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ЗМІСТ (Contents)

Редакційна рада (Editorial Board)

C. (P.)

Зміст (Contents)

1

2-7

ОГЛЯДИ (REVIEWS)

МОЛЕКУЛЯРНЫЕ МИШЕНИ НЕФРОТОКСИЧЕСКОГО ДЕЙСТВИЯ ПОЛИМИКСИНОВ
Лисняк Ю. В.

8-24

MOLECULAR TARGETS OF NEPHROTOXIC ACTION OF POLYMYXINS

Lisnyak Yu. V.

The rapid spread of multidrug-resistant gram-negative bacterial strains has necessitated the search for the more efficient antimicrobial agents and prompted a renewed interest in polymyxins which have been invaluable for the therapy of serious nosocomial pathogens but withdrawn due to their nephrotoxicity. Polymyxins are nonribosomal cyclic lipopeptides isolated from *Paenibacillus polymyxa*. Several distinct groups of polymyxins have been structurally identified, each group is characterized by the unique amino acid sequence and the chemical structure of the fatty acyl group. But, only two polymyxins, polymyxin B and colistin (polymyxin E), have been clinically used. The revival of polymyxins into clinical practice has stimulated further thorough investigations of their toxicity. During the last decade, the toxicity of polymyxin B and colistin has been thoroughly studied (taking into account the chemical purity, homogeneity, dosing regimens and other factors) and appeared to be not as high as reported earlier. Nevertheless, it still may substantially complicate therapy and even result in its stoppage. Thus, the development of less toxic polymyxin derivatives remains to be a topical problem. Understanding of molecular mechanism(s) of polymyxin's toxicity based on detailed knowledge of the peculiarities of their intermolecular interactions with the targets of their toxic action is a prerequisite of a purposeful search for such polymyxin-based compounds. Nephrotoxic effect of polymyxins is determined by their accumulation in the epithelial cells of the kidney proximal tubules. The main factor of the accumulation of these antibiotics considered to be their interaction with megalin, the giant receptor of cell surface, which is the most abundant in the apical membrane of renal proximal tubules. Megalin is a representative of the low-density lipoprotein receptor (LDL-receptor) family which contains several structurally homologous receptors. Megalin is the largest member of the family; molecular weight of rat megalin is about 600 kDa. Its amino acid sequence involves about 4660 amino acid residues and is identical to one of human megalin by approximately 77%. All members of this receptor family have a modular structure, in particular, they contain clusters of two or more cysteine-rich complement-type repeats (CR modules) which are the binding sites of the most ligands of LDL-receptors. Each of CR domains consists of approximately 40 amino acid residues. The binding site of the cationic ligands on the LDL-receptors shown to contain a common structure motif, so called DXDXD motif, which consists of three negatively charged aspartic acid residues coordinated by Ca²⁺ ion and a hydrophobic residue. As a rule, the cationic part of a ligand is represented by lysine residue. The binding occurs mainly due to electrostatic interactions between positively charged lysine residue and negatively charged residues of aspartic acid. The binding is enhanced by hydrophobic interactions between aromatic residue of CR module and aliphatic region of lysine. Structural data on megalin are rather limited now: there is known a structure of CR12 domain of rat megalin and a structure of CR10 domain of human megalin (as well as its complexes with gentamicin) both solved by NMR in solution. Polymyxin binding site on the megalin is not yet determined experimentally, there are absent as well any structural models at atomic level for polymyxin interaction with megalin. However, based on an analysis of available data on the structure of ligand-target complexes for the members of LDL-receptors family, there are reasons to suppose that polymyxin's molecular fragment which binds to megalin is represented by cationic residues of di-amino-butyric acid (an analogue of lysine), and the structural DXDXD motifs of CR domains of megalin compose binding sites for polymyxin as well. In the review, the available data on the structure and three-dimensional organization of megalin and other members of LDL-receptors family are represented; the peculiarities of their ligand-target intermolecular interactions are considered. It is concluded that the weakening of polymyxins binding with megalin may be an effective preventive measure against polymyxin-induced nephrotoxicity.

Key words: polymyxins, megalin, nephrotoxicity, molecular target, LDL-receptors.

БІОФІЗИЧНА ХАРАКТЕРИСТИКА ВПЛИВУ ЕЛЕКТРОМАГНІТНИХ ТА УЛЬТРАЗВУКОВИХ ХВИЛЬ НА БІОБ'ЄКТИ

25-36

Калініченко С.В., Антушева Т.І., Коротких О.О., Бабич Є.М., Ківва Ф.В., Коваленко О.І., Рижкова Т.А., Балак А.К.

BIOPHYSICAL CHARACTERIZATION OF THE IMPACT OF ELECTROMAGNETIC AND ULTRASONIC WAVES ON BIOLOGICAL OBJECTS

Kalinichenko S.V., Antusheva T.I., Korotkykh O.O., Babych E.M., Kivva F.V., Kovaneko O.I., Ryzhkova T.A., Balak A.K.

In recent years the physical mechanisms of action of electromagnetic fields on biological objects are actively studied. Extensive research about the effect of weak electromagnetic waves on biological objects were launched in the second half of the 60-ies of XX century. It has been suggested that the millimeter waves to biological objects are natural and are used by cells to control basic physiological functions. This review is summarizes the data of foreign and domestic literature related to influence of electromagnetic and ultrasonic waves on biological objects. The concepts of sound waves and electromagnetic field are sanctified. Several hypotheses and points of view of mechanisms of action of microwave radiation on biological objects were discussed in details. One of the hypotheses suggests that the acoustic vibrations of extremely power range can stimulate the cell plasma membrane. Other hypothesis of the mechanism of influence weak signals on biological systems are associated with the discovery of the phenomenon of stochastic resonance. It is assumed that the initiation of acoustic-electrical waves in the lipid-protein membrane of the cell activates the transport of water, ions and various substances through it. The data about the effect of electromagnetic millimeter waves on photosynthetic organisms have been summarized by several scientists. They established that exposure in the microwave range is realized on the membrane level and is

connected with the change of oscillation spectrum. Peculiarities of influence of ultrasonic waves on microorganisms and cell membranes have been analysed. Scientists have discovered that detonation of dichloride nitrogen takes place under the influence of ultrasonic waves. Dichloride nitrogen causes breakdown of protein particles. Cavitation effect is one of bactericide action of ultrasonic waves. The mechanism of action of ultrasonic waves in the literature is explained by two theories: cavitation-mechanical, cavitation-electrochemical. According to the first theory of ultrasonic waves propagating in a dense environment, causing it to alternating compression and decompression. Cavitation-electrochemical theory explains the ionization liquid vapor and the presence in it gases in the formation of cavitation bubble. An electric discharge occurs at break bubbles. The electrical discharge accompanied by a sharp rise in temperature and in the formation of cavitation bubble electric high voltage charge. Then the vapor of the liquid and high molecular weight compounds in the cavitation chamber are split into hydrogen and hydroxyl groups with formation of active oxygen, hydrogen peroxide, nitrous acid and nitric acid, resulting in inactivation of enzymes and proteins coagulate. All this causes the death of microbial cells. As well ultrasonic vibrations can be used to initiate chemical reactions, the commission of a number of new methods of synthesis and accelerate the slow reactions in organic systems. One of the actions of the ultrasonic waves on microorganisms is a change of concentration various substances in the cytoplasm due to a change of the equilibrium concentration of substances inside and outside the cell. It was found that the effects can be different. On the one hand, researchers observed an increase in the agglutination and the loss or total loss of virulent bacteria. On the other hand there was opposite effect - increasing the number of viable cells. Application of ultrasound in medical microbiology is theoretically proved in this article. Ultrasonic techniques in microbiology used not only as an effect on biological objects and the ability to change the physico-chemical characteristics of substrates for culturing microorganisms. Application of such techniques is important for controlling the composition of artificial culture medium, in particular the concentration and the activation of molecular oxygen by ultrasonic degassing culture medium.

Key words: electromagnetic waves, ultrasonic waves, biological objects

ДОСЯГНЕННЯ ОСТАННІХ РОКІВ У ПОШУКУ ПОТЕНЦІЙНИХ АНТИКОНВУЛЬСАНТІВ СЕРЕД ПОХІДНИХ АЗАГЕТЕРОЦИКЛІВ

37-45

Перехода Л.О.

THE PROGRESS OF RECENT YEARS IN A SEARCH OF POTENTIAL ANTICONVULSANTS AMONG THE DERIVATIVES OF AZA-HETEROCYCLES

Perekhoda L. O.

Introduction. Epilepsy is one of the most common diseases of the nervous system that affects all aspects of life. This fact stimulates an in-depth analysis of the problem of epilepsy from medical and social points of view. The social significance is determined by the prevalence of epilepsy, the possibility of personality and psyche changes in 1/3 patients, and complex social, legal and economic issues associated with the disease. Thus, it is vital to constantly improve the anticonvulsant drug therapy in order to find the most pharmacologically active and safe anticonvulsants. In addition, one of the priority areas of the pharmaceutical industry in Ukraine is the import substitution, which in turn requires the introduction of production that is not only generic drugs, but also original ones. Analyzing the published data we found that five-membered di(three) aza-heterocycles is quite promising matrix based on which the search of these anticonvulsants can be done. In this paper is introduced the structures of the synthesized in recent years 1,2,3-triazoles derivatives, 1,2,4-triazoles, 1,3,4-oxadiazoles and 1,3,4-thiadiazole that possess an anticonvulsant effect with bringing of specific models of a convulsive state. Analyzed and summarized published data regarding the qualitative and quantitative relation "structure-activity" (SAR and QSAR analysis) in the series of anticonvulsants that are now widely used - benzodiazepine, barbiturates and hydantoin, and a number of new compounds that are under preclinical trials. Introduced the correlation dependencies in the form of regression equations that connect anticonvulsant effect with structural characteristics of anticonvulsants that are expressed by means of molecular descriptors of different types. These descriptors consistently affect the amount of anticonvulsant activity, in particular, dipole moment, molecular weight, Gammet's constant, Taft's steric parameter of substituents in certain atoms, atomic charges, electron density, and lower energy of free molecular orbital. Also, there is demonstrated an important role of a lipophilicity for manifestation of anticonvulsant activity and are given equations of its correlation with retention time, determined by HPLC. There was proved an availability for further study of anticonvulsants' activity based on derivatives of 1,2,3-triazoles, 1,2,4-triazoles, 1,3,4-oxadiazoles and 1,3,4-thiadiazole and common areas of chemical modification have been identified in order to search for perspective anticonvulsants. Promising methods included an introduction to the structure of benzylamine residue (substituted and unsubstituted), carboxamide group, ester groups, free amino and carboxyl groups, sulfonyl groups, aromatic rings and heterocyclic π -redundant heterocyclic systems. In paper there was outlined a question, which is not resolved yet of the described in the literature SAR and QSAR models that are built only for narrow homogeneous samples of compounds that usually belong to the same chemical class. At the same time, the creation of reliable model for various sample compounds that allow using one correlation equation to predict the anticonvulsant properties of compounds of not only homologous, but different chemical structure has not been resolved. In addition, methodological rules for constructing such models are not explicitly formulated. However, in most scientific papers are used either pre-selected descriptors by the authors, or an automatic descriptor selection from a sufficiently large number of them (usually with descriptors implemented in a particular program).

Conclusion. A detailed study of the prospective structures in the series of the five-membered derivatives of di(three) aza-heterocycles and summarizing the progress of recent years in SAR and QSAR analysis will further allow to choose the most promising ways of modification in order to improve the search efficiency of new anticonvulsants.

Keywords: epilepsy, anticonvulsant agent, SAR, QSAR.

ЕКСПЕРИМЕНТАЛЬНІ РОБОТИ Experimental papers

THE STUDY OF THE ANTI-NEURAMINIDASE AND INTERFERON INDUCING ACTIVITY OF ALTABOR SUBSTANCE

46-48

Rybalko S. L., Krutskykh T. V., Shalamay A. S.

Influenza is acute respiratory disease of viral etiology characterized by the phenomena of general intoxication and respiratory tract lesions. Hemagglutinin and neuraminidase, surface antigen of influenza virus are the factors of aggression. Hemagglutinin is a polypeptide, a surface protein of the viral envelope, due to it the virus attaches to the surface of the host cell. Neuraminidase is an

enzyme, a glycoprotein complex that determines the enzymatic activity and is responsible for the ability of a viral virion to penetrate the host cell and leave it after propagation. In other words, neuraminidase helps viral particles to penetrate the secretions of the mucous membranes to achieve epithelial cells of the respiratory tract by virions. In addition, the properties of hemagglutinin determine the intensity of intoxication in disease, and neuraminidase exhibits a marked immunosuppressive effect. Therefore, for prevention and treatment of influenza diseases it is advisable to use two schemes, which would affect two elements of the infectious process, namely the use of drugs that would affect influenza virus destroying it, and medicines for strengthening the body's defences by stimulating the interferon inducing activity. Thus, the search for drugs that would have a combined scheme of action is appropriate and promising. The aim of the work was to study the anti-influenza activity of Altabor substance obtained from the collective fruit of black alder (*Alnus glutinosa*) and grey alder (*Alnus incana*) in order to determine its effect on the virion of influenza virus concerning its destruction and on a human in relation to increase of the body defences. The research of the antiviral activity of Altabor substance was conducted by studying its effects on isolated neuraminidase and different strains of the influenza virus. The effect of the drug on interferon production was also studied. The antineuraminidase activity of Altabor substance was studied on the example of inhibition of neuraminidases of different types of influenza viruses (A/Hong Kong/68/H3N2/, A/Victoria/75/H3N2, A/Khabarovsk/H1N1/) and neuraminidase isolated from *Astrobacter ureafaciens* 1 unit Calbiochem, Hoest in the concentrations of 105 and 51.5 γ /ml/min with Altabor solution in the concentration of 1 mg/ml. The interferon inducing activity of Altabor was studied in the culture of human leukocytes. The solution of the drug in different doses was added to 3 mln of human leukocytes and cultured at 37°C for 24 hours. After that the supernatant was collected, and the pH value was adjusted to 2.0, left at 4°C for 48-72 h, then the pH of the liquid was restored to 7.3, and the level of interferon was determined. Thus, based on the results it can be concluded that Altabor substance in the dose of 1 mg/ml completely inhibited 1/6 enzyme units or 51.5 γ /ml/min of neuraminidase (*Astrobacter ureafaciens* 1 unit Calbiochem, Hoest), and there is 84.2 and 85.0 % reduction in the activity of neuraminidase of A/Hong Kong/68/H3N2/, A/Victoria/75/H3N2, A/Khabarovsk/H1N1/ influenza viruses. These data show that Altabor dose-dependently stimulates the production of interferon in human leukocytes indicating the activity of the drug and its prospects as an interferon inducer. The results of the studies conducted on the antiviral activity of Altabor substance have shown that this substance effectively inhibits the reproduction of influenza viruses. The mechanism of the anti-influenza action of Altabor is due to inhibition of the neuraminidase activity of influenza viruses with simultaneous induction of interferon by human cells.

Key words: altabor, antiviral activity, influenza, neuraminidase, interferon inducing activity.

ХАРАКТЕРИСТИКА ЦИТОКІНОВОГО ОБМІНУ У ХВОРИХ НА ГОСТРІ ВІРУСНІ МЕНІНГІТИ

49-52

Нартов П. В.

FEATURE CYTOKINE METABOLISM IN PATIENTS WITH ACUTE VIRAL MENINGITIS

Nartov P. V.

Introduction. The urgency of the problem of viral meningitis etiology is due to the high frequency of severe forms of the disease, significant levels of mortality, spread spectrum etiopathogenia, the difficulty of differential diagnosis. The leading role in the pathogenesis of CNS is given to pathological processes that occur in Sabar mi area, i.e. the blood-brain barrier and causes changes intrathecal homeostasis. The height of the inflammatory syndrome and release of anti-inflammatory cytokines (CK), as well as alternative Pro-inflammatory patients of acute viral meningitis (AVM) associated with the penetration into CSF infectious pathogen or molecular patterns, which act as trigger cells of the immune response.

Material and methods. 26 patients were examined with a diagnosis of GUM who was admitted on an emergency basis in regional clinical infectious diseases hospital, Kharkov. The age of patients ranged from 17 to 65 years, and was dominated by persons younger than 40 years (the average age of women was 37 years, and 25 for men). The majority of patients with GUM was identified enterovirus and herpesvirus etiology of the disease: in 9 patients were diagnosed with enteroviral meningitis, and 12 patients herpesviruses meningitis (HSV-1/2-7, VZV-1, EBV-1, CMV-2, HHV-6-1). The diagnosis was verified on the basis of clinical and Iquiroga, serological and molecular genetic studies. The research material was cerebrospinal fluid (CSF) of patients GUM which was received in early disease and in the recovery period. The concentration of GC (IL-1, 2, 4, 6, 10 and TNF- α) in CSF were determined by ELISA method. The control group consisted of 11 individuals with intact CSF. The results are statistically processed on a computer using standard computer programs (Excel and Statistica 6).

Results and discussion. In the acute period of the disease, patients AVM GC content that were studied, were significantly higher compared with the control group: TNF- α – 8.7-fold ($p < 0.05$), IL-1 – 6.7-fold ($p < 0.05$), IL-2 – 10.4 times ($p < 0.05$), IL-4 – 3.5 times ($p < 0.05$), IL-6 – 2.3 times ($p < 0.05$) and IL-10 – 4.4-fold ($p < 0.05$). Regarding the concentration of GC in the period of convalescence: NEF- α was higher than the control value 5.8-fold ($p < 0.05$), IL-1 – 3.7 times ($p < 0.05$), IL-2 – 6.2 times ($p < 0.05$), IL-4 2.7 times ($p < 0.05$), IL-6 2.2 – fold ($p < 0.05$), IL-10 – 2.7 times ($p < 0.05$). In connection with monodirectional changes in cytokines exchange in the form of increased levels of both inflammatory and anti-inflammatory cytokines, to assess the nature of compensatory mechanisms between inflammatory and anti-inflammatory was used normalized indicator – t-test. The degree of activity of inflammatory CK in the acute period of the disease was only 15% higher in comparison with indicators of the Central Committee of anti-inflammatory. This indicates a balance of both classes of the Central Committee and on the functioning of cytokine exchange in the direction normocapnic. To understand the compensatory capacity of cytokine exchange in the period of convalescence was also assessed the balance between inflammatory and anti-inflammatory activity of the Central Committee of the inflammatory activity of the Central Committee slightly (15.9%) was higher than the rates of inflammatory CK. So, inflammatory and anti-inflammatory CK in CSF of patients GUM in the period of convalescence also balanced, and their functions are normocapnic.

Conclusion. Patients with AVM in the dynamics of the disease, the increase in the levels of proinflammatory (IL-1, IL-2, IL-6, TNF- α) and antiinflammatory (IL-4, IL-10) cytokines compared with control, and also pathogenetic balance (normocapnia) cytokines. The increase in the GA concentration in the acute phase GUM evidence of both the existence of laws neurontina processes and peculiarities, caused by the pathogen, and an increase in the stage of convalescence – preservation of intrathecal inflammation. Research in CSF in the CNS allows not only to clarify the pathogenesis of the disease, but in the long term to make a differential diagnosis between serous and purulent nature of inflammation of the meninges, to predict the severity of disease.

Key words: acute viral meningitis, cerebrospinal fluid, cytokines.

КЛІНІКО-ЕПІДЕМІОЛОГІЧНЕ ОБґРУНТУВАННЯ КОМБІНОВАНОЇ СТРАТЕГІЇ СКРИНІНГУ ЗАХВОРЮЮВАНЬ ШИЙКИ МАТКИ СЕРЕД ЖІНОК УКРАЇНИ

53-58

Соловійов С.О., Артемчук Г.П., Ковалюк О.В., Дзюблик І.В.

CLINICAL AND EPIDEMIOLOGICAL BACKGROUND OF COMBINED STRATEGY FOR CERVICAL SCREENING OF WOMEN IN UKRAINE

Soloviov S.O., Artemchuk H.P., Kovalyuk O.V., Dzyublyk I.V.

Background: cervical cancer (CC) ranks second place among cancers of women of reproductive age in Ukraine, accounting for more than 2000 deaths annually. Virtually all cases of CCs are caused by persistent infection with Human Papillomaviruses (HPVs). Two main methods of diagnosis in screening for cervical pathology are cytological test (Pap-test) and molecular-genetic (HPV-test) tests. Implementation of efficient cervical cancer screening programs, based on Pap- and HPV-tests, might significantly decrease CC incidence rates. Issue of effective combination of the two diagnostic methods and development of optimal screening strategies is essential. The primary need is the development and application of pharmacoeconomic models to compare two or more diagnostic techniques (methods). This approach could be used as a tool to select more appropriate strategy given its cost and effectiveness, or utility. The main criterion of the "cost - utility" method is utility function of each diagnostic technology. The first step in constructing this model should be the definition of its basic parameters, that is the probability of HPV infection and the presence of subclinical or clinical manifestations in women at a certain age and to identify the utility function for each diagnostic test and their combinations thereof. Objective of the study was the definition of utility functions of combined strategy using HPV- and cytological tests based on clinical and epidemiological research data.

Materials and methods: 1257 cervical smears from Ukrainian women aged 19 – 65 were tested (HPV typing test and cytological testing). "Cost-utility"-based analysis and evaluation are based on the results of own clinical and laboratory studies of 1257 cervical samples (HPV DNA tests and cytological diagnostics) from women aged 19 – 65 (mean age 30.68 (± 7.72)), living in different regions of Ukraine. Statistical and mathematical methods are used for modeling the utility function of a number of diagnostic strategies. Obtained results of laboratory testing formed the basis for developing screening strategies, with HPV test only, cytological test only or both tests depending on the differences in clinical and epidemiological history of the patient were evaluated.

Results: According to the results of clinical and epidemiological studies defined utility function for each test as components of a comprehensive strategy, the first step in developing an optimal algorithm for screening of HPV-associated cervical diseases in Ukraine. Simulation shows maximum of diagnostic utility for HPV test, as a single screening strategy, in women aged 29-30 years. Screening with cytological test only increases its utility with the increase of patients' age. Combined screening strategy based on both HPV and cytological tests shows maximum of utility for HPV test when using it among younger women (<21 years old) and for cytological test when using it with women aged >21. The results will be the basis for further comprehensive assessment of screening strategies for HPV-associated lesions of the cervix.

Conclusions: Obtained results compose the first step in the development of an optimal algorithm for HPV-associated cervical screening in Ukraine. The results will form the basis for further comprehensive assessment of screening strategies.

Key words: diagnostic screening strategy, cervical cancer, utility function, HPV testing, cytological smear.

МЕТОДИ СУЧАСНОЇ ДІАГНОСТИКИ БАБЕЗІОЗА. КРИТЕРІЙ ЯКОСТІ. ПЕРЕВАГИ ТА НЕДОЛІКИ (Повідомлення перше)

59-65

Похил С. І., Торяник І. І., Тимченко О. М., Чигиринська Н. А., Костирия І. А.

METHODS OF THE BABESIOSIS MODERN DIAGNOSTICS. QUALITY CRITERIUM. ADVANTAGES AND LIMITATIONS (First report)

Pokhyl S. I., Torianik I. I., Tymchenko O. M., Chygyrynska N. A., Kostyria I. A.

Introduction. Babesia spp. is a tick-transmitted protozoan parasite, one of the causative agents of piroplasmiasis (babesiosis). The disease is characterized by signs of malaise, inappetence, fever, hemolytic anemia and hemoglobinuria. The parasite has a wide distribution and occurs on all continents, except Australia. The life cycle of the parasite in animals organism (equine hosts, cattle, raveling dogs, cats) comprises two intracellular stages: sporozoites inoculated by infected ticks develop into schizonts within lymphocytes where they multiply and subsequently transform into microzoites, which then invade erythrocytes. Purpose and tests was to study the retrospective analysis of scientific reports about classic and modern microscopic and culture technique of blood smear staining. Materials and methods The objects of this investigations are the retrospective analysis of Ukrainian, European and pan American scientific papers (reports) about using classic and modern microscopic and culture technique of blood smear staining. Results of the study. As the result of the study (theoretic and patent literature analysis) it was revealed that the used microscopic (by Romanovsky, Write, Papengaim, Nocht) and culture technique of blood smear staining in healthy domestic dogs and those ill with Babesia infection had certain advantages. This method is simple, undemanding, short-term and not labour-consuming; these features make it promising for use in field conditions of expeditions, etc. The method is reasonable for its pricing; the ingredients are actively used in research practice by Ukrainian scientists and widely represented on the domestic market. The presence of stains, which contrast by their wide colour spectrum, facilitates the integral awareness of the morphological plot of changes in studied tissues. From the technical viewpoint there are typical appeals of clear visualization in the structural differentiation of nuclei, cytoplasmic components and membrane. In conditions of analysis of blood preparations (smears), which belonged to control animals, the expressiveness and saturation of staining of blood cells, well-outlined borders of each of them and absence of any grey and blur in their contours were striking. Erythrocytes were bright braun, characterized by a round shape and presence of clarification in their centre (discocytes). Single sajes were well discerned; determination of their quantity was not a problem. The cytoplasm of leukocytes was notable for its gray and the nuclei for their violet-red tints. The colour of the granules was saturated, up to blue. The intracellular components were visualized, their differentiation was obvious. In case of observation of affected cells the qualitative properties of the stains remained unchanged. Erythrocytes were red-grey, with a well-outlined border and evident changes of the superficial architectonics (echino-, dacryo-, stomato-. ovalo- and microspherocytes). Poikilocytosis was rather strongly pronounced in 60 % of preparations, anisocytosis in 25 %. The phenomenon of formation of "coin columns" was more widespread than in controls. The intracellular inclusions were lilac and red-violet. The character of colour of the leukocyte population did not change. Some cases demonstrated numerous aggregations of thrombocytes in the immediate proximity to erythrocytes. These blood platelets were crimson, their granules (with different shape and size according to the degree of thrombocyte maturity) were notable for their dark blue tint. The revealed phenomena were attributed to pronounced adsorptive properties of the above stains against a background of absorptive ability of blood cells (connective-tissue ones according to typology) and the result of summation of the wide colour spectrum. Through diffusion of chemical components of the used stains facilitated long-term preservation of the preparations within the established period of observation as well as qualitative representation of major elements of cell structures and intracellular inclusions. Conclusions: investigations of the scientific reports about the declared technique (microscopic and culture) of staining of blood smears from domestic dogs and cattie have been proved that is a qualitative, economically sound and technically available method of rapid diagnosis of haemoparasitic diseases like babesiosis.

Keywords: retrospective analysis scientific reports, classic and modern microscopic methods, culture technique, blood smears staining, domestic dogs, cattie, babesiosis.

ИССЛЕДОВАНИЕ ПРОТИВОМИКРОБНОЙ АКТИВНОСТИ ЛЕЧЕБНО-КОСМЕТИЧЕСКИХ СРЕДСТВ ДЛЯ ПРИМЕНЕНИЯ В СТОМАТОЛОГИИ

Бойко Н. Н., Зайцев А. И., Осолодченко Т. П., Мельник А. Л., Долгая И. Н.

STUDY OF ANTIMICROBIAL ACTIVITY OF MEDICAL-COSMETIC PRODUCTS USED IN DENTISTRY

Boyko, N.N., Zaytsev, A.I., Osolodchenko, T.P., Melnik, A.L., Dovga, I.N.

Introduction. According to statistics, the incidence of caries, gingivitis and periodontal disease in children and adults, both Ukraine and the world in general, is increasing. For medical and medical-cosmetic purposes in dentistry used medications on the basis of synthetic and natural substances that complement the basic therapy in the form of mouthwashes, toothpastes and elixirs. The purpose of this work is to study antimicrobial activity of commercially available medical-cosmetic products (mouthwashes, elixirs, and toothpastes) used for treatment of teeth and gums and comparison of integrated indexes with those of water-ethanol extracts from some plant raw material.

Materials and methods. Microbiological method of studying antimicrobial properties of eleven products, agar well diffusion method has been applied on standard microorganism test strains: *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; specific mathematic method for comparison of antimicrobial properties of products, vector theory, has been used. The microbial burden was 10^7 CFU/mL of the medium and was determined in accordance with McFarland standard. 18-24-hour microorganism culture was taken into work. Mueller-Hinton agar and MPA were used for bacteria. Agar Saburo was used for *Candida albicans*. For comparison of the antimicrobial activity preparations between themselves, calculating an average value group of identical preparations and preparation selection of the most active among these authors used vector theory. Vector theory makes it possible to present antimicrobial activity of the medications on the basis of single test strains of microorganisms as a unified vector in n-dimensional space. In this case, the vector is characterized by the following: A - integrated index of antimicrobial activity (describes the "strength" of antimicrobial activity of the medication); r^2 - the square of the correlation coefficient (describes the spectrum of action of the medication on test strains of microorganisms studied), its range of values is from 0 to 1. **Results and discussion.** High level of antimicrobial activity has been demonstrated by all medical-cosmetic products studied (mouthwashes, elixirs, and toothpastes). The averages of the complex index of antimicrobial activity for mouthwashes and elixirs are $A=1.83$, $r^2=0.93$ and the ones for toothpastes are $A=1.98$, $r^2=0.98$. It has been shown that some extracts from raw materials containing different kinds of hydroquinones, naphthoquinones, anthraquinones, alkaloids, and tannins have high potential to be used in complex phytochemical medications for dentistry as their main antimicrobial components.

Keywords: antimicrobial activity, therapeutic and preventive medicines, dentistry.

ІСТОРІЯ МЕДИЦИНИ (HISTORY OF MEDICINE)

КЭРИ МУЛЛИС – ЯРКОЕ ИМЯ В ИСТОРИИ НАУКИ

Короваева И.В., Попова Н.Г.

72-74

KARY MULLIS - BRIGHT NAME IN THE HISTORY OF SCIENCE

Korovaeva I.V., Popova N.G.

The article is devoted to the Karry Mullis – American biochemist, winner of Nobel Prize in Chemistry and to the history of discovery of polymerase chain reaction thanks to which rapid release of large number of specific DNA fragments becomes possible. This discovery made real revolution in molecular biology. The scientists had to work his hard way up from the discovery of the PCR to the recognition. Today, the polymerase chain reaction has been widely used in medicine, veterinary medicine, biology, criminology, history, archeology and other fields of human activity. PCR amplification of DNA is used around the world, becoming routine and daily tool in every molecular biology laboratory. This method is the most sensitive and specific method for diagnosis of infectious diseases. Thanks to PCR many modern scientific problems, genotyping of organisms, diagnosis of genetic diseases and the identification of predisposition to them are successfully solved. Application of PCR makes possible accurately to establish family ties, identify the person and made an analysis of ancient remains and identify the genetically modified organisms. And all this is giving the benefit to the fact that our contemporary Kerry Mullis joined previous achievements by his sharp analytical mind and discovered creative approach to the solving of existing scientific problems in biochemistry and molecular biology. **Keywords:** Kary Mullis, polymerase chain reaction, DNA amplification, oligonucleotide primers.

НАУЧНЫЕ ИДЕИ МЕЧНИКОВА И СОВРЕМЕННОСТЬ

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MECHNIKOFF'S SCIENTIFIC IDEAS AND MODERNITY

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On May 15, 2015, we celebrated the 170th anniversary of the Ilya Metchnikoff, the author of the hypothesis about parenchymella, the father of cellular immunology and inflammation theory, founder of gerontology, aging and longevity science. He was at the vanguard of the probiotics use in elderly therapy. In his work "Essays about the Origin of Species" Metchnikoff critically reviewed the history of evolution ideas development. He rejected the idea that the struggle for existence occurs primarily among member of the same species and is the starting place of the new species development and complexity. There is still dispute about the mechanisms of speciation about whether evolution is punctuated rather than smoothly gradual about the role of natural selection in the new species creation and generation of life complexity.

Metchnikoff devoted many years to studying the comparative development of the embryonic layers of lower animals. He proved that in their development the lower animals follow a plan similar to that of the higher animals. Ilya Ilyich demonstrated that ciliarians gastrulate by introgression of cells which move from the blastula wall into the interior blastocoel and formed parenchymella or phagocytella. Also he assumed that invagination arose as a secondary mechanism of gastrulation. In the paper we discussed the contemporary views about parenchymella and multicellular animal origin. Metchnikoff is rightly famous for his theories of phagocytosis and inflammation. He proposed that macrophages evolved first to regulate development, and that these function are the stage for their evolution into the cells of innate immunity. He revealed of phagocytic cells functions in clearing infections and inflammation. He described of phagocytosis as an active process and its role in host defence, across a wide range of organisms digestion. It is very importantly that cells and microorganisms were taken up by an active process, involving living, and not only dead organisms. He demonstrated killing by leukocytic enzymes ("cytase"). The humoral theory claimed that the phagocytes caused the spread of disease in the body and thus would harm the host, rather than defend it, against bacterial invasion. Metchnikoff

devoted much of his scientific work to the development and defense of the role of phagocytosis in inflammation. He observed diapedesis through vessel walls, aggregation of leukocytes at sites of inflammation. Phagocytosis not only destructs of infectious microbes including bacteria, spirochaetes and yeasts, but uptake of host cells, e.g. erythrocytes, from diverse species as well. More broadly phagocytes are the cells which preserving the integrity and defining the identity of the organism. In the article we summarized the new data and concepts about the development and functions of phagocytes in innate immunity, inflammation, oncogenesis and tissues repair. Metchnikoff believed that the disabilities of old age are the work of phagocytes transformed from defenders against infection into destroyers of tissues by autotoxins derived from putrefactive bacteria residing in the colon. According to his concept, senile degeneration of the nervous system, for example, is the work of macrophages induced by autotoxins cause them to atrophy. Such degenerative changes, he believed were nearly always premature and potentially prevented by procedures directed against the putrefactive bacteria. Metchnikoff hypothesized that diminishing the number of putrefactive bacteria in the gut with probiotics could prolong life. Until recently it was generally assumed that phagocytic removal of neurons occurs only after neuronal death. But now it has been convincingly proved that stressed but viable neurons reversibly exposed the "eat-me" signal leading to their phagocytosis by microglia; this neuronal loss was prevented in the absence of microglia. As a result these data breathe life into the Metchnikoff ageing theory.

Keywords: evolution, natural selection, parallel evolution, complexity, parenchymella, phagocytella, gastrula, phagocytes, inflammation, innate immunity, M1 macrophage, M2 macrophage, probiotics, ageing theory

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