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ЗМІСТ (Contents)

Редакційна рада (Editorial Board)

Зміст (Contents)

ОГЛЯДЫ

Rewiews

C. (P.)

1

2-11

МЕТОДИ КОНТРОЛЮ БЕЗПЕЧНОСТІ ДИФТЕРІЙНОЇ ВАКЦИНИ

Ісаєнко О. Ю., Бабич Є. М., Єлисеєва І. В., Ждмарова Л. А., Білозерський В. І., Колпак С.А.

12-17

METHODS OF CONTROL DIPHHTHERIA VACCINE SAFETY

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Vaccination success depends not only on the timely coverage of threatened contingents, but also on the quality of vaccines. Every day, the requirements for security guarantees vaccines and their use guarantees of security increases. For the fast, reliable and independent scientific assessment of vaccine safety issues, WHO in 1999 created the Global Advisory Committee on Vaccine Safety. To enhance the capacity of pharmaceutical supervision in relation to vaccines in 2012 it was developed the Global Vaccine Safety Initiative. The main directions of the Global Vaccine Safety programs are considered in this review. It's noted more strict requirements of Ukrainian pharmaceutical industry to produce public immunization drugs regulated Supplements to the State Pharmacopoeia of Ukraine, in comparison with other countries. This review considered diphtheria vaccine safety monitoring in the process of production according to the recommendations of the World Health Organization (WHO), described a subcutaneous method for determining the specific toxicity of the combined purified toxoid, characterized an intracutaneous method of determining of the presence of diphtheria toxin in each sample of the combined purified toxoid, that additionally used by some manufacturers. The definition of diphtheria toxin in dilutions of purified toxoid is presented. This review considered diphtheria vaccine safety monitoring in the process of production according to the recommendations of the World Health Organization (WHO), described a subcutaneous method for determining the specific toxicity of the combined purified toxoid, characterized an intracutaneous method of determining of the presence of diphtheria toxin in each sample of the combined purified toxoid, that additionally used by some manufacturers. The definition of diphtheria toxin in dilutions of purified toxoid is presented. As methods for determination of diphtheria toxin must be able to detect even a small amount of toxin it's examined the WHO's proposal to use an intradermal method on guinea pigs and tests on cell cultures. Attention is drawn to the fact that the determination of specific toxicity in cell culture can be carried out at presence of the approval of this method of a national control authority and sensitivity rates no less than in experiments on guinea pigs. The determining of specific toxicity of ready vaccine by subcutaneous method is described. The publication gave a test for elevated toxicity of the final product by intraperitoneal infection of mice and guinea pigs. It's cited the WHO recommendations aimed at removing the possibility of recovery of the refined toxin toxicity. Checking vaccines toxicity, pyrogenicity, sterility, allergenicity, teratogenicity, mutagenicity and immunogenicity mainly performed on laboratory animals. The review examined the unreliability of animal experiments and the need to find alternative methods for determining the toxicity without their use particularly in light of the "3R" concept. Methods for determining diphtherial toxin using cell cultures is considered, namely, colony overlay test (COT), tests using a monolayer of HeLa cell culture, a culture of Vero cells (kidney cells of african green monkeys), a culture of CHO cells (cells of Chinese hamster ovary), which are based on the toxin cytopathic effect on sensitive cell culture. Their advantages and disadvantages are listed. An alternative method for the quantitative detection of C. diphtheriae toxin using the polystyrene plate coated with monoclonal antibody to the part of the diphtheria toxin which defines its binding to the cell, is described. It's regarded the cytotoxic effect of diphtheria toxin on cells of the immune system of mice and guinea pigs: splenocytes, adhesive phagocytes і B- lymphocytes.

Keywords: diphtheria vaccine, safety, diphtheria toxin, toxoid, cell culture, laboratory animals.

АНАЛІТИЧНЕ ОБҐРУНТУВАННЯ ВКЛЮЧЕННЯ ПРОТИВІРУСНОГО ПРЕПАРАТУ В СХЕМУ ЛІКУВАННЯ ПАЦІЄНТІВ З ПІДОЗРОЮ НА ГОСТРЕ ВІРУСНЕ ЗАХВОРЮВАННЯ

Соловйов С. О., Дзюблик І.В., Заліська О.М., Сахно Г. О.

18-26

ANALYTICAL JUSTIFICATION OF INCLUDING THE ANTIVIRAL DRUG INTO TREATMENT SCHEME FOR PATIENTS WITH SUSPECTED VIRAL DISEASE

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Background: Viruses play a leading role in human pathology development, causing a large number of infectious diseases in acute, persistent or chronic forms. Although the number of deaths caused by viral infections have decreased significantly today, they continue to be a significant factor in reducing of the population overall productivity. Viral diseases cause additional losses in community related to the duration of the course or disease or its chronization, increased use of health care, loss of working hours, premature death etc. Introduction of the new antiviral drugs into medical practice is accompanied by the emergence of questions to assess its effectiveness and including into existing clinical protocols. So the aim of this work is the development of methodology of choosing and justification of optimal treatment strategy for viral diseases that could be included into certain clinical protocols for managing patients with certain viral diseases. Methodology justification: The methodology based on the method of pharmacoeconomic analysis "cost of illness", takes into account the economic burden of viral diseases: direct costs for treating of disease, indirect costs related to the disease and intangible costs. Algorithm of treatment scheme choice depends on the cost of treatment for the patient without viral disease also as for patient with viral disease. It was proposed to use lower limit priori probability (critical prevalence) of viral disease as decision rule in the choice of treatment scheme. Results: Examples of the proposed methodology use show that the choice of the optimal therapeutic scheme for patients with suspected viral disease depends on the current prevalence of this disease among patients with similar clinical symptoms of the disease and its cost, depending on the chosen strategy of therapy. The proposed methodology determines the critical level of viral infection prevalence, which comparing to the current prevalence level is the criterion for inclusion of certain antiviral drug into treatment

scheme. Conclusions: The implementation of developed methodology requires a dynamic and updated database of patients with similar clinical symptoms, which will include information of etiological agent, chosen treatment scheme, number of bed days and costs associated with viral disease for each patient.

Keywords: viral infection, treatment, cost of illness, pharmaco-economic analysis, decision making.

СОВРЕМЕННЫЕ МЕТОДЫ ИССЛЕДОВАНИЯ БИОМАРКЕРОВ РАССЕЯННОГО СКЛЕРОЗА

27-33

Коляда Т.И., Зеленская А.Д., Тупотилов А.В.

CURRENT APPROACHES FOR RESEARCH OF MULTIPLE SCLEROSIS BIOMARKERS

Kolyada T.I., Zelenskaya A.D., Tupotilov O.V.

Current data concerning features of multiple sclerosis (MS) etiology, pathogenesis, clinical course and treatment of disease indicate the necessity of personalized approach to the management of MS patients. These features are the variety of possible etiological factors and mechanisms that trigger the development of MS, different courses of disease, and significant differences in treatment efficiency. Phenotypic and pathogenetic heterogeneity of MS requires, on the one hand, the stratification of patients into groups with different treatment depending on a number of criteria including genetic characteristics, disease course, stage of the pathological process, and forms of the disease. On the other hand, it requires the use of modern methods for assessment of individual risk of developing MS, its early diagnosis, evaluation and prognosis of the disease course and the treatment efficiency. This approach is based on the identification and determination of biomarkers of MS including the use of systems biology technology platforms such as genomics, proteomics, metabolomics and bioinformatics. Research and practical use of biomarkers of MS in clinical and laboratory practice requires the use of a wide range of modern medical and biological, mathematical and physicochemical methods. The group of "classical" methods used to study MS biomarkers includes physicochemical and immunological methods aimed at the selection and identification of single molecular biomarkers, as well as methods of molecular genetic analysis. This group of methods includes ELISA, western blotting, isoelectric focusing, immunohistochemical methods, flow cytometry, spectrophotometric and nephelometric methods. These techniques make it possible to carry out both qualitative and quantitative assay of molecular biomarkers. The group of "classical methods" can also include methods based on polymerase chain reaction (including multiplex and allele-specific PCR) and genome sequencing techniques (including full genome resequencing, targeted resequencing of the genome). The results obtained with these techniques became the basis for the further development of screening technologies. Disadvantages of the "classical" methods are associated not only with their resolution or other technical limitations but also to the fact that the range of pathological processes in MS may vary significantly from patient to patient and single biomarkers suitable for one group of patients may be inappropriate for another group of patients. Due to the complexity of MS the reflection of pathological changes may be determined not by single biomarkers but by isolated biomarkers panel from different compartments. The solution of this problem seems to be possible due to the development of microarray methods including biochips technology. Biochips are used for screening of MS patients and allow determining the rare MS-associated gene variants that have a significant impact on the development of the disease. In conjunction with the "classical" methods, microarrays allowed to apply systems biology approaches (i.e. genomics, transcriptomics, proteomics, metabolomics, epigenomics) in the study of MS biomarkers. Addition of bioinformatics methods to "classical" and microarray laboratory methods allows not only to find new biomarkers but to identify complex patterns of biomarkers while single biomarkers informative value is not sufficient. To date, the use of genome-wide association study (GWAS) revealed more than a hundred genetic variants associated with the development of MS, while the total number of investigated genetic variants including the candidate ones exceeded two hundred. GWAS is used to identify correlations of genetic variants with the disease, including the identification of variants associated with a risk of developing MS, but cannot answer the question of the causal links between specific genes polymorphism and the pathogenesis of MS. Current studies of biomarkers of disease severity, progression, pathogenetic type and treatment efficacy are based on transcriptomics, proteomics and metabolomics technologies. Transcriptomics includes genome-wide research of RNA sequences based on the results obtained with comparative genomic hybridization on biochips, massive parallel RNA sequencing, and measuring the amount of mRNA by real-time PCR. This technology is actively used in studies of gene expression profile of peripheral blood mononuclear cells from MS patients aimed at identifying molecular markers of disease status suitable for clinical use. Proteomics is a large-scale expression and protein distribution studies in patients with MS based on the results obtained via microarray and mass spectrometry, liquid and gas chromatography methods. In recent years, a growing number of MS proteomic studies using 2DE-MS method (two-dimensional electrophoresis coupled with mass spectrometry). Metabolomics studies of low-molecular-weight metabolic profiles based on the results obtained by mass spectrometry, liquid and gas chromatography, nuclear magnetic resonance. However, unlike other «-omics»-technologies, in metabolomics microarray-techniques are not used. Conclusion. Search, verification and clinical application of biomarkers for multiple sclerosis are one of the most challenging medical and biological problems. Its solution requires an interdisciplinary approach, organization of large-scale research and engagement of new research methods. In recent years, a significant amount of data received allowed to reveal hundreds of candidate biomarkers. Some of these biomarkers have significant potential for the monitoring of disease activity and assessment of therapy efficiency. However, the verification is required for a widespread clinical application; it implies further large-scale studies in different countries. The development of personalized medicine in Ukraine, the application of its principles to the management of multiple sclerosis patients, along with the use of advanced "classic" biomarker research methods requires the introduction of modern methods including the use of new-generation biochips, genomics technologies, proteomics and metabolomics.

Keywords: multiple sclerosis, biological markers, mononuclear phagocytes, genomics, proteomics, metabolomics

ЕКСПЕРИМЕНТАЛЬНІ РОБОТИ

Experimental papers

COMPOSITE MATERIALS BASED ON ZINC SULFIDE AND ZINC OXIDE: STRUCTURAL AND BIOCIDAL PROPERTIES

34-39

Sukhodub L.B., Khrystian G.E., Sukhodub L.F., Shulga N.M., Meshkov A.M., Kazmirchuk V.V., Martynov A.V.

Introduction. The widespread use of drugs with antimicrobial action has led to the formation of microorganism resistance against wide range of antibiotics. One of the approaches to dissolving this problem is the substances modification by inorganic bioactive ions in order to initiate a controlled reaction in the bone tissues and provision of antimicrobial activity. It is known that ZnO-based materials have a pronounced biocompatibility, they are characterized by high limit strength, absolute mechanical hardness, as well as the ability to withstand the harsh operating conditions. The aim of this work is the study of structural and biocidal properties of composite material

based on zinc oxide and zinc sulfide (ZnS-ZnO) and its complex with an organic substance - sodium alginate (ZnS-ZnO-Alg) for use in biomedical purpose. Materials and methods. For the synthesis of ZnS-ZnO composite 50 ml 0.2M solution zinc nitrate was added to the 50 ml 0.2M thiourea CS (NH₂)₂ solution and stirred in a shaker for 60 minutes. The formation of the compound took place when added to a mixture of 25 mas.% solution of ammonia with the subsequent heating at 80 oC for 30 minutes. Synthesis of the metalorganic complex of ZnS-ZnO-Alg was performed by above mentioned procedure, but to the thiourea solution was previously added 1 ml of 3 mas.% solution of sodium alginate under ultrasonic mixing.. For the next research composites were dried or lyophilized. Study of antibacterial activity of the ZnS-ZnO and ZnS-ZnO-Alg particles was carried out with the use of nutrient mediums: Muller Hinton, meat-pepton nutrient (MPN). As the reference cultures were used E. coli ATCC 25922, S. aureus ATCC 25923, S. aureus ATSS 29213, S. aureus ATSS-6538, C albicans ATCC 885-653. Determination of the minimum bactericidal concentration (MBC) was carried out by a modified serial diluted method in liquid nutrient broth followed plating on solid Muller Hinton nutrient medium. In addition, the study of the sensitivity of the above listed microorganisms to the experimental samples was carried out by the method of diffusion in Agar in the modification of the wells. The crystallinity and structure of precipitates were examined using an X-ray diffractometer DRON 3. The elemental composition of synthesized samples was studied by an X ray fluorescence (XRF) analysis using ElvaX Light SDD spectrometer. Results and discussion. X-ray structural analysis indicate that in the composite material, synthesized both in the presence of sodium alginate (ZnS-ZnO-Alg) and without sodium alginate adding (ZnS-ZnO) exist two phases: ZnS and ZnO. Based on RFA calculations show that ZnS-ZnO sample contains up to 50 wt. % zinc oxide. Zinc oxide content in the ZnS-ZnO-Alg is about 25 wt.%. The MBC was determined by above described method placing the liquid from each tube with a sample on a Mueller-Hinton solid culture medium. MBC of ZnS- ZnO-Alg samples against all studied microorganism strains was 1,25 mg/ml. MBC of ZnS- ZnO samples ranged from 5 mg/ml for C. albicans to 12.5 mg/ml for E. coli. It is possible that due to small sample solubility in experimental conditions and small ion diffusion of the active substance there was no full contact with the whole bacterial cell volume. As ZnS-ZnO-Alg samples differ from ZnS- ZnO samples by smaller crystallite size and greater solubility, they exhibit a marked antimicrobial effect. At the same time in direct contact of the entire surface of the sample with bacterial cells under condition of the modifying method of diffusion into agar, both types of samples showed high antimicrobial activity. Obtained data can be explained by two major mechanisms of the antimicrobial action ZnO and ZnS: a) toxic effects of zinc ions in the cell membrane of bacteria; b) toxicity ROS (reactive oxygen species), formed with the participation of ZnO and ZnS, on components of the bacterial cell. Antibacterial activity is the result of the formation such ROS, as hydrogen peroxide (H₂O₂), peroxide anion (O₂⁻), hydroxyl radicals (OH⁻). These particles damage cellular components such as DNA, lipids and proteins. Conclusion. The composites ZnS- ZnO and ZnS-ZnO-Alg have been obtained by the applied method of synthesis. X-ray structural analysis of samples proved the presence of ZnO and ZnS phase with defined structure: ZnS has a cubic crystal structure type sphalerite (JCPDS 5-566) with average crystallite size of 23 nm and ZnO - hexagonal structure (JCPDS 80-75) with an average size of about 35 nm. The introduction of sodium alginate to the reaction mixture during synthesis reduces the size of ZnS and ZnO crystallites to 10 nm and 12 nm, respectively. In the ZnS-ZnO-Alg samples, synthesized in presence of sodium alginate, the ZnS phase content increased for 25wt.% compared with the ZnO phase, which was confirmed by XRF. Microbiological studies have shown the presence of antimicrobial activity of samples against Gram-positive S. aureus, Gram-negative E. coli and fungi C. albicans. The estimated values for the integral antimicrobial activity, calculated by the vector theory, are for ZnS-ZnO and ZnS-ZnO-Alg 1,57 and 1,9 respectively. It means that both types of samples have average antimicrobial activity.

Keywords: zinc sulfide, zinc oxide, antimicrobials, structural properties

CYTOKINE DISBALANCE AT HERPESVIRUS MYOCARDITIS

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Viral myocarditis is a heterogeneous group of diseases not only by etiologic factors, which belong to different families of Vira kingdom, but is also characterized by a unique mechanism of inflammatory process and cytokine levels specific for each of them. According to numerous researches in cardio-immunology, at herpesvirus infection of the cardiovascular system occur both systemic and localized violations of the immune response. Unfortunately, the accessible literature did not provide the data analysis of complex cardio-immunological research that would take into account the features of herpesvirus myocarditis clinical course. This grounds relevance of immunodiagnosis directed on the exposure of dysimmunities by study of indices of general and local immunity with the estimation of the immune status in patients depending on the stage of exasperation or relapse of chronic herpetic infection in the complex of diagnostic tests. The purpose of our research was to determine features of the state of the immune system with the complex analysis of cytokine profile data, immune and interferon statuses in subacute and chronic forms of herpesvirus myocarditis. Materials and methods. 87 myocarditis patients who were receiving inpatient treatment in medical establishments of Kharkiv were examined. The average age was (M ± m) 36 ± 3,46 years old. The diagnosis of myocarditis was established according to the order № 436 by the Ministry of Healthcare of Ukraine from 03.07.2006 of clinical findings protocol. In accordance with the term of myocarditis clinical course, the patients were divided in two sub-groups: 44 patients with subacute (from 2 to 6 months), and 43 patients with chronic (over 6 months) clinical course of viral myocarditis. The control group correlated with patients of basic group by age and gender and consisted of 40 practically healthy persons without implications of cardiac pathology. Definition of cytokine concentration: IL-2, IL-4, IL-6, IL-10, INF-γ, TNF-α in blood serum was conducted by the method of solid-phase enzyme-linked immunosorbent assay, population structure of lymphocytes with different antigenic determinants (CD3+, CD4+, CD8+, CD16/56, CD19+, CD95+) was determined by monoclonal antibodies by cytofluorimetric assay. Obtained data processing was conducted with the use of parametric and non-parametric methods of biostatistics by programs EXEL-2003® and Biostatistics 4.03. Results and discussion. The data obtained indicates the disbalance in their system, which above all is characterized by a considerable level increase of pro-inflammatory IL-6 up to 134,09 ± 22,72 pg/ml (control level 11,83 ± 1,64 pg/ml) and in relation to moderate growth of levels of IL-2 та TNF-α at subacute myocarditis. Such increase in level of IL-6 can take place due to the change of pro-inflammatory effect to anti-inflammatory in a remote period. In a complex with IL-10 IL-6 limits the secretion of TNF-α. For this reason, its level remains high at chronic herpesvirus myocarditis and exceeds the level of the control group by over 8 times. In addition, there is an increase of levels of anti-inflammatory IL-4, IL-10 cytokines at the chronic form of herpesvirus myocarditis course by 2,9 and 3,1 times respectively. At the same time, the level of IL-10 increased not only in comparison with the level of the control group but also almost 2 times exceeded the proper index at subacute myocarditis. Instead of the predicted INF-γ level rise, its decline was discovered, in patients with subacute course the index value was the lowest. This phenomenon can be the result of mast cells activity and in its turn influences the synthesis of collagen and processes of myocardium remodeling. Analysis of sub-population composition of lymphocytes discovered the increase in number of CD3+CD95+ lymphocytes of peripheral blood at myocarditis, especially in the group of patients with subacute herpesvirus myocarditis with its level exceeding the index of the control group by 3,3 times, and at chronic course – by 2,7 times. We consider that determination of CD95+ expression already has a prognostic value during the first signs of cardiac insufficiency. An increase in level of the indicated receptor is the evidence of active rejection process of defective and infected cardiomyocytes, which clinical displays are signs of cardiac insufficiency and decline of myocardium retractive ability. Conclusion. Thus, determination of cytokine in blood serum at infectious myocarditis of herpesvirus nature has a high diagnostic value and can compete with invasion and instrumental methods of diagnostics. Disbalance in the system of cytokines at herpesvirus myocarditis is a universal reaction of the immune system which is characterized by the increased levels of pro-inflammatory cytokines against the moderate decline of anti-inflammatory, and the increase in concentration of IL-10 in combination with the level of lymphocytes of membrane phenotype CD3+CD95+ can be used as a diagnostic criterion of chronization course of

disease. Understanding the pathogenesis of viral myocarditis at cellular level matters for the development and optimization of methods of laboratory diagnostics and forms a basis for determining prognosis of a disease course and choice of treatment tactic.

Keywords: herpesvirus myocarditis, immunological disbalance.

VECTOR THEORY AND OPTIMAL CHOICE OF ANTIMICROBIAL DRUG FOR LOCAL WOUND TREATMENT

46-52

Boyko, N. N., Osolodchenko, T. P., Zhilyakova, E. T.

Introduction. One of important problems in the field of medicine and pharmacy is an optimal choice among several alternatives. For example, the choice of drugs for treatment among several analogs, selection of excipients among analogs for development of pharmaceutical forms with optimal pharmacological, technological and economical parameters, etc. The aim of the work is to show the possibility of vector theory use for optimal choice of antimicrobial drugs for local wound treatment among analogs taking into account several criteria at the same time. **Materials and methods.** For our investigation we have chosen ten drugs with antimicrobial properties for local wound treatment in different pharmaceutical forms (ointment, liniment, water and glycerin solution, tincture). We have determined antibacterial activity of drugs by agar well diffusion method on six test-stain microorganisms: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 4636, *Bacillus subtilis* ATCC 6633, and *Candida albicans* ATCC 885-653. Well diameter was 10 mm, the volume of drug in the well was 0.27 ± 0.02 ml, microbial burden of agar upper layer was 107 CFU/ml, and total layer height in Petri dish was 4.0 ± 0.5 mm. In order to integrate various qualitative and quantitative parameters into one index (vector object in multidimensional factors' space) we modify these parameters to non-dimensional normalized values. For this purpose we use a desirability theory. We have chosen the following criteria for optimal choice of the drug: antimicrobial activity (integrated index of drug's antimicrobial activity), drug's price, pharmacological and technological index, spectrum of drug's action on test strains of microorganisms studied. Results and their discussions. Using vector and desirability theory, we have obtained the following range of drugs in decreasing order: Laevomecol ointment, Ioddicerinum, Tincture of Sophora japonica, Povidone-Iodine liniment, Methyluracilum cum Myramistino ointment, Laeosinum ointment, Tincture of calendula, Ranostop ointment 10%, Myramistinum-Darnitsa ointment, and Decasanum water solution 0.02 %. **Conclusions.** In this paper we have shown the possibility of vector theory use for optimal choice of antimicrobial drugs for wound treatment among analogs by taking into account several criteria at the same time. This mathematical method together with desirability theory gives us a possibility to determine low values of desirability function (weak points) for the drug and to predict its rating when we change values of original parameters.

Keywords: vector theory, optimal choice of wound treatment drug.

HPLC FOR CONTROL STABILITY OF QUERCETIN INJECTABLE DOSAGE FORM

53-57

Martynov A.V., Batrak E.A., Kabluchko T.V.

Introduction. Quercetin is a flavone derivatives which known like a substances with vitamin activity, high antioxidant, antimutagenic and anticarcinogenic activity and many other types of biological activity. Wide usage of quercetin prevents their polyphenolic nature structure which does not allow a high bioavailability of pure quercetin when administered orally. This is associated with a wide spectrum variety of chemical reactions for the phenolic groups: from interaction with amino acid residues in proteins to reactions with amine heterocyclic alkaloids and polysaccharides. In our days Corvitin – one from the number of quercetin based drugs with sufficiently low levels all types toxicity, allergenic and has no irritating action on intravenous administration. In the same time quercetin cannot be used in full measure because of the limited number of publications with analysis methods, especially HPLC. Determining the stability over time of concentrate quercetin solution, as well as determining the stability of the concentrate to the original autoclave sterilization conditions is a promising direction in creating new drugs. **Materials and methods** The objective was to research quercetin soluble formulation samples in different conditions: 1) fresh dilute concentrate (0.9% sodium chloride); 2) the original dilute concentrate, which was stored at room temperature for 14 days in light and 3) similar to the first sample dilute concentrate, which went before breeding in autoclaving at 120 0 C for 20 minutes. The objects used in the studies were industrial drug-substance quercetin (Sinkea manufactured (China)), the original pharmaceutical composition as the soluble form of quercetin for injection and aerosol applications, glycerol (Sigma), Polysorbat 80 (Merk), ethanol 96 %. For the HPLC – analysis, chromatograph "Milichrom A-02" (SiChrom, Knauer) (Econova, Novosibirsk, Russia) was used. Results and discussion Quercetin was identified using information on its retention time and spectral relations in the UV region from the database of DB-2003 spectrums. HPLC analysis results show that the quercetin is stable in ampouled form under autoclave and storage and freshly diluted quercetin concentrate for infusion are identical. Quercetin aqueous solution which was stored at room temperature for 14 days in the light, turned out to be unstable. It was found that aqueous solutions of polysorbate-80 was full hydrolyzed to the initial compounds. **Conclusions** In this work the ability of quercetin's perspective concentrates to be stable were checked. The stability of concentrates was determined by HPLC chromatograph "Millichrome A - 02» (SiChrom, Knauer). It is shown that the HPLC methods can be used to establish the smallest difference in the samples. The quercetin's non-aqueous concentrate is capable of withstanding retorting and remains in standard indestructible state in nonaqueous media (glycerol, ethanol, polysorbate 80). Quercetin is unstable in aqueous solutions and are destroyed during prolonged storage. HPLC- chromatogram is presented in the article and show that gradient HPLC with UV- detection can be used for quality control of quercetin.

Keywords: quercetin, stability, HPLC, autoclave sterilization, polysorbate-80.

ВИВЧЕННЯ БЕЗПЕЧНОСТІ ЕКСПЕРИМЕНТАЛЬНИХ ЛІПОСОМАЛЬНИХ ВІРУСНИХ ВАКЦИН

58-64

Романова О. А., Давидова Т. В., Клыса Т. Л., Клыса А. О.

EXPERIMENTAL LIPOSOMAL VIRAL VACCINE SAFETY

Romanova O.A., Davydova T.V., Klysa T.L., Klysa A.O.

Introduction. With the transport links development there is rather important issue respiratory viral infections spread, especially influenza. The only method controlling influenza is vaccination. Search and development effective and safe vaccines is important. **Material and methods.** In base SO "Mechnikov Institute Microbiology and Immunology National Ukrainian Academy Medical Sciences" in the scientific theme "Developing new approaches to creating viral vaccines and study specific activity depending of type and degree component's modification" was created several experimental influenza vaccine with subsequent component's modification for selecting the most optimal pattern of safety and immunogenicity. In assessing the influenza vaccine safety is using a few criteria, including, reactivity, as measured by the frequency of local and systemic adverse (negative) effects, which due to its introduction, and for lipid content drugs, ability to influence oxidation processes. At present study phase was determined: a) systemic reaction and local reaction of delayed-type hypersensitivity (foot pad swelling assay); b) lipids and proteins peroxidation processes after administration official and experimental vaccines (content protein's carbonyl groups, lipid's hydroperoxides, activity of glutathione-peroxidase). Study objects were trivalent seasonal influenza vaccine, "Vaxigrip" (Sanofi Pasteur, S.A., France), "Inflexal V" (Biotech Ltd. Berne, Switzerland) and experimental vaccine samples. Highest immunogenicity vaccines had undergone improvements and modifications using adjuvant

systems and acylation influenza proteins. Liposomes 2 – the experimental influenza vaccine with a liposome negative charge and antigenic composition like split vaccines "Vaksihryp". Liposomes 2.1 - the adjuvant experimental influenza vaccine with modifications liposomal components (etony and chlorophyllipt molecules embedded in liposomal membrane). Liposomes 2.2 - the adjuvant experimental influenza vaccine further modification through acylation antigenic component. Results and discussion. Among the vaccines with the antigenic component modification and addition of adjuvants, the highest production of specific influenza antibodies was observed after administration liposomes №2.2 sample, which was made on the basis of antigen Vaxigrip with negatively charged liposomal formulation, the addition of adjuvants and modification antigenic composition, the second ranked liposomes №2.1, without antigenic modification. The study identified regarding the frequency of local reactions, assessed by visual observations, among experimental animals in injection site after legalizations vaccines or newly samples weren't characterized by the formation of swelling, hardening of tissue hyperemia or painful local reactions throughout the observation time. Experimental mice also haven't fever for the 5 days after manipulation, which is the main criterion of systemic adverse reactions after they administered vaccine preparations. Also after use of experimental drugs and drug comparison, subjective, wasn't happened abnormalities in general condition animals, including a decrease in appetite, digestive disorders, changes in activity and more. These observations, however, do not allow to conclude the complete safety newly created experimental vaccine and require additional evaluation tests. As base component for building experimental liposomal vaccine used the fosfatydlholin (FH). FH is a substrate for activation lipid peroxidation. Lecithin liposomes, that are liposomal vaccine structural and functional components, are exposed to a variety number of physical and chemical factors. One of biochemical events, that happen to them, are lipid peroxidation, accompanied by free radicals appearance in the system and, ultimately, causes phospholipid bi-layer membranes degradation by a violation of their permeability and lysis. In this regard, system safety control and liposomal drug efficacy should include the definition the content lipid peroxidation products. Conclusion. Thus, experimental samples influenza liposomal vaccine (without modification and with its for liposomal and antigenic components) haven't found increased levels primary products lipid peroxidation – lipid hydro peroxides and protein oxidation products – carbonyl protein and haven't significant effects inhibition anti-oxidant enzymes in rat's serum. More results the study stage the safety most effective vaccine samples will be present in the text.

Keywords: influenza, liposomal vaccine, safety, adjuvants

ОРГАНІЗАЦІЯ ДОСТУПНОСТІ ОБІГУ АНТИДІАБЕТИЧНИХ ЛІКАРСЬКИХ ЗАСОБІВ НА ЗАСАДАХ ФАРМАЦЕВТИЧНОГО ПРАВА В УКРАЇНІ **Зброжек С.І., Шаповалова В.О., Шаповалов В.В.**

65-71

ORGANIZATION OF AVAILABILITY OF THE CIRCULATION OF ANTIDIABETIC MEDICINES BASED ON PHARMACEUTICAL LAW IN UKRAINE

Zbrozhek S.I., Shapovalova V.A., Shapovalov V.V.

Introduction. In recent years as the global problem of healthcare in many countries acts diabetes, number of patients with this disease is growing and is already 4–6% of the population in developed countries. These indicators enable WHO experts include diabetes to one of the four priority non-infectious diseases and non-infectious epidemic of the 21st century. Because of chronic disease of diabetes decreases the quality of life of citizens, develops related diseases such as stroke, heart attack, blindness, kidney failure, amputation of the lower extremities causing deaths. Therefore, programs to combat diabetes and its prevention is a priority for national healthcare systems without exception countries. Materials and methods. Circulation of the registered antidiabetic medicines in Ukraine during pricing and delivery (example); forensic and pharmaceutical practice of the complaints and appeals on the availability for them of the antidiabetic medicines; pricing characteristics of the antidiabetic medicines over the period of 2012–2015. Methods: normative and legal, documentary, bibliographic, statistical, comparative, forensic and pharmaceutical, graphical analysis. Results and discussion. The study of organization of circulation of the antidiabetic medicines requires a systematic approach from the organizational, legal and forensic and pharmaceutical research. Today in Ukraine the arsenal of drugs for the treatment of diabetes presented with more than 85 registered antidiabetic drugs for trade names, of which 60% – insulin, and the remaining 40% – oral hypoglycemic drugs offered in a 210 release forms. Given forensic and pharmaceutical example shows that the barrier, which reduces the availability of antidiabetic medicines for diabetics at discounted prescription is mandatory registration of wholesale prices, because that price mechanism of registration by the Ministry of Healthcare of Ukraine interferes with the right of privileged contingent of citizens to timely access to vital antidiabetic drugs in full and the required range. It was also studied the availability of treatment antidiabetic medicines by pharmacoeconomic, forensic and pharmaceutical indicators and found the changes to the average price of antidiabetic drugs for the period from 2012 to 2015 according to changes of the US dollar exchange course, which reduced the availability of antidiabetic drugs for patients with diabetes. In difficult conditions, healthcare financing of urgent issues needs to achieve effective supplement of antidiabetic drugs with optimal use of funds. At the national and regional levels consistently implemented organizational and legal measures to improve the availability of antidiabetic drugs in the healthcare system for patients with diabetes: the development of a system of recovery of insulin; pharmaceutical needs for support from the targeted expenditure of regional budgets; targeted distribution of expenditures among local budgets of administrative units; control over pricing for antidiabetic drugs; optimization of implementation of the statement of the Cabinet of Ministers of Ukraine № 73 "Questions of the realization of the pilot project concerning the introduction of state regulation of prices for insulin" by drafting the amendments thereto. Conclusions. Our studies indicate the need for in-depth reform of the healthcare system by providing subsidies from the state budget of Ukraine, the increase the accessibility of antidiabetic medicines for diabetics.

Keywords: pharmaceutical law, forensic pharmacy, circulation, pharmacies, antidiabetic medicines.

ТЕОРЕТИЧЕСКИЕ И ПРАКТИЧЕСКИЕ АСПЕКТЫ ПОЛУЧЕНИЯ РЕКОМБИНАНТНЫХ АНТИГЕНОВ ДЛЯ ДИАГНОСТИКИ ЛЕЙКОЗА КРУПНОГО РОГАТОГО СКОТА **Шаповалова О.В.**

72-78

THE THEORETICAL AND PRACTICAL ASPECTS OF THE RECOMBINANT ANTIGENS FOR THE DIAGNOSIS OF BOVINE LEUKEMIA PRODUCTION

Shapovalova O.V.

Introduction. Nowadays the problem of bovine leukemia (EBL) effective diagnosis in countries where EBL is registered and the disease-free areas remains actual. The main diagnostic tests are immunodiffusion reaction (AGID) and enzyme-linked immunosorbent assay (ELISA), which allow the identification of infected animals by the presence of antibodies to bovine leukemia virus (BLV) both in serum and in milk samples. The effectiveness of these methods depends on the quality of diagnostic test systems used and determines by the cultural and recombinant virus antigens specificity. EBL recombinant antigens have certain advantages as they are more active and cheap. Purpose of the work. The analysis of theoretical and practical approaches in the bovine leukemia virus recombinant antigens development and its diagnostic potential evaluation. The article contains data from the literature on the recombinant antigens of bovine leukemia virus construction and use. Analysis of the literature showed that the recombinant proteins are widely used in the serological

diagnosis of bovine leukemia. Numerous protocols of BLV gp51 and p24 immunodominant antigens preparation has been developed in heterologous systems (*Saccharomyces cerevisiae*, *E. coli*, vaccinia virus, baculovirus). In order to obtain recombinant antigens, the BLV provirus genome regions isolated from FLK-BLV cell culture, lymphocytes or tumor cells from naturally infected cattle are typically used. For the recombinant antigens labeled by hexahistidine or Srept II purification one-step immobilized-metal affinity chromatography IMAC and highly selective Strep-Tactin affinity chromatography methods are carried out. The end products activity and specificity are studied in the immunoblotting, ELISA and AGID diagnostic reactions. The ukrainian scientists' publications are devoted to the clone *E. coli* HB101-2 transformed by the recombinant plasmid containing fully functional BLV env and gag genes nucleotide sequence construction. The efficiency of BLV gp51 and p24 encoding regions fusion-sequence integration was confirmed by the screening with the specially designed oligonucleotides. The recombinant antigen expression was induced by addition of IPTG. To isolate the antigen bacterial mass was destroyed by defrostation and ultrasonic disintegration in the experimentally selected modes. The activity and specificity of the antigen was determined by AGID with the use of the bovine fetal serum, positive and negative reference diagnostic serum by unified method in comparison with the standardized cultural BLV antigen in AGID. The antigen specificity was increased by adsorption with commercial anticolibacillosis serum. The antigen activity was confirmed by AGID. Conclusions. Nowadays the most promising BLV antigens expressing genetic constructions with *E. coli* and baculovirus. *E. coli* recombinant strains are the most available and effective using expressing system, which allows to get an active and specific antigenic product if an optimal vector constructions and commercially available systems of metal affinity chromatography purification and control with appropriate Mab are used. As an cultural and recombinant antigens alternative the mimicking critical BLV antigenic epitopes synthetic peptides were tested. In recent times many scientific works formed the basis for the bovine leukemia diagnostic test systems' creation, which are now widely available on the biotechnological products market. Although the majority of manufacturers prefer the recombinant antigens of the pathogen, pilot studies on more improved and cheaper ways to obtain different diagnostic antigens preparations shall not lose relevance. Keywords: bovine leukemia virus, bovine leukemia, recombinant antigens, diagnosis.

ОСОБЛИВОСТІ РОЗПОДІЛУ HLA У ХВОРИХ НА ПІСЛОНЕФРИТ Гайсенюк Ф.З., Дриянська В.Є., Круглік В.Т.

79-83

PECULIARITIES OF HLA-PHENOTYPES IN PATIENTS WITH PYELONEPHRITIS

Haiseniuk F., Driyanska V., Kruglikov V.

Introduction. Of great interest are the studies on the role of human leucocyte antigens (HLA) in pathogenesis of a disease. The kidneys is vulnerable to injury in the context of inflammatory responses, with the potential involvement of a number of different inflammatory processes. There are shown the associative links of the HLA antigens which stipulate the relative and attributive risks of some autoimmune diseases, with immune disorder and high production of pro-inflammatory cytokines, that confirms their important role in immunopathogenesis. The aim was to determine the value of some HLA in the development of such important disease as pyelo- and glomerulonephritis. Material and Methods. The distribution of HLA-A, B, DR antigens in 364 patients with kidney diseases (120 – pyelonephritis and 244 - glomerulonephritis) was analyzed. HLA antigens were defined using a standard microlymphocytotoxic test on the Terasakirs planchette with special panels of anti-HLA serums (20 antigens of locus A, 31 – B and 9 – DR). The control group consisted of 350 healthy donors – students from Kiev. The HLA antigen frequencies in normal and diseased subjects were compared taking each antigen separately, using χ^2 test. The etiologic fraction (attributive risk \square more 0.1) was counted using the formula: $\square = (x - y)/(I - y)$, where x is frequency of antigen in patients and y – frequency in healthy. The \square reading was considered reliable when it exceeded 0.1. Results and discussion. It is advisable to associate PN are A10, A11, B14, B16 и B17 (RR > 2); the causal role ($\sigma > 0.1$) was determined for A10, A11, B14, B16; antigens-protectors - A2, B21, B35, B40. Associated with CGN, NS (RR > 2) are with antigens HLA- A23, 24, 28; B8, 38, 44 in patients; the causal role ($\sigma > 0.1$) was determined for A24, 28; B8; antigens-protectors – B12, B16. The analysis of the associative features of HLA-phenotype and identified pathogens in patients with PN is carried out. HLA-A2 и B35 as protectors of PN associate with smaller frequency of presence the *E. Coli* in urine of patients. Conclusion. The article analyzes the peculiarities of HLA-phenotypes in patients with pyelo- and glomerulonephritis, which allowed to establish a correlation between certain genes of histocompatibility complex and susceptibility to develop some diseases of the kidneys in humans. The HLA-phenotype analysis and infection activators for PN allows to take into account the additional prognostic markers not only of disease but also of its course, that provokes more individualized approach to the therapy of patients.

Key words: HLA-phenotype, pyelonephritis, glomerulonephritis, *E. Coli*

АНТИМИКРОБНАЯ АКТИВНОСТЬ СУБСТАНЦИЙ, ПОЛУЧЕННЫХ ИЗ СЫРЬЯ РАСТЕНИЙ СЕМЕЙСТВА БЕРЕЗОВЫЕ

Федченкова Ю.А., Савинова Е.М.

84-87

ANTIMICROBIAL ACTIVITY OF THE SUBSTANCES RECEIVED FROM RAW MATERIALS OF BIRCH FAMILY PLANTS

Fedchenkova Yu.A., Savinova E.M.

Introduction. In accordance with the last events in Ukraine (considering military operations in anti-terrorist operation in the Luhansk and Donetsk regions) the domestic medicine is in great need in preparations with antimicrobial activity. Our attention as the sources of receiving biologically active substances with antimicrobial activity was drawn with birch *Betulaceae* family plants – hazel ordinary *Corylus avellana* L. and black alder *Alnus glutinosa* (L.) Gaertn. It is known that in medicine the leaves of hazel ordinary are used as antiseptic, anti-inflammatory, vesselrestorative drug, and the leaves of black alder reveal the antiinflammatory, astringent, wound healing, spasmolytic and choleric action. However, the drugs with antimicrobial action received from the leaves of these plants are absent on the market of Ukraine. Therefore the studying of antimicrobial activity of this type of raw materials received from hazel ordinary and black alder, for creation of new medicines, is now one of the main directions in pharmacy. For this purpose we have revealed tinctures, spirit, lipophilic and polysaccharid fractions received from the leaves of hazel ordinary and black alder. The purpose of our research is studying of antimicrobial activity of revealed substance received from the leaves of black alder and hazel ordinary. Materials and methods. There were being examined tinctures, lipophilic, spirit and polysaccharid fractions received from the leaves of hazel ordinary and black alder. The test of antimicrobial effect of substances was carried out by means of serial dilution concerning the following six reference cultures: *Staphylococcus aureus* ATCC 6538-P, *Candida albicans* ATCC 885-653, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6833, *Bacillus cereus* ATCC 10702, *Pseudomonas aeruginosa* ATCC 9027, according to the State Pharmacopoeia of Ukraine, in the Department of Microbiology and Immunology of KMAPE. For the experiment there was prepared tenfold dilution of extracts on meat-peptone broth 1:10 and 1:100 to which there were added the referential cultures which are grown up on appropriate differential diagnostic medium depending on a type of cultures. The crops were incubated in the thermostat at t - 35-37 °C within 24-48 hours. For identification of test-strain growth of *E. coli* there was used Endo medium, for *S. aureus* – Chistovich medium, for *B. subtilis* and *B. cereus* - meat-peptone agar, for *P. aeruginosa* - 5% blood agar and for *C. albicans* – Saburo medium. As the solutions remained muddy after incubation, for the assessment of antimicrobial action of the received fractions, there were being

made the cloning on differential and diagnostic mediums. Results and discussion. Data of the conducted researches of antimicrobial activity of various substances of hazel ordinary and black alder leaves – tinctures, lipophilic, spirit and polysaccharide fractions are given in the table. The obtained data demonstrate that lipophilic fractions of hazel ordinary and black alder leaves possessed the bactericidal activity when it was diluted in the ratio of 1:10 and 1:100 concerning *P. aeruginosa* and *E. coli*. These fractions also have antimycotic activity concerning *C. albicans*. Concerning *E. coli* it is revealed that the spirit fractions from black alder leaves had the bactericidal activity, and concerning *P. aeruginosa* and *C. albicans* – all studied spirit fractions possessed. Among polysaccharide fractions the antimicrobial activity is revealed only for this substance of hazel ordinary leaves in both dilutions concerning *E. coli*, *S. aureus*, *P. aeruginosa*. As concerning *B. subtilis* and *B. cereus*, bacteria of these strains were resistant to all studied fractions. Conclusions. Antimicrobial activity of a number of substances of hazel ordinary and black alder leaves is studied. As a result of the conducted researches it is established that lipophilic and spirit fractions of hazel ordinary and black alder leaves, tinctures from these types of raw materials revealed antimicrobial activity concerning *P. aeruginosa*, *C. albicans*, lipophilic fraction of hazel ordinary leaves, spirit fraction and tincture of black alder leaves – also concerning *E. coli*. The polysaccharide fraction of black alder leaves doesn't possess antimicrobial action, and the polysaccharide fraction of hazel ordinary leaves has shown the activity only concerning *E. coli*, *S. aureus* and *P. aeruginosa*.

Keywords: antimicrobial activity, leaf, alder, hazel, substance.

ВИВЧЕННЯ КІНЕТИКИ УТВОРЕННЯ ГРАНУЛ ЦЕОЛІТУ ПРИРОДНОГО ПРИ РІЗНИХ СПОСОБАХ ГРАНУЛЮВАННЯ

88-96

Рибачук В.Д.

THE STUDY OF THE KINETIC OF NATURAL ZEOLITE GRANULES GROWTH AT DIFFERENT WAYS OF GRANULATION

Rybachuk V.D.

Introduction. Active substances and excipients used in the manufacture of medicines in tablet form, in most cases, have poor technological properties. This fact determines the need for prior granulation of mass before compression. Granulators of various sizes and designs, running on different modes, made the formation, growth and consolidation of the powder particles that lead to obtain pellets of different shapes and sizes. From the literature it is known that granulation leads to two forms of granules: isodiametric and nonisodiametric. The first group of particles forms has globular shape with a smooth surface and the proportion in which the length, thickness and height are about the same. They are usually made by fluidized bed granulation, spray drying, pelletizing and granulation in dragee pan. Granules of nonisodiametric form in which length is several times the width and height are made mostly by extrusion and compacting. The geometrical parameters of obtained granules are affected by the properties of raw materials, the granulation modes, type and amount of added humidifier and so on. The shape and size of granules, from a technological point of view, are the key factors that contribute, except organoleptic characteristics of the product, its technological properties such as particle size distribution, bulk volume, the ability of the material to shrinkage, porosity, fluidity, mechanical strength and so on. Properly selected for specific conditions granulation method is able to provide the finished product with the specified technological parameters depending on the needs. The aim of this work was to study the effect of granulation method and its conditions on the kinetics of growth of the natural zeolite granules and some quality characteristics of obtained granules. Material & methods. As objects of study served the natural zeolite pellets produced using 3%, 5%, 7% and 10% potato starch paste and solution of polyvinylpyrrolidone (PVP). Natural zeolite granules were prepared by wet granulation using a laboratory rotary granulator NG-12, laboratory extruder, laboratory high-speed mixer-granulator and laboratory dragee pan NSD A-0.25. Microstructure and form of granules were established by electron microscope REM-106. Fractional composition and average grain size was determined using a standard set of sieves with the diameter of the holes 2.0; 1.0; 0.5 and 0.25 mm. Porosity determined on the basis of tapped density and the real density, which were defined on the device Pharma Test PTF PT-TD200, Germany by the method. Form factor calculated as the ratio of the share of grains in its width. The hardness of the granules was assessed by their ability to withstand compression. Results & discussion. The resulting granules have heterogeneous porous surface. Granules obtained using rotary granulator and extruder have elongated form of particles with form factor 1,6-1,7 and 2,5-2,8 respectively. Granules obtained in high speed mixer-granulator and dragee pan have nearly round shape with form factor 1.1-1.2. The size of the granules is affected by the concentration of the humidifier and the way of granulation. The increasing of concentrations of potato starch paste from 3% to 10%, lead to an increase of the average size of granules according to the method in average 3-5 times, the last was proved by the increase of fractions with a larger particle size. The largest average size of granules was obtained in the laboratory dragee pan, and the smallest - in the high-speed mixer-granulator. When using PVP solution of different concentrations the average grain size varied depending on the method of granulation within 120-1464 μm . The highest binding capacity showed PVP solutions, as evidenced by higher values of grain size at the same concentrations of humidifiers. Increased concentrations of both humidifiers more than 10% is inappropriate, because results in large particle size of granules. Study of influence of quantities of added humidifier on grain size showed that the granulation process is absent in mixtures containing humidifying substance in quantities of 15-20%. The best concentration of humidifier should be 25-30%, which give granules with an average size of 450-1350 μm , and a further increase of its content results in a viscous mass that is difficult granulated and undesirable. Experimental data of granule porosity indicates that increasing the concentration of binders increases porosity within 50-70%. The influence of granulation mode on porosity of both substances is different. The data of hardness of granules showed that this parameter is in inverse proportion to their porosity. Pellets made with using PVP solution demonstrated greater mechanical strength compared to granules containing starch potato paste. Conclusion. The influence of type and concentration of the humidifier and the method of granulation on the kinetics of growth of the natural zeolite granules were studied and its influence of these variables on the size, porosity and mechanical strength of the pellets were proven. The optimum concentration of potato starch paste and solution of polyvinylpyrrolidone as binders for granulation of natural zeolite at level 5-10% and optimal content of humidifier in the total mass at level 25-30% were established. The results of experimental studies used in the development of technology and composition of tablets containing natural zeolite as the main active component..

Keywords: natural zeolite, granules, granulation, porosity, hardness

ВИВЧЕННЯ БІОПЛІВКУТВОРЕННЯ ЕНТЕРОКОКІВ ФОТОМЕТРИЧНИМ МЕТОДОМ З МОДЕЛЮВАННЯМ БІОПЛІВОК НА АБІОТИЧНИХ ПОВЕРХНЯХ З ПОЛІСТИРОЛУ

97-101

Мироненко Л.Г., Перетятко О.Г., Ягнул Ю.А.

STUDY OF ENTEROCOCCI BIOFILM FORMATION USING PHOTOMETRIC METHOD WITH BIOFILM MODELING ON ABIOTIC POLYSTYRENE SURFACES

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Introduction. Static photometric method is widely used to determine the ability of biofilm formation. The essence of this method is to measure the optical density of the dye, eluted with a solvent from the biofilm. The aim of the study was to conduct a comparative

analysis of the results of determining the enterococci biofilm formation using photometric method with biofilm modeling in 96-well microtiter plates and 4-section Petri dishes. Material and methods. The study was conducted using 61 strains of microorganisms genus *Enterococcus*, taken from the clinical material of patients with purulent-inflammatory infections. To perform the study of biofilm formation using 96-well microtiter plates technique, 100 μ l of TSB with 1% glucose and 10 μ l of enterococci daily culture suspension with density of 0.5 units by Mc Farland, prepared using the Densi-La-Meter II device, were placed in each well. The plates were incubated at 37 °C for 48 hours, washed three times in phosphate-buffered saline (pH 7.2-7.4) of 200 μ l volume, dried at 37 °C for 15 minutes. The biofilms were stained with crystal violet 1% aqueous solution, washed with distilled water. 100 μ l of ethanol/isopropanol mixture (1:1) were added to the wells. The optical density of wells content was measured with the AIF-C-01S reader at a wavelength of 570 nm and 620 nm. The results were analyzed using Stepanovic S. et al. recommendations, according to which enterococci were divided into strains with high, medium and low degree of biofilm formation. To study the enterococci ability of biofilm formation in 4-section Petri dishes, 1.75 ml of TSB with 1% glucose and 0.25 ml of enterococci microbial suspension with density of 0.5 units by Mc Farland were added in each sector, 0.25 ml of medium were added to the control sector. The dishes were incubated at 37 °C for 48 hours, washed three times in phosphate-buffered saline (pH 7.2-7.4), dried at room temperature for 30 minutes. The biofilms were stained with crystal violet 1% aqueous solution. The dye elution from the biofilm was performed twice with 2 ml of ethanol/isopropanol mixture (1:1) for 20 minutes. The optical density of eluates was measured with the SF-56L spectrophotometer at a wavelength of 590 nm. Results and discussion. Analysis of the results of the study on enterococci ability to form biofilms with biofilm modeling in 96-well polystyrene microplates revealed that, when using light filters with a wavelength of 570 nm, optical density rate ranged from 0.001 to 1.256 OD570 with an average value of (0.264 \pm 0.04) OD570. It was found that the number of strains capable of biofilm formation amounted to (68.8 \pm 5.9)% of all strains used in the study. Enterococci distribution by intensity of biofilm formation was as follows: number of strains with high degree equaled (90.4 \pm 4.5)%, share of strains with medium and low degree was significantly lower – each amounted to (4.8 \pm 3.3)% (p <0.05). When measuring the optical density of eluates using the AIF-C-01S reader with light filter at a wavelength of 620 nm, it was found that the indicators ranged from 0.001 to 0.436 OD620 with an average value of (0.077 \pm 0.01) OD620. While analyzing the results with consideration of the above criteria, it was found that the share of strains capable of biofilm formation equaled (60.7 \pm 6.3)%. The number of strains with high degree of biofilm formation amounted to (75.6 \pm 7.1)%, with average and low degree – (13.5 \pm 5.6)% and (10.9 \pm 5.1)% respectively. The performed statistical analysis showed no significant difference between the number of strains capable of biofilm formation and intensity of biofilm formation when applying light filters with a wavelength of 570 nm and 620 nm (p >0.05). When modeling the enterococci biofilms in 4-section Petri dishes, it was found that the eluates optical density rates ranged from 0.161 OD590 to 3.294 OD590 with an average value of (1.387 \pm 0.14) OD590. Analysis of the results showed that the number of enterococci strains capable of biofilm formation equaled (62.3 \pm 6.2)% and did not differ significantly from the corresponding indicator in the study with 96-well polystyrene microplates (p >0.05). Conclusion. Static photometric method is widely used to determine the enterococci ability of biofilm formation due to its convenience, high performance, and clearness. Microbial biofilms are mostly modeled in 96-well polystyrene microtiter plates. Our study showed the feasibility of also using 4-section polystyrene Petri dishes for enterococci biofilm modeling. It was confirmed that it is necessary to use light filters with a wavelength range from 570 nm to 620 nm when measuring the optical density of the enterococci eluates with a reader, if using the crystal violet dye.

Keywords: enterococci, biofilm modeling, 96-well microplates, polystyrene Petri dishes.

АНТИБАКТЕРІАЛЬНІ ВЛАСТИВОСТІ ГЕПАТОПРОТЕКТОРІВ ФАРМАЦЕВТИЧНОЇ КОМПОЗИЦІЇ «ЛАВАФЛАМ»

102-106

Асланян М. А., Бобрицька Л. О., Осолодченко Т. П.

ANTIBACTERIAL PROPERTIES OF PHARMACEUTICAL COMPOSITION OF HEPATOPROTECTORS

Aslanian M. A., Bobrytska L.O., Osolodchenko T.P.

Pathology problem of hepatobiliary system (HBS) of contagious origin becomes relevant with the increase of disturbance and complications of lipid metabolism and bile pigments among young people. Leading role in prophylaxis and treatment in the context of this pathology belongs to antibacterial hemotherapeutic agents. However, along with antimicrobial activity, numerous side effects can be observed during treatment with antibacterial agents. According to the recent study on hepatobiliary system (HBS), therapeutic effect can be seen only while using herbal medicine with choleric action as well as their various compositions. Amount of medicine with the appropriate effect is insufficient, that is why the study was conducted towards finding effective combinations of plant substances of different groups for the purpose of creating an effective medicine for treatment of hepatobiliary system (HBS) of contagious origin. Aim of the work The purpose of study was to examine antibacterial properties of different combinations with flamin and lavender oil in combined medicine during the course of treatment of cholecystitis and cholangitis. Materials and methods. It the result of study 61 microbial strains were distinguished and identified from the pathological material taken from 53 patients with cholecystitis and cholangitis. All distinguished clinical microbial strains taken from the patients were tested for sensitivity to combined medicine in the form of tablets with flamin and lavender oil. Minimal inhibitory concentration for *S. aureus* ATCC 25923 amounted to 250-350 μ g/ml, for *E. coli* ATCC 25922 - 350 \pm 50,0 μ g/ml, for *P. aeruginosa* ATCC 27853 750 \pm 100,0 μ g/ml, for *P. vulgaris* ATCC 4636 - 850 \pm 100,0 μ g/ml, minimal inhibitory concentration to the tablets № 2 та № 3 amounted to > 1000 мкг/мл. Minimal inhibitory concentration for *B. subtilis* ATCC 6633 amounted to 250 \pm 50,0 μ g/ml, for *C. albicans* ATCC 885/653 300 \pm 50,0 μ g/ml. Compared to the tablets No. 2 and No. 3, where minimal inhibitory concentration was higher and amounted to 350-550 μ g/ml. Results and discussion. Minimal inhibitory concentration for *S. aureus* amounted to 250-300 μ g/ml, for *S. epidermidis* 150-250 μ g/ml, for *S. agalactiae* and *E. faecalis* 300-400 μ g/ml, for *E. coli*, *K. pneumoniae*, *E. cloacae* – 400-750 μ g/ml. Minimal inhibitory concentration for anaerobic bacteriae (*P. niger*, *P. anaerobius*, *Fusobacterium* spp, *B. fragilis*) -300-550 μ g/ml, minimal inhibitory concentration to the tablets № 2 та № 3 amounted to 450-650 μ g/ml. Minimal inhibitory concentration for *C. albicans* 300-400 μ g/ml. Compared to the tablets No. 2 and No. 3, where minimal inhibitory concentration was higher and amounted to 450-600 μ g/ml. To sum up the results of the conducted experimental studies of combined capsules with flamin and lavender oil we can make a conclusion that the most effective against the different clinical microorganism and fungi strains is a combination of flamin 0.05 g, 0.02 g lavender oil (tablets No. 1), which makes it possible to prepare combined medicine for pathologies of hepatobiliary system (HBS). Conclusions. To sum up the results of experimental studies of combined capsules which consist of flamin and lavender oil we can make a conclusion that the most effective remedy for different clinical microorganism and fungi strains is a combination of flamin 0.05 g, 0.02 g lavender oil (tablets No. 1), which gives the opportunity to prepare combined medicine for pathologies of hepatobiliary system (HBS).

Keywords: antibacterial properties, hepatoprotectors, lavender oil, flamin, tablets

ВТОРИННА ЛАКТАЗНА НЕДОСТАТНІСТЬ ТА ЇЇ КОРЕКЦІЯ У ДІТЕЙ РАНЬОГО ВІКУ, ХВОРИХ НА РОТАВІРУСНУ ІНФЕКЦІЮ

107-111

Кірсанова Т.О., Кузнєцов С.В.

SECONDARY LACTASE DEFICIENCY AND ITS CORRECTION IN INFANTS ILL WITH ROTAVIRUS INFECTION

Kirsanova T.A., Kuznetsov S.V.

Introduction. Cardinal changes in medicine during recent years have made the problem of disorders in digestion and carbohydrate absorption one of the most crucial. Lactose intolerance (lactase deficiency) is a clinically revealed inability of intestinal enzymatic systems to break down lactose, where secondary lactase deficiency results from damage of erythrocytes against a background of some disease, including that of an infectious origin, particularly in viral intestinal infections. Purpose of the study. To study the efficacy of taking lactase preparations by infants during the first year of their life, who are breast fed and ill with rotavirus infection. Materials and methods. The study involved 28 naturally fed infants of the first year of life with rotavirus infection. The diagnosis was made by revealing the virus antigen in the patients' faeces and antibodies to it in their blood. Besides the standard methods of examination the faeces were analysed for carbohydrates and pH values. The patients were divided into two groups: the first group of infants did not receive lactase-containing drugs in their combined therapy; the second group took them. Results. Damage of the gastrointestinal tract of the retrovirus aetiology was characterized by the following signs: vomiting, abdominal distention, diarrhoea with watery frothy stool having sour odour and undigested boluses. In the group of infants, whose combined therapy used lactase-containing drugs, regression of their clinical signs passed reliably more rapidly than in the group of infants, who did not receive the above medicines. Conclusion. The use of lactase-containing preparations in the treatment of infants, who are breast fed and ill with rotavirus infection, is undoubtedly effective in order to correct lactase deficiency, since it causes a more rapid disappearance of clinical manifestations of the disease, thereby making it possible to examine the possible inclusion of these drugs into the scheme of combined therapy for the above category of infants.

Keywords: lactase deficiency, infants, rotavirus infection, treatment.

ИЗУЧЕНИЕ АНТИМИКРОБНОГО ДЕЙСТВИЯ КОМБИНИРОВАННОЙ ЛЕКАРСТВЕННОЙ КОМПОЗИЦИИ ДЛЯ ЛЕЧЕНИЯ КИШЕЧНЫХ ИНФЕКЦИЙ 112-116

Фарес Р., Бобрицкая Л. А., Осолодченко Т. П., Гриценко В. И.

STUDY OF ANTIMICROBIAL ACTION OF COMBINED DOSAGE FORM FOR THE TREATMENT OF INTESTINAL INFECTIONS

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Intestinal infection (II) of various etiologies is among to the most widespread diseases in the world. The treatment regimen bacterial etiology involves the suppression of pathogenic and conditionally pathogenic with the restoration of the normal intestinal microflora. For effective antibiotic pharmacotherapy of intestinal infections are widely used drug combinations with the addition of nifuroxazide, as well as enzymatic and normalizing bowel motility broad-spectrum drugs. Intestinal antiseptics nifuroxazide characterized by broad spectrum of antibacterial action against *Staphylococcus* spp, *Clostridium* spp, *E. coli*, *Salmonella* spp, *Shigella* spp, *Proteus* spp, *Klebsiella* spp, *Enterobacter* spp, *V. cholerae*, *H. pylori*, *Yersinia* spp, and also the lack of effect on the normal intestinal flora, high safety profile. Recently, for the treatment of intestinal infections nifuroxazide often combined with pre- and probiotics for complex correction of the intestinal microflora disorders. For complex therapy of intestinal infections, we have developed an original combined medicine "Diaplant", in the form of capsules, comprising as active ingredients nifuroxazide (200 mg) in combination with plant substance plantaglucide (200 mg). Plantaglucide drug obtained from *Plantago major* has spasmolytic, antimicrobial and anti-inflammatory activity, normalizes bowel peristalsis, while reducing the tone of smooth muscles of the stomach and intestines, reduces swelling folds of the gastric mucosa, and contained therein polysaccharides in the form of pectins have properties of prebiotic and have immunostimulatory effects. Aim of the work – study of antibacterial action of combined drug "Diaplant" containing nifuroxazide and plantaglucide in regard to test strains and clinical strains of microorganisms allocated from patients with bacterial diarrhea. Materials and methods. Estimation of antimicrobial activity was performed under conditions in vitro by method of serial dilutions. The object of research is a combined drug "Diaplant", a reference drug "Enterofuril" manufacture of ("Bosnalijek" Bosnia and Herzegovina). For the evaluation of drugs samples activity used the following test strains: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Proteus vulgaris* ATCC 4636, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans* ATCC 885/653. Clinical material comes from the Center of medical and environmental research to the bacteriological laboratory. Was allocated and identified 109 strains of conditionally pathogenic microorganisms. Results and its discussion. For clinical and test strains *S. aureus* MIC indicators for combined drug «Diaplant» were 15.0 mg / l, whereas the reference drug "Enterofuril" MIC equaled 30.0 mg / l. For *E. coli* - MIC indicators for combined drug «Diaplant» was 7.5 mg / l, for an "Enterofuril" - 15.0 - 30 mg / l. MIC for combined drug "Diaplant" against different clinical strains of *Enterobacteriaceae* species ranged from 15.0 mg / l to 30.0 mg / l in comparison with "Enterofuril", where the MIC was 30mg / l - 60mg / l. For yeasts and clinical strains *C. albicans* MIC combination drug "Diaplant" product is 15.0 mg / l, whereas equal to 30.0 mg / l for the control drug "Enterofuril" MIC. For test strains, such as *P. aeruginosa* ATCC 27853, *P. vulgaris* ATCC 4636, MIC "Diaplant" drug was 100.0 mg / l, whereas control drug "Enterofuril" MIC was above > 100.0 mg / l. For clinical strains of *P. mirabilis* and *P. vulgaris* MIC "Diaplant" was 30.0 mg / l, whereas the MIC "Enterofuril" equal to 60.0 mg / l. For clinical *P. aeruginosa* strains MIC "Diaplant" was 100.0 mg / l, whereas control drug "Enterofuril" MIC was above > 100.0 mg / l. It has been established that 80-95% of clinical microbial strains showed sensitivity to combined preparation "Diaplant". The highest index of sensitivity has next strains: *S. aureus* - 92,8% of strains, *S. epidermidis* - 100% of the strains, *E. coli* (lactose-negative) - 100% of the strains, *K. pneumoniae*, and *K. mobilis* 85-87% of the strains. Conclusions. 1. As a result of the conducted research was found, that the combined drug "Diaplant", containing as active ingredients nifuroxazide in combination with herbal substances plantaglucide, has a pronounced antibacterial activity against the reference strains and clinical strains of microorganisms, the main pathogens of bacterial diarrhea. 2. Plantaglucide substance which is part of the capsules is enhances the antimicrobial effect nifuroxazide at the expense of its own antibacterial properties of phenolic compounds, polysaccharides and their possible synergies.

Keywords: intestinal infection, nifuroxazide, plantaglucide, capsules.

ВЛИЯНИЕ КОМПЛЕКСНОЙ ТЕРАПИИ С ИСПОЛЬЗОВАНИЕМ ИММУНОМОДУЛЯТОРОВ НА СОСТОЯНИЕ МЕСТНОГО ИММУНИТЕТА БОЛЬНЫХ ХРОНИЧЕСКИМ ГЕНЕРАЛИЗОВАННЫМ ПАРОДОНТИТОМ I-II СТЕПЕНИ ТЯЖЕСТИ С ЭНТЕРОБИОЗОМ 117-122

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STUDY OF THE INFLUENCE OF COMPLEX TREATMENT USING IMMUNOMODULATORS ON THE STATE OF LOCAL IMMUNITY IN PATIENTS WITH CHRONIC GENERALIZED PERIODONTITIS I-II SEVERITY ON ENTEROBIOSIS

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Introduction. Due to the high prevalence of chronic generalized periodontitis there is a need for a broader analysis of the causes and development of diseases, as well as the search for effective treatments for etiopathogenetical. The aim of this work was to study the

effect of newly developed therapy on local immunity in patients CGP I and II severity with enterobiasis. Material & methods. The main group consisted of 32 people with CGP I degree and 60 people with CGP II severity who were treated according to our scheme. The control group consisted of 30 people with CGP I degree and 58 people with CGP II severity, treated with conventional treatment. The control group consisted of 30 people without periodontal disease and chronic diseases of other systems. All patients were studied the main group and the comparison group conducted a basic local therapeutic treatment of periodontal disease, including professional oral hygiene, temporary splinting of teeth, selective prishlifovyvanie teeth. For medical treatment of periodontal tissues using 0.05% - 0.2% solution of chlorhexidine bigluconate. Further treatment of patients of the main group carried out in 2 stages. At the first stage the main group received: irrigation and instillation of periodontal tissue in periodontal pockets antiseptic preparation "Dekasan" application keratoplastic drug "Katomas". Systemically administered drug tonic "Sage oil" probiotic "Kvertulin" immunomodulator "Erbisol". In the second phase, patients received: applications on the gums periodontal gel "Lizomukoid" systemically complex preparation "Oil extract from pumpkin seeds." All patients of the main group used toothpaste "Lacalut flora" and rinse "grapefruit". In the comparison group, patients received applications in periodontal pockets (drug Dalatsin C) application to the gums (keratoplastic drug Aekol) system - a probiotic Linex, immunomodulator "Echinacea compositum C". All patients with the comparison group used toothpaste and rinse "Forest Balsam" for the duration of treatment and 1 month after the end of therapy. The effect of the proposed and conventional therapy on local immunity judged on the content of lysozyme, sIgA, mIgA, IgG, total protein, extracellular peroxidase activity and bactericidal oral secretions. Statistical processing of materials made using mathematical statistical methods for data analysis. Results & discussion. Under the influence of the proposed therapy in patients of the group increases the activity of local immunity factors and attenuates the inflammatory process in the periodontium. On the 1st day of the end of therapy in this group of patients significantly increases the content of lysozyme in the oral secretions and sIgA to normal values, with elevated levels of IgG and mIgA, which differed before treatment, reduced to normal values ($p < 0,05$) and remain so the entire period of observation for 6 months. Patients comparison group in the application of traditional therapy of total protein content in the oral secretions to the 6-month observation is not reduced to normal, but there was a positive trend in the growth of the content of sIgA and lysozyme and decreased total protein levels in the oral secretions. The level in the oral secretions mIgA and IgG in patients with the comparison group were not significantly changed the whole period of study. Patients of the main group under the influence of the therapy took place dynamic normalization of extracellular peroxidase activity, whereas in patients with comparison groups to restore the values of the rate of extracellular peroxidase activity did not occur. Between indicators peroxidase activity of patients of the main group and the comparison group, respectively, in all periods of the study were significant differences ($p < 0,05$). Study of the bactericidal activity of saliva showed that the study group patients who received the combined treatment immunokorregirujushchej therapy by the end of the treatment of bactericidal action of saliva increased to values of norms and remained so the entire period of observation. In the comparison group, the bactericidal action of saliva significantly increased from 30 days last treatment. Conclusion It was found that the proposed two-stage combined therapy with the use of immunomodulators has a significant normalizing effect on the activity of local immunity of patients CGP I and II degree of severity of the disease with enterobiasis. Under its influence there is dynamic recovery by the end of the treatment the activity of lysozyme, sIgA content, Miga and bactericidal oral secretions. It is shown that such action does not have a common set of traditional therapy. These results demonstrate the high efficiency of this method of treatment of chronic generalized periodontitis I and II of Article severity in patients with enterobiasis and point to the possibility of its application in clinical practice.

Keywords: Dekasan, Katomas, Lizomukoid, Lacalut flora, immunomodulators, periodontitis

МИКРОФЛОРА НАДГОРТАННИКА У ВЗРОСЛЫХ БОЛЬНЫХ ОСТРЫМ ЭПИГЛОТТИТОМ 123-127

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EPIGLOTTIS MICROFLORA OF ADULT PATIENTS WITH ACUTE EPIGLOTTITIS

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Introduction. Nowadays acute infectious-inflammatory processes of upper respiratory tract, including acute epiglottitis retain a high proportion among human pathology. In the literature acute epiglottitis is allocated into an independent nosology as severe acute phlegmonous bacterial inflammation of the epiglottis and hypopharynx. There are currently no clear guidelines on how to classify an acute epiglottitis, as well as protocols for patients at various stages of the pathological process. According to common belief, Haemophilus influenzae type -B (Haemophilus influenzae type b (Hib)) is the most common cause of epiglottitis. At present, the main etiological role in the genesis of acute epiglottitis in children belongs to haemophilus influenzae. In adults the causes of the disease are beta hemolytic streptococci groups A, B, pneumococcus, Klebsiella, Pseudomonas, Staphylococcus aureus, herpes simplex virus (type 1) and parainfluenza, and others. The aim of this work is to study: the mucosal microflora of the epiglottis in adult patients with acute epiglottitis and to study sensitivity of certain isolates to antimicrobial agents. Material & methods. 86 adult patients with acute epiglottitis were observed: 36 with abscess form of epiglottitis and 50 - with infiltrative. Microbiological analysis of mucosal swab samples taken from hypopharynx were conducted by the conventional technology: for seeding solid or liquid nutrient medium, followed by allocation of isolith and its microscopic, biochemical and serological identification. Microorganisms were classified according to schemes of Bergy. Antimicrobial susceptibility of each strain was determined in accordance with the guidelines. We used discs with antibacterial drugs. The availability of sensitive and resistant strains of microorganisms to antibiotics was assessed. A mucous membrane of the epiglottis was analyzed through microbiological investigation in 86 patients with acute epiglottitis. As a result, 169 strains of microorganisms were sowed from mucous membrane of epiglottis. Results & discussion. In patients with acute epiglottitis Streptococcus progenies dominated in 33 (23.7%) of cases, H. influenza was detected in 27 cases (19.4%), Streptococcus pneumonia strain was in 21 (12.4%) of cases, 4th place -. Staphylococcus aureus -9.3%. There are different types of: staphylococci strains -29, of Enterococcus spp - 11, gram-negative bacilli -41. Noteworthy fact is the high degree of microbial contamination of patients 107-109 CFU. Conclusion. Acute epiglottitis highest sensitivity was observed to the following medications as cephalosporin, namely cefotaxime while it is variable within the same group (1,2,3 cephalosporin's generation). The isolated strains showed almost 99,4±1,2% sensitivity to fluoroquinolones, especially to levofloxacin and others (S. aureus, S. pyogenes, S. pneumoniae, H. Influenzae). There was a high sensitivity to lincosamides - clindamycin; fluoroquinolones- levofloxacin. In 50% of cases, a resistance to the antibiotic penicillin; and macrolide antibiotic, especially the last generations was observed. According to the obtained findings on the sensitivity of microflora to antibiotics cephalosporin's II- III generation with moderate and severe degrees of severity in combination with fluoroquinolones (all administered parenterally) are used in acute epiglottitis. Use of fluoroquinolones (levofloxacin, ciprofloxacin, and others) is recommended in case of Cephalosporin intolerance, as well as lincosamides (clindamycin, dalatsin, lincomycin).

Keywords: acute epiglottitis, microflora.