_{16}+ 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 contest 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

Кучма И.Ю., Овчаренко С.В., Коляда О.Н., Почуева Т.В., Ямпольская Е.Е.

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

Kuchma I.U., Ovcharenko S.V., Pochyeva T.V., Kolyada O.N.,Yampolskya K.E.

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 discutions. 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

Волянский А. Ю., Конорева Е. С.

THE PREVALENCE OF INFECTION WITH HERPES VIRUSES AMONG FREQUENTLY ILL CHILDREN

Volyansky AY, Konoreva E.S.

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 ± 0,92 episodes a year. The average duration of an episode of disease was 9,12 ± 2,75 days. The complicated course occurred in 32% of cases, the average duration of a complicated episode grew to 12,37 ± 3,91 days.

56-59

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

АЛЕРГІЯ І ПРОТИТУБЕРКУЛЬОЗНИЙ ІМУНІТЕТ В ПРАЦЯХ М. М. ЦЕХНОВІЦЕРА

Кучма І.Ю., Моісеєнко Т.М.

UDC 616-002 .5:612.017.1:616-056 .3(091)

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

Kuchma Y.U., Moiseenko T.M.

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.tuberculosis*i 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.tuberculosis*i 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.tuberculosi* 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 tuberculous 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.

60-65

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

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АНТИМІКРОБНА АКТИВНІСТЬ СТЕРЕОІЗОМЕРІВ ПОХІДНИХ ХОЛАНОВОЇ КИСЛОТИ В ПОРІВНЯННІ З ХОЛЕВОЮ КИСЛОТОЮ

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У статті представлено результати вивчення антимікробної активності ацильованих похідних 3α та 3 β аміно холанових кислот. Сполуки виявили вищу антимікробну активність у порівнянні з їх попередниками. Наявність та спектр антимікробної активності виявляли методом серійних розведень у щільному поживному середовищі та показали як бактеріостатичний так і бактеріцидний ефекти.
Ключові слова: жовчні кислоти, 3-ацильовані α- та β стереоізомери 3-аміно-7α,12α-дигідрокси-5β-холанової кислоти, антимікробна активність.

Поява багатьох штамів бактерій з множинною лікарською стійкістю загострила актуальність створення новітніх протимікробних препаратів. Катіонні пептидні антибіотики, що були виділені з різних організмів, отримали значну увагу через їх широкий спектр дії [1]. Як правило, мембрани бактерій були об'єктами дії цих антибіотиків [2]. Серія мембрано-активних катіонних похідних холевої кислоти з антимікробною дією можуть мати спільні аспекти механізму дії з раніше знайденими катіонно-пептидними антибіотиками [3,4]. Бактерицидна дія деяких із цих сполук була виявлена для широкого спектра грам-негативних і грам-позитивних мікроорганізмів, інші похідні холевої кислоти були слабо активні відносно грам-негативних мікроорганізмів, але ефективно проникали через зовнішні мембрани і підвищували чутливість бактерій до гідрофобних антибіотиків, таких як еритроміцин і рифампіцин. Для охарактеризування сполук групи хоанової кислоти було визначено антибактеріальну активність проти бактерій з множинною лікарською стійкістю, у тому числі як грам-негативних, так і грам-позитивних мікроорганізмів. Крім того, було охарактеризовано здатність усіх похідних жовчних кислот за різним ступенем сенсибілізації грам-негативних бактерій з множинною лікарською стійкістю. Вищезгадана здатність холевих кислот може бути використана для збільшення сили дії антибіотиків проти цих організмів [5].

Негативною особливістю мембрано-активних протимікробних агентів є частий прояв гемолітичної властивості, що може бути перешкодою для їх широкого практичного застосування [2]. Рівень гемолітичності похідних жовчних кислот варіював від високо гемолітичної до слабко гемолітичної [3].

Метою роботи було вивчити нові стереомерні сполуки — похідні жовчної кислоти з слабкою гемолітичною активністю та потенційно потужною антимікробною дією [3].

Жовчні кислоти мають супресивну дію на мікрофлору, тому було цікаво дослідити активність похідних раніше синтезованих стереоізомерів α/β-аміно похідних жовчних кислот і з'ясувати вплив таксономічно різних представників мікрофлори[1-9]. Згідно раніше проведеного дослідження відносно оптичної будови і фармакологічної дії α/β-аміно стереоізомерів похідних сполук, було виявлено, що жовчні кислоти змінюють свої фармакологічні властивості в залежності від стерео-конфігурації, через це для продовження аналізу жовчних кислот було взято похідні вже досліджених стереоізомерів амінофункціоналізованої холевої кислоти [10]. 3D структури 3β-аміно-7α,12α-дигідрокси-5β-холанової кислоти і 3α-аміно-7α,12α-дигідрокси-5β-холанової кислоти представлені на рис. 1 та рис. 2, побудовані за допомогою програми “ChemBioOffice ChemBio3D Ultra 12.0”.

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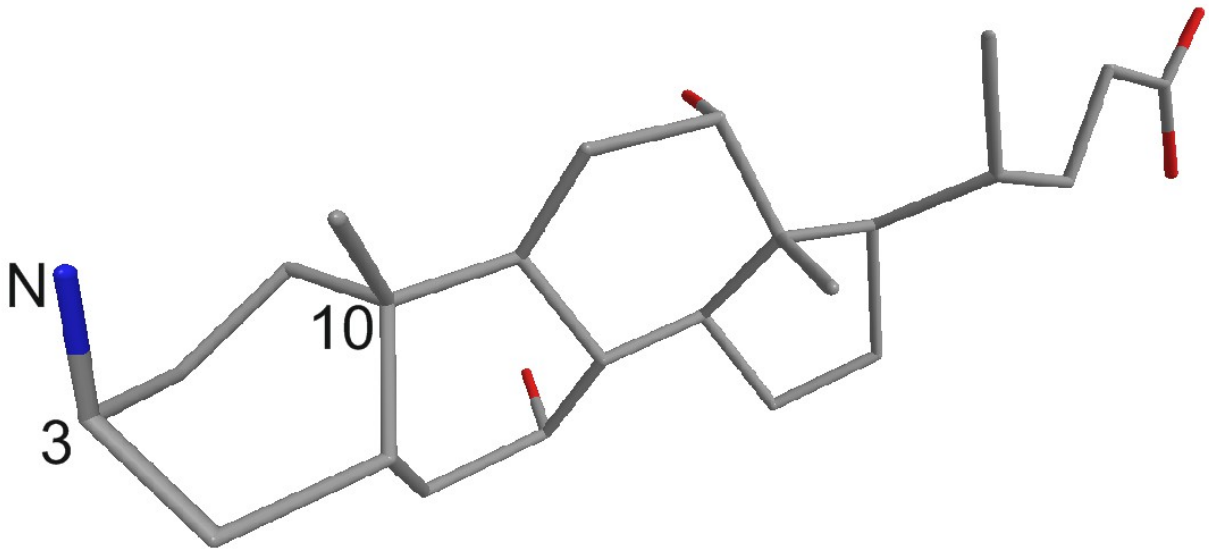

Рисунок 1. Просторова структура 3β-аміно-7α,12α-дигідрокси-5β-холанової кислоти

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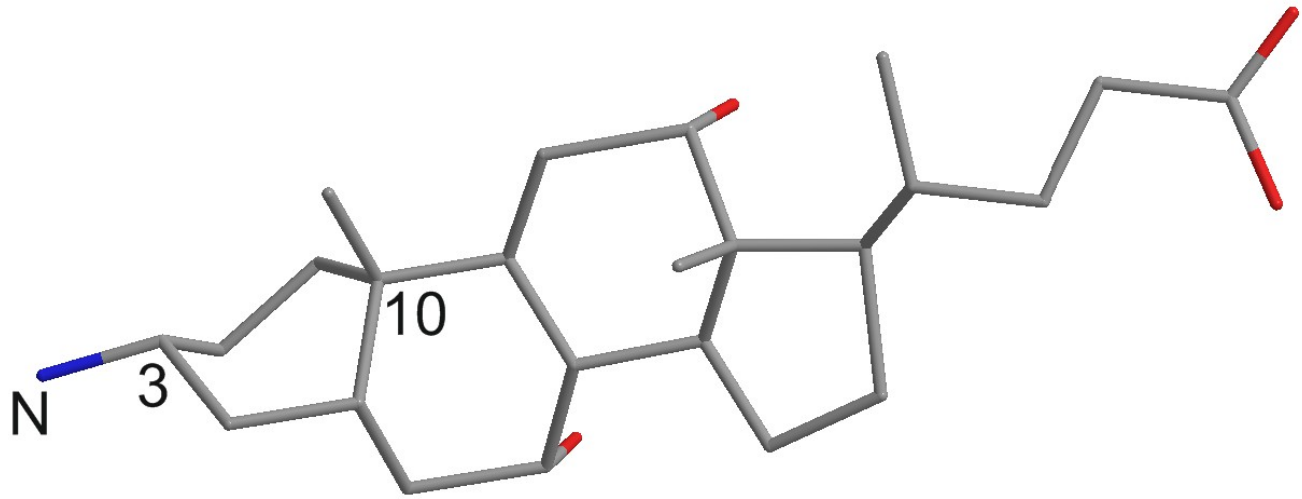


Рисунок 2. Просторова структура 3α-аміно-7α,12α-дигідрокси-5β-холанової кислоти

В 3D-моделі 3β-аміно-7α,12α-дигідрокси-5β-холанова кислота має більш несприятливе положення аміногрупи при 3-му атомі карбону та потребує більш великої кількості енергії активації та жорсткіших умов реакції, ніж 3α-ізомер, що вірогідно може обумовити блокування бактеріального метаболізму. Так реакції за участю 3β-аміногрупи ускладнені через її екранування об'ємним замісником у положенні 10 стеранового фрагменту – групою CH_3 (рис. 1) на відміну від 3α-аміногрупи, яка спрямована у протилежний бік.

Матеріали та методи

Вивчення антибактеріальних властивостей сполук проводили методом дифузії в агар в лабораторії кафедри мікробіології Національного фармацевтичного університету. Відповідно до рекомендацій ВООЗ для оцінки активності препаратів використовували референтні тест-штами: *Staphylococcus aureus* ATCC 26923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Candida albicans* ATCC 885/653, *Proteus vulgaris* ATCC 4636. Мікробне навантаження складало $1{,}5x10^8$ мікробних клітин на 1 мл середовища та встановлювалось за стандартом (0.5)McFarland. В роботі використовували 18-24 годинну культуру мікроорганізмів. Для досліджень використовували агар Мюллер-Хінтона (Дагестанський НДІ поживних середовищ). За методикою «колодязів» проводили визначення антибактеріальної активності на двох шарах щільного живильного середовища, розлитого в чашки Петрі. У нижньому шарі використовували «голодні» не засіяні середовища, складові: агар-агар, вода, солі. Нижній шар представляє собою підкладку висотою 10 мм, на яку горизонтально встановлювали 3-6 тонкостінних циліндрів з неіржавкої сталі діаметром 8 мм і висотою 10 мм. Навколо циліндрів заливали верхній шар, що складався з живильного агаризованого середовища, розплавленого та охолодженого до 40-45 ^{0}C, в яке вносили відповідну дозу добової культури тест-мікроба. Попередньо верхній шар добре перемішувався до утворення однорідної суспензії. Після застигання верхнього шару циліндри стерильним пінцетом витягали з лунки, що утворилась, вносили випробувану речовину з урахуванням її об'єму. Діаметр середовища для верхнього шару коливався від 14 до 16 мм. Чашки підсушували 30-40 хвил. при кімнатній температурі та ставили в термостат при температурі 37±0,5 ^{o}C впродовж 18-24 годин для бактерій, для дріжжоподібного гриба *Candida albicans* – протягом 24-48 годин. Вихідний «стандарт» мікробної суспензії становив 1,5 х 10^8 мікробних клітин в 1 мл. При оцінці антибактеріальних властивостей враховували наступні критерії:

- відсутність зон затримки росту мікроорганізмів навколо лунки, а також зони затримки до 10 мм оцінювали як показник нечутливості мікроорганізмів до внесеного в лунку зразку речовини;
- зони затримки росту діаметром 10-15 мм оцінювали як малу чутливість культури до випробовуваної концентрації антибактеріальної речовини;
- зони затримки росту діаметром 15-25 мм оцінювали як показник помірної чутливості мікроорганізму до досліджуваного зразку речовини;
- зони затримки росту, діаметр яких перевищує 25 мм, оцінювали як показник високої чутливості мікроорганізмів до досліджуваного зразку речовини.

Кількісну оцінку антимікробної дії визначали методом серійних розведень. Сутність методу полягає у визначенні мінімальної пригнічуючої концентрації (МПК), що характеризує бактеріостатичні властивості об'єктів дослідження. В першу пробірку вносили концентрацію досліджуваної речовини у бульоні, яка складала 1000,0 мкг/мл, потім потім методом послідовних розведень знижували кожну наступну концентрацію. Експериментальна концентрації речовин складали 1) 500,0 мкг/мл; 2) 250,0 мкг/мл; 3) 125,0 мкг/мл; 4) 65,5мкг/мл; 5) 31,2 мкг/мл; 6) 15,6 мкг/мл; 7) 7,8 мкг/мл;8) 3,9 мкг/мл; 9) 2,0 мкг/мл; 10) 1,0 мкг/мл. В кожну пробірку вносили 0,1 мл мікробних клітин тест-штамів (*S. aureus* ATCC 26923, *E. coli* ATCC 25922, *B. subtilis* ATCC 6633, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 653/885, *P. vulgaris* ATCC 4636). Культивували при температурі (37±0,5)^{0}C впродовж 24-48 год. В пробірках із найбільшим розведенням речовини, де був відсутній ріст (немає помутніння), визначали їх МПК. З останніх 3-х пробірок, в яких не було ознак росту тест-щтама, робили висів на

поживний агар і визначали МБК (мінімальну бактерицидну концентрацію) [11-13].

Вихідні речовини (похідні 3α- та 3β–аміно-7α-12α-дигідрокси-5β-холанові кислоти) були отримані шляхом послідовного стереохімічного синтезу у лабораторії Національного фармацевтичного університету. Основні структурні фрагменти подані на рисунку 3.

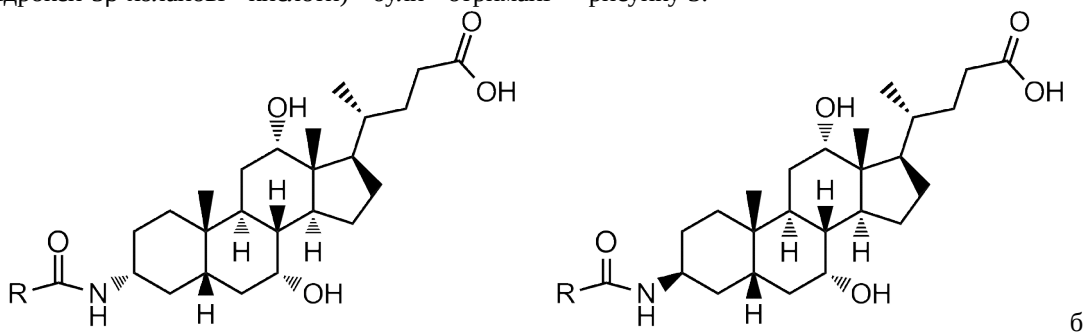
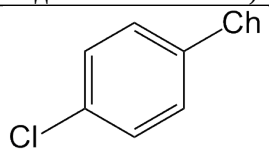
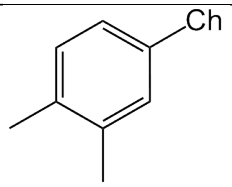
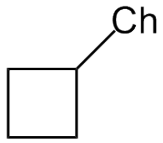
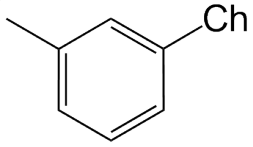


Рисунок 3. Основні структурні фрагменти похідних 3α-амінохоланових кислот(а) та 3β-амінохоланових кислот.

Назви речовин та структури замісників подані у таблиці 1. Статистичну обробку проводили за допомогою програми Microsoft Excel 2007

Таблиця 1-Будова R-3α/β-аміно-7α,12α-дигідрокси-5β-холанових кислот з поданими замісниками

Назва сполук	Структура замісника R(Ch - місце з'єднання з кислотою)
N(4-хлорфеніл)-3β– та N(4-хлорфеніл)-3α-аміно-7α,12α-дигідрокси-5β-холанова кислоти(β4Cl-Ph, α4Cl-Ph)	
N(3,4–диметілфеніл)-3β– та N(4-хлорфеніл)- 3α-аміно-7α,12α-дигідрокси-5β-холанова кислоти(β3,4(Me)$_2$Ph, α3,4(Me)$_2$Ph)	
N(циклобутил)-3β та N(циклобутил)-3α–аміно-7α,12α-дигідрокси-5β-холанова кислоти(βCyclBut, αCyclBut)	
N(3-метилфеніл)-3β– та N(3-метилфеніл)- 3α–аміно-7α,12α-дигідрокси-5β-холанова кислота(β3Me-Ph, α3Me-Ph)	

Результати і обговорення

Для порівняння впливу просторової конфігурації на антимікробну дію речовин було використано 8 сполук (4 α-стереоізомери та 4 β-стероізомери) з однаковими замісниками, що були отримані синтезом відповідної α/β-аміно холанової з відповідними ацилхлоридами. На рис 3 наведено структури самих 3 α/β аміно-7,12-дигідроксихоланових кислот.

Подані у табл. 2 результати антимікробної активності свідчать, що усі досліджувані препарати характеризуються широким спектром антибактеріальних властивостей у відношенні штамів різних таксономічних груп бактерій та грибів *C. albicans* та вищим ступенем затримки росту порівняно з їх 3α/β попередниками [10]. Всі досліджувані зразки виявили різні спектри дії, але абсолютно превалюючої субстанції встановлено не було.

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Таблиця 2-Антимікробна активність субстанцій щодо бактерій та грибів

Субстанції	**Тест культури, Діаметри зон затримки росту мікроорганізмів, мм**					
	Staphylococcus aureus ATCC 26923	*Escherichia coli* ATCC 25922	*Pseudomonas aeruginosa* ATCC 27853	*Proteus vulgaris* ATCC 4636	*Bacillus subtilis* ATCC 6633	*Candida albicans* ATCC 653/885
β4Cl-Ph	24.40±1.10	23.30±1.2	16.40±1.1	21.50±1.2	22.70±1.1	21.60±0.45
β3,4(Me)$_2$Ph	31.30±0.8	28.20±1.0	15.40±0.7	21.30±1.2	23.40±1.2	22.00±0.5
βCyclBut	26.40±1.0	26.10±1.1	16.70±1.1	27.10±1.0	26.50±1.2	24.60±1.1
β3Me-Ph	32.30±1.3	24.70±0.9	18.40±0.9	23.60±0.9	27.10±1.2	25.10±1.1
α4Cl-Ph	22.00±0.9	25.30±1.2	17.10±0.95	19.60±0.95	17.50±1.05	20.10±1.15
α3,4(Me)$_2$Ph	20.10±1.1	26.50±1.1	17.90±1.0	20.20±0.9	19.10±0.7	23.40±1.0
αCyclBut	29.30±1.1	28.70±0.7	19.50±1.1	24.70±1.1	20.40±0.9	27.60±0.9
α3Me-Ph	21.40±1.1	24.90±1.1	16.10±1.0	21.50±0.9	20.70±0.9	23.30±0.8
холева кислота	16.00±0.57	18.20±0.1	17.00±0.7	6.50±0.76	08.50±0.29	09.85±0.46

Примітка. n=3

Майже усі α ізомери виявили слабшу антимікробну дію відносно *P. vulgaris, B. subtilis, S. aureus* порівняно з їх β аналогами та у абсолютному значенні. Розмір зон затримки росту сполук-ізомерів мав суттєву різницю, їх розбіг коливався у межах 2-9 мм.

Відносно інших штамів ізомери виявили співставний ступінь дії, але слід зауважити, що спостерігалась тенденція зростання активності за відповідну активність холевої кислоти. Дані щодо антибактеріальної дії холевої кислоти співпали з раніше проведеними дослідженнями інших дослідників[9]. Як і було вже раніше продемонстровано, існує різниця у впливі різних ізомерів на мікрофлору[16].

Згідно таблиці 2, на якій проілюстровано вплив різних зразків на тестові штами мікроорганізмів, було встановлено, що до *Candida albicans, Escherichia coli* та *Pseudomonas aeroginosa* αCyclBut виявив більший вплив на затримку росту, β3Me-Ph — що до *Bacilus subtilis* та *Staphylococcus aureus,* а βCyclBut— що до *Proteus vulgaris.*

Визначені значення МПК (табл. 3) ілюструють бактеріостатичні властивості наведених сполук по відношенню до аеробних бактерій та грибів, що узгоджується з даними літератури про антимікробну активність похідних жовчних кислот [10].

Таблиця 3-Визначення МПК відносно аеробних бактерій та грибів

Субстанції	**Тест культури, МПК, мкг/мл**					
	S. aureus	*E. coli*	*P.aeruginosa*	*P.vulgaris*	*B. subtilis*	*C.albicans*
β4Cl-Ph	400±21.2	350±11.5	550±23.5	450±10.8	450±14.5	450±18.3
β3,4(Me)$_2$Ph	250±18.4	300±17.5	500±21.5	400±16.0	400±21.5	400±15.7
βCyclBut	200±17.4	250±19.6	400±14.4	350±15.7	350±22.0	350±18.4
β3Me-Ph	250±23.5	200±19.3	350±21.5	400±15.7	300±18.0	350±15.4
α4Cl-Ph	450±15.8	350±17.5	550±19.1	600±17.4	550±16.2	400±21.1
α3,4(Me)$_2$Ph	450±13.5	400±18.1	600±17.5	450±18.4	500±16.9	450±17.4
αCyclBut	200±21.5	350±13.5	450±17.4	350±14.4	300±17.4	350±14.8
α3Me-Ph	300±17.5	450±16.0	700±21.5	550±13.5	500±18.3	450±21.5
холева кислота	550±17.4	350±15.3	500±18.5	>1000	>1000	850±13.5

Примітка. n=3

За результатами наших досліджень вдалося підтвердити літературні данні відносно МПК холевої кислоти [9]. Усі зразки проявили бактеріостатичну дію до *P. vulgaris* та *B. subtilis* на відміну від холевої кислоти, а до *C. albicans* значення МПК для всіх речовин були у 2 рази нижче ніж у вихідної холевої кислоти з якої вони були отримані.

Згідно значення МПК для деяких зразків досліджених ізомерів антимікробна дія в 2 рази перевищила дію холевої кислоти. Зважаючи на доволі велику різницю у масах молекул речовин складно порівнювати їх абсолютну ефективність, найменша молекулярна маса у βCyclBut та αCyclBut, відповідно їх МПК був менший. Для усіх сполук, якщо провести порівняння МПК та молекулярної маси,

прослідковується залежність: чим більша маса тим більше МПК. Тому можемо припусти що в перерахунку на кількість речовини, їх антимікробна дія може бути співставною.

Висновки

1. Всі сполуки виявили широкий спектр антимікробної дії та можуть буди використані як потенційні антибактеріальні препарати
2. αCyclBut, βCyclBut, β3Me-Ph викликали більшу затримку росту мікроорганізмів

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

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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 expressionof hemolytic properties that may be an obstacle to their regular use. Level haemolyticity of bile acids 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-7α, 12α-dihydroxy-5β-cholanic 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.

Materials and methods. The antimibicrobial activity was meseared 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

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obtained by synthesis of corresponding α/β-amino cholanic 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 3α/β predecessors. αCyclBut showed a greater influence on growth delay of *Candida albicans, Escherichia coli and Pseudomonas aeroginosa.* 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.

Conclutions. All compounds have showed a wide range of antimicrobial activity and might be used as potential antibiotics. αCyclBut, βCyclBut, β3Me-Ph caused the greatest growth delay of microorganisms.

Keywords: **bile acid, 3-acylated α- stereoisomers and β 3-amino-7α, 12α-dyhydroxy-5β-cholanic acid antimicrobial activity.**