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Contents

TUBERCULOSIS UREASE

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Introduction. *M. tuberculosis* urease (MTU) is an attractive target for chemotherapeutic intervention in tuberculosis by designing new safe and efficient enzyme inhibitors. A prerequisite for designing such inhibitors is an understanding of urease's three-dimensional (3D) structure organization. 3D structure of *M. tuberculosis* urease is unknown. When experimental three-dimensional structure of a protein is not known, homology modeling, the most commonly used computational structure prediction method, is the technique of choice. This paper aimed to build a 3D-structure of *M. tuberculosis* urease by homology modeling and to study its stability by molecular dynamics simulations. **Materials and methods.** To build MTU model, five high-resolution X-ray structures of bacterial ureases with three-subunit composition (2KAU, 5G4H, 4UBP, 4CEU, and 4EPB) have been selected as templates. For each template five stochastic alignments were created and for each alignment, a three-dimensional model was built. Then, each model was energy minimized and the models were ranked by quality Z-score. The MTU model with highest quality estimation amongst 25 potential models was selected. To further improve structure quality the model was refined by short molecular dynamics simulation that resulted in 20 snapshots which were rated according to their energy and the quality Z-score. The best scoring model having minimum energy was chosen as a final homology model of 3D structure for *M. tuberculosis*. The final model of MTU was also validated by using PDBsum and QMEAN servers. These checks confirmed good quality of MTU homology model. **Results and discussion.** Homology model of MTU is a nonamer (homotrimer of heterotrimers, $(\alpha\beta\gamma)_3$) consisting of 2349 residues. In MTU heterotrimer, sub-units α, β, and γ tightly interact with each other at a surface of approximately 3000 $Å^2$. Sub-unit α contains the enzyme active site with two Ni atoms coordinated by amino acid residues His347, His349, carbamylated Lys430*, His459, His485, Asp 573, Gly490. Helix-turn-helix motif (residues 524-545) forms a mobile flap that covers the active site and is in closed conformation impeding access to the enzyme active site. The structural stability of MTU model was checked by molecular dynamics simulation in explicit water at 300 K and pH 7,4. During the simulation, root mean square deviations of C_α atoms (RMSD C_α) and root mean square fluctuations (RMSF) of amino acid residues of MTU were monitored for 60 ns. Also, the distance between the loop that covers the active site and the dinickel center was monitored. Analysis of MD trajectory indicate that the enzyme global structure is stable and the flap covering the active center remains in closed state during the simulation time. **Conclusion.** Predicted three-dimensional structure of *M. tuberculosis* urease can be used in the studies of structure-function relationships of the enzyme, in designing new safe and efficient enzyme inhibitors aimed to struggle with infectious diseases promoted by urease activity.

Key words: *Mycobacterium tuberculosis* urease, three-dimensional structure, homology modeling, molecular dynamics simulations

LIGAND-BINDING SITES ON THE *MYCOBACTERIUM TUBERCULOSIS* UREASE 37-46

Lisnyak Yu. V., Martynov A. V.

Introduction. *Mycobacterium tuberculosis* is the causative agent of tuberculosis that remains a serious medical and social health problem. Despite intensive efforts have been made in the past decade, there are no new efficient anti-tuberculosis drugs today, and that need is growing due to the spread of drug-resistant strains of *M.tuberculosis. M. tuberculosis* urease (MTU), being an important factor of the bacterium viability and virulence, is an attractive target for anti-tuberculosis drugs acting by inhibition of urease activity. However, the commercially available urease inhibitors are toxic and unstable, that prevent their clinical use. Therefore, new more potent anti-tuberculosis drugs inhibiting new targets are urgently needed. A useful tool for the search of novel inhibitors is a computational drug design. The inhibitor design is significantly easier if binding sites on the enzyme are identified in advance. This paper aimed to determine the probable ligand binding sites on the surface of *M. tuberculosis* urease. **Methods.** To identify ligand binding sites on MTU surface, computational solvent mapping method FTSite was applied by the use of MTU homology model we have built earlier. The method places molecular probes (small organic molecules containing various functional groups) on a dense grid defined around the enzyme, and for each probe finds favorable positions. The selected poses are refined by free energy minimization, the low energy conformations are clustered, and the clusters are ranked on the basis of the average free energy. FTSite server outputs the protein residues delineating a binding sites and the probe molecules representing each cluster. To predict allosteric pockets on MTU, AlloPred and AlloSite servers were applied. AlloPred uses the normal mode analysis (NMA) and models how the dynamics of a protein would be altered in the presence of a modulator at a specific pocket. Pockets on the enzyme are predicted using the Fpocket algorithm. To model the reduction in flexibility of allosteric pocket on modulator binding, the unperturbed normal modes are first calculated for the protein. The calculation is then repeated, each time perturbing one of the pockets in the protein. These results are combined with output from Fpocket in a support vector machine (SVM) to predict allosteric pockets on proteins. The AlloSite server is similar to the AlloPred method in that it uses the Fpocket algorithm to elucidate allosteric pockets, whereas AlloPred uses an approach that combines flexibility with the Fpocket output. **Results and discussion.** By computational solvent mapping method FTSite, we have explored *M.tuberculosis* urease nonamer surface to find sites that tend to bind small organic molecular probes representing fragments of drug molecules with diverse hydrophobic and hydrophilic properties. The predicted three top ranked binding sites were situated at the interfaces between chains C and A, and chain G of neighbour trimer (and at equivalent locations in symmetrical trimers as well). A mapping of enzymes generally yields the most probable sites situated in a subsite of the enzyme active site. This was not the case for MTU which active sites were inaccessible for probes due to the closed conformation of the covering flap, and predicted binding sites were located not far from them at the entrance into a deep pocket. To explore their possible structural and functional role, we correlated the locations of predicted MTU binding sites and its ancillary pockets (which remain open and solvent exposed even while the flap is closed) and indicated their partial overlapping. This overlapping may suggest that predicted sites are likely the intermediate binding sites responsible for recruiting a ligand to another binding site deeply buried in the protein. To examine the possibility that predicted binding sites are the sites for allostery binding we carried out the search for probable sites of allostery binding on MTU surface by AlloPred and AlloSite servers. Predicted probable allosteric sites overlapped with binding sites revealed by FTSite suggesting their possible function as sites for allosteric binding. **Conclusions.** On the surface of *M.tuberculosis* urease, there were revealed the probable ligand binding sites that appear to be the sites of allosteric binding. They may serve as promising targets for designing novel allosteric modulators as receptor-selective anti-tuberculosis drugs.

Key words: *Mycobacterium tuberculosis* urease, anti-tuberculosis drugs, allosteric binding, computational drug design.

SECRETORY IMMUNOGLOBULIN A AND ITS ROLE IN FORMATION OF CLINICAL COURSE OF SHIGELLOSIS IN CHILDREN INFECTED WITH HELICOBACTER PYLORI 47-50

Kurlan N.Yu.

Introduction. Secretory immunoglobulin A (sIgA) is a crucial factor in protection of the gastrointestinal (GI) tract mucosa directly providing the first line of defense of the intestine from the impact of foreign antigens. At the same time, sIgA is able to form immune complexes not only with infectious agents and their constituent elements, which are found in the mucous membrane, but also with those, which for some reasons overcome the epithelial barrier and directly penetrate lamina propria. The release of sIgA from plasma cells occurs influenced by IL-4, IL-5, IL-6, IL-10 cytokines. Formation of long-term infectious processes as well as chronic pathology associated with increased local immunity, sIgA in particular, is considered in a range of studies. The local immunity factors are of great importance in combination with two or more pathogenic causative agents that can be present in the intestine for a long time. Taking into consideration that Shigellosis mortality rate among children is still high up to now, the issue focused on local immunity state in children with Shigellosis, infected with Helicobacter pylori is currently of concern. **Purpose of the study** is to explore local immunity competence in children with Shigellosis, infected with

Helicobacter pylori by means of sIgA level assessment. **Materials and methods.** The study involved 68 children aged from 1 to 3, who were diagnosed with Shigellosis Sonnei of medium severity. Additionally, the determination of H. pylori in feces and the level of sIgA in coprofiltrate were provided. **Results and discussion.** Significantly higher sIgA level, in comparison with the same values of the control group, was revealed in coprofiltrates of all children in acute period. At the same time, in children of Group 2 sIgA content was significantly higher than the values of patients with background infection. The qualitative sIgA content restored and significantly did not differ from the values of the control group in early convalescence period of Shigellosis in children without background infection. However, significant difference was observed with acute period value. sIgA concentration in children infected with H. pylori in the period of clinical recovery was significantly decreased in comparison with acute period values. It was significantly different from the data of healthy children and the children of Group 2. The findings obtained are indicative of imbalance of local immunity competence of the intestine in Shigellosis in children infected with H. pylori, especially impaired sIgA production. Insufficient sIgA secretion in coprofiltrates of patients with Shigellosis, that has been revealed, is not contrary to the hypothesis of some scientists concerning the capacity of pathogenic H. pylori strains to carry out their cytotoxic effect associated with decrease of local protective mechanisms of the GI tract mucosa as well as system immunity, the ability to spin out of control of specific immunity mechanisms up to development of immune-dependent inflammation forms. Taking the revealed differences of sIgA content in coprofiltrates in Shigellosis in patients with and without background infection with H. pylori into consideration, we have carried out the study of correlational connection concerned with assessment of Pearson coefficient of this value with basic clinical laboratory values. Present correlational interactions between sIgA values in coprofiltrates of children suffering from Shigellosis, infected with H. pylori, and frequency of development of specific symptoms along with their duration, are indicative of sIgA role in formation of pathogenic mechanisms of the disease course. **Conclusion.** Therefore, Shigellosis in children infected with H. pylori is accompanied by substantial impairment of local immunity competence that influences the frequency of manifestations of some clinical symptoms and pathologic changes of laboratory values, their duration. The data obtained allow to assume as the perspective direction in perfection of therapy of such patients use of complex immunoglobulin medications should be aimed primarily at inflammatory processes stopping.

Keywords. Shigellosis, immunoglobulins, children, Helicobacter pillory

INFLUENCE OF GENTAMICIN ON ENTEROCOCCI BIOFILM FORMATION 51-55

Myronenko L.G., Peretyatko O.G., Iagniuk J.A., Martynov A.V.

Introduction. Today, it is well established that almost 80% of all infectious diseases are caused by microorganisms that exist in the form of biofilms. Microorganisms in the biofilms acquire signs of increased resistance to antibiotics, disinfectants and other aggressive environmental factors, complicate the course of infectious diseases and play an important role in their chronicity. Formation of biofilms by hospital strains of bacteria poses a serious threat to the practical medicine. Enterococci, foremost *Enterococcus faecium* and *Enterococcus faecalis*, are the third most common cause of hospital infections, most of which involve the use of permanent medical equipment. Internal hospital infections gain particular importance in intensive care units and in surgical hospitals, since the formation of biofilms is the cause of severe catheter and fan associated infections, sepsis, pneumonia and endocarditis. It should be noted that ineffective antibiotic therapy of infections, accompanied by the formation of biofilms, also leads to significant economic losses. The aim of the work was to study the effects of gentamicin and gentamicin in combination with a penetrator on the processes of enterococci biofilm formation. **Materials and methods.** The objects of the study included 3 strains of bacteria genus Enterococcus, obtained from the bacteria museum of the Mechnikov Institute of Microbiology and Immunology National Academy of Medical Sciences of Ukraine: E. faecalis ATCC 29212, E. faecalis IMI (X) 49 p, E. faecium IMI (X) 80. The biofilms modelling was performed in 4-section polystyrene Petri dishes. To study the influence of compounds on biofilm formation, a photometric method was used. The optical density (OD) of eluates from enterococci biofilms, stained with crystal violet, was measured with the SF-56L spectrophotometer at a wavelength of 590 nm. Statistical processing of the obtained data was carried out by means of nonparametric statistical methods using Microsoft Excel 2007 and STATISTICA 6.0 programs. The validity of the differences between the two related samples was assessed by the Wilcoxon test and the Sign test. The effect of the compound was evaluated using biofilm inhibition index (BII), which was calculated according to the formula: [(OD positive control - OD tested) / OD positive control] × 100%. Reduction of the OD value by more than 25% in the experiment relative to OD positive control was considered as a positive effect. **Results and discussions.** Analysis of the results allowed to conclude that gentamicin at concentration of 8 mcg/ml is capable of preventing the formation of biofilms taken in experiments with enterococci. Further increase in the concentration of gentamicin to 64 mcg/ml did not lead to an increase in its activity relative to biofilm formation. When applying gentamicin at concentrations of 8 mcg/ml, 16 mcg/ml, 32 mcg/ml and 64 mcg/ml, the index of inhibition equaled 53,8%, 56,6%, 49,9% and 49,6% respectively. A higher inhibitory effect of gentamicin was identified for the formation of *E. faecium* biofilms than for *E. faecalis* ones. Thus, when applying gentamicin at concentrations of 8 mcg/ml, 16 mcg/ml, 32 mcg/ml and 64 mcg/ml for *E. faecalis* biofilm formation, the inhibition index was equal to 45,5 %, 46,7 %, 49,6 % and 48,5 %, for *E. faecium* – 54,8 %, 53,5 %, 65,3 % and 60,2 % respectively. One way of facilitating the transportation of biocides through the extracellular matrix of biofilms to the target of action may be the use of so-called penetrators. Polyethylene glycol was used as a penetrator (PNT) in our studies. Inhibition index analysis showed a statistically significant increase in the suppressing effect of the combination of gentamicin and PNT on the enterococci biofilm formation compared to the effect of gentamicin without PNT ($p<0,05$). The inhibition index of gentamicin at concentartions of 8 mcg/ml, 16 mcg/ml, 32 mcg/ml and 64 mcg/ml with PNT amounted to 73,5 %, 74,4 %, 77,2 % and 78,2 % respectively. Also, a higher suppressing effect of gentamicin with penetrator on enterococci biofilm formation was found for *E. faecium* compared to *E. faecalis*. Thus, the results obtained suggest that polyethylene glycol can increase the penetration of gentamicin through the biofilm glycocalyx. **Conclusion.** Gentamicin at concentrations of 8 mcg/ml, 16 mcg/ml, 32 mcg/ml and 64 mcg/ml shows an inhibitory effect on enterococci biofilm formation (inhibition index varied from 49,6 % to 56,6 %). Inhibitory effect of gentamicin at concentrations of 8 mcg/ml, 16 mcg/ml, 32 mcg/ml and 64 mcg/ml on enterococci biofilm formation is enhanced under the influence of polyethylene glycol (inhibition index – from 73,5 % to 78,2 %).

Keywords. Gentamycin, Enterococcus, biofilm formation, Polyethylene Glycol -400

INTEGRAL EVALUATION OF THE CYTOKINE SYSTEM IN VIRAL MYOCARDITIS 56-61

Peremot S. D., Volyansky A. Y., Smelyanskaya M. V., Kashpur N. V., Yudin I.P., Klysa T.L.

The need for an individual approach in the choice of means for the prevention of complications in inflammatory processes in cardiomyocytes, the course of which unfolds against a persistent viral infection, dictates the need to determine the general mechanisms for maintaining and progressing of the pathological process and an objective evaluation of immunological changes. **The aim of the study** was to determine changes in the system of inflammatory mediators in patients with subacute and chronic herpesviral infectious myocarditis on the basis of an integral assessment of the levels of opposing groups of cytokines. **Materials & methods.** To achieve this goal, we conducted a determination and analysis of changes in the cytokine profile in 87 patients with subacute (from 2 to 6 months) and chronic (more than 6 months) myocarditis due to an integral assessment of the mediator levels of inflammation of opposing groups in patients with herpesviral myocarditis on treatment in medical institutions of the Kharkov city. The average age of the patients was (27 ± 7.4) years. The control group was attracted to 40 people without clinical manifestations of cardiovascular diseases and in whose anamnesis there were no data on the transferred inflammatory diseases of the myocardium. Both groups of subjects were comparable in age and gender. The main group of subjects was divided into two subgroups. The first was 44 patients with subacute flow, the second - 43 patients with chronic infectious myocarditis. The diagnosis was established in accordance with the recommendations of the Association of Cardiologists of Ukraine and

experts of the European Society of Cardiology, according to the formation of definitions of diseases in the International Classification of Diseases (ICD-10) of the tenth revision. The removal of material from patients was carried out according to the rules for the collection of infectious material. The concentration of cytokines: IL-2, IL-4, IL-6, IL-10, INF-γ, TNF-α in serum was measured by enzyme-linked immunosorbent assay using commercial enzyme immunoassay kits for Thermo Scientific ™ (IL-2R IL-4, IL-6, IL-10, TNF alpha, IFN gamma ELISA Kit, Human, USA) and Stat Fax 303 Plus spectrophotometer. Statistical processing of all received data was carried out on a personal computer using the program Statistica, version 6.1 (StatSoft Inc., USA) [1]. **Results & discussion.** Analysis of levels of pro- and anti-inflammatory cytokines in patients indicates an imbalance in their system, which is characterized primarily by a significant increase in the level of IL-6 prophylaxis to (134.09 ± 22.72) pg / ml (control level 11.83 ± 1, 64 pg / ml) and a relatively moderate increase in IL-2 and TNF-α levels in subacute myocarditis. Such an increase in the level of IL-6, in our opinion, is due to the dualism of the action of this interleukin, the proinflammatory nature of its action at the final stage of inflammation changes to anti-inflammatory. As a consequence, in combination with IL-10, it limits the secretion of TNF-α. That is why its level remains high and with chronic herpesviral myocarditis and exceeds the level of the control group more than 8 times. In addition, in the chronic form of the course of herpesviral myocarditis, an increase in the levels of anti-inflammatory IL4 and IL-10 cytokines is observed in 2.9 and 3.1 times, respectively. And the level of IL-10 increased not only in comparison with the level of the control group, but also exceeded by 1.5 times the corresponding index for subacute myocarditis. In order to optimize the analysis of cytokine imbalance, an integral assessment of the levels of inflammatory mediators from opposing groups was carried out. Calculation of the integral indicator (II) of the cytokine balance was performed by determining the values of cytokine indices as the ratios of the levels of proinflammatory and anti-inflammatory sera in the examined patients to the reference values of the control group and the arithmetic mean for each opposing group of cytokines expressed in conventional units (c.u.). The optimal balance of cytokines corresponded to the level of II ≤ 1 c.u. and indicated the absence of inflammation, but the activity of the inflammatory process was characterized by exceeding the level of more than 1 condition. In the group of patients with subacute myocarditis, II was 6,27 c.u., exceeding the corresponding calculated indicator of a group of patients with chronic course in more than 1.6 times (3,82 c.u.). Therefore, the higher the deviation of the II from 1 cu is, the deeper the violation of immunological homeostasis. **Conclusion.** It was found that imbalance in the cytokine system in subacute and chronic herpesviral myocarditis is a universal immune system response, which is characterized by an increase in the levels of proinflammatory cytokines against a background of moderate growth of anti-inflammatory ones. The level of the integral cytokine index is more than 1 cu indicates the dysfunction of the immunological status of the patients being examined and can be used as an additional diagnostic criterion for the unfavorable course of the disease with a propensity to progress. Calculation of II defines a personalized diagnosis of cytokine imbalance with the ability to determine on its basis therapeutic approaches and the choice of immunorehabilitation tools, and also allows evaluating the effectiveness of selected anti-inflammatory agents for treatment of infectious herpesvirus myocarditis.
Keywords. Cytokine, viral myocarditis, herpesviruses.

NEW FORMYL PEPTIDE HAS THE STIMULATING PROPERTIES IN POINT OF IMMUNE SYSTEM CELLS **62-66**

Martynov A. V., Romanova E. A., Pohorila M. S., Shcherbak O. M., Sidorenko T. A., Igumnova N. I., Yukhimenko V. I.

Introduction Tuberculosis infection (TBI) is still one of the major health problems, despite of global intensive medical and pharmaceutical efforts as it is known, in the majority of immunocompetent individuals TBI is repressed by immune system, and as a result we can observe the latent TBI. The main danger hides in unpredictable activation process of latent TBI determining the spreading of infection among population. Now we investigate the ability of new formyl peptide to stimulate phagocytosis completion in vivo. This strategy is explained by the key role of the phagocytosis completion in preventing long-term persistence of *M. tuberculosis* in macrophage. Formyl peptides are released by microbes and damaged tissues that are perceived as danger signals and is recognized by the innate immune system by formyl peptides receptors expressed on neutrophil granulocytes. Recent studies show that activated formyl peptide receptors (FPR1 and FPR2) trigger a variety of functions, including chemotaxis, degranulation, ROS (reactive oxygen) production and phagocytosis. **Materials and methods** The ability of new formyl peptide to activate the completeness of phagocytosis by peritoneal macrophages absorbed by them was evaluated *in vivo*. For reaching the aim of the study we have used peritoneal macrophages obtained from white laboratory male mice 2 months of age, and weight - 22 ± 2 g. Total of 36 animals were randomized on 4 groups:1 Group – (Control) - mice with NaCl solution (0,9 %) injection, (n=11), 2 Group – mice with dexamethasone injection, (n=11), 3 Group - mice with dexamethasone and BCG injection, (n=11), 4 Group - mice with dexamethasone, BCG and formyl peptide injection, (n=11). Animals were kept in vivarium of "Mechnikov institute of Microbiology and Immunology of NAMS of Ukraine" on a standard diet with specified conditions of animal management. Work with laboratory animals was performed according to the rules. The peritoneum macrophages functional activity was assessed by using Staphylococcus phagocytosis test, proliferative activity of lymphocytes - by the level of their spontaneous and FGA-induced transformations in vitro (RBTL), level of receptor's expression on lymphocytes was examined in reaction of lymphocytes rosette formation with sheep red blood cells. The number of different types of cariocytes in the leukogram was counted morphologically using the light microscope «PrimoStar» (Carl Zeiss, Germany), taking into account not less than 200 cells in the preparation stained with Romanowsky-Gimza stain. Statistical significance was determined by using unpaired *t* test and one way ANOVA. $p < 0,05$ was taken as the level of significance. **Results and discussion** The introduction of the new formyl peptide to mice injected with BCG and dexamethasone promoted increasing of the phagocytic activity of peritoneal macrophages. What is demonstrated by significantly growing of phagocytic and lytic indexes in this group compared to the group of mice that has not obtained the formyl peptide after BCG and dexamethasone administration, ($p<0,05$). Also, was observed the *ex vivo* enhancing of FGA-stimulated (by 1,4 times) and spontaneous lymphocyte transformation (by 1,8 times) after the formyl peptide's administration compared to the appropriate control group, ($p<0,05$). New formyl peptide is able to promote the expression of receptors on lymphocytes. The percent of receptors expression on lymphocytes has raised by 2,7 times after the formyl peptide's administration in mice with BCG and dexamethasone injections. The formyl peptide administration has led to the normalization of blood cells count, when their depletion after dexamethasone and BCG injection has taken place. Conclusions The new formyl peptide administration to mice injected with BCG and dexamethasone promotes increasing of the phagocytic activity of peritoneal macrophages, enhance the FGA-stimulated and spontaneous lymphocyte transformation, enhances the level of receptors expression on lymphocytes compared with to the group of mice that has not obtained the formyl peptide after BCG and dexamethasone injection, ($p<0,05$). The formyl peptide administration normalizes the blood cells count compared to the appropriate control group, where the total count of leucocytes was decreased and ratio of neutrophils, lymphocytes and eosinophils were characterized by a disproportion.
Key words: tuberculosis infection, formyl-peptides, immune system cells, phagocytosis, immunosuppression, experiment.

ANALYSIS OF THE EFFECTIVENESS OF INDIVIDUALIZED TREATMENT REGIMENS FOR NONTUBERCULOUS MYCOBACTERIOSES **67-70**

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Despite the fact that in Ukraine, as well as worldwide the incidence of nontuberculous mycobacterioses is growing, in our country there are still no standardized protocols for their diagnosis and treatment, which makes it impossible to prescribe adequate chemotherapy and worsens the prognosis of treatment. We have retrospectively studied medical histories of 26 patients who were diagnosed with "pulmonary non-tuberculous mycobacteriosis" during 2014-2016. The diagnosis of "non-tuberculous mycobacteriosis" was established based on the growth of non-tuberculous mycobacteria (NTMB) in BACTEC system, and then verified by the absence of Cord-factor formation, negative immunochromatographic test, negative GeneXpert MTB / RIF. Based on the results of the studies, it was determined that 17 patients had slow-growing chromogenic NTMB and 9 patients had slow-growing non-chromogenic NTMB. With the use of 2HRZE regimen, 83.3% of

patients with slow-growing chromogenic NTMB underwent laboratory recovery, and only 41.7% of patients had clinical and X-ray recovery. However, patients who received the individual regimen (2R(Rfb)Z(E)LfxKm) had a rapid positive dynamics, clinical, radiological and laboratory recovery until the end of intensive phase. In patients with slow-growing non-chromogenic NTMB, all three regimens (2HRZE, 2R(Rfb)Z(E)LfxKm, 2R(Rfb)Z(E)LfxClr) proved to be effective. We believe that it is necessary to improve level of identification of NTMB for the timely appointment of an adequate chemotherapy regimen.
Keywords. Nontuberculosis mycobacterium, chemotherapy

SYNERGISTIC EFFECTS OF ETHANOL MEDICINAL PLANT EXTRACTS WITH ERYTHROMYCIN AGAINST SKIN STRAINS OF STAPHYLOCOCCI WITH INDUCIBLE PHENOTYPE OF MLS-RESISTANCE

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Introduction. One of the main ways to control microorganisms' resistance to antibiotics is to find substances that are able to overcome it and potentiate antibiotics action, in particular to neutralize the antibiotic-inactivating enzymes or block the active efflux of antibiotic from microbial cells. Every year there is a growing interest in the therapeutic potential of herbal active compounds as modifiers of antibiotic resistance including MLS-resistance (macrolide-lincosamide-streptoramin B). It should be emphasized that a number of biologically active substances of plant origin can potentiate antimicrobial activity of erythromycin (ERY) against MLS-resistant staphylococci. The present study was designed to investigate the antibacterial and synergistic effects of eight Ukrainian ethanol medicinal plant extracts with erythromycin against skin strains of staphylococci with inducible phenotype of MLS-resistance. **Material & methods.** *S. aureus* and *S. epidermidis* strains were tested for susceptibility to antibiotics of MLS-group by disk diffusion test. Effective antimicrobial concentrations of plant extracts and erythromycin were determined by two-fold serial dilution in nutrient agar and broth. Combinatory effects between organic extracts and ERY were assessed using the checkerboard assay against tested strains to evaluate culture growth in the presence of two antimicrobials with different concentrations. **Results & discussion.** The *Alnus incana L*. fruits extract was the most potent inhibitor against tested strains (MIC 40.625-162.5 μg/mL); while *Geranium pratense L*. rhizomes extract exhibited the least antimicrobial activity (MIC 650-2,600 μg/mL). The *Alnus incana L*. fruits extract and the *Geranium pratense L*. rhizomes extract showed synergistic effect with erythromycin against 100% strains of staphylococci (average FICI 0.028 – 0.057; $p<0.001$). In the presence of $^1/_4$ MIC of ERY *Alnus incana L*. fruits extract antimicrobial concentration was decreased in 32-64 times and Geranium pratense L. rhizomes extract antimicrobial concentration was decreased in 64-256 times. Ethanol extracts of *Betula verrucosa L*. buds (average FICI 0.473±0.20), *Arctostaphylos uva-ursi (L.) Spreng*. leaves (average FICI 0.143±0.18) and *Tamarix ramosissima Ledeb*. leaves (average FICI 0.189±0.29) showed synergic action with erythromycin against 71.4-85.7% tested strains. Ethanol extracts of *Sanguisorba officinalis L*. roots showed non-interactive action with antibiotic against 42.8% isolates of staphylococci. Additive interaction with erythromycin for this extract was observed against 28.6% and synergic action against 28.6% strains (average FICI 0.812±0.52). *Biota orientalis (L.) Endl*. (*Platycladus orientalis (L.) Franco*) fruits extract and *Cotinus coggygria Scop*. (*Rhus cotinus R*.) leaves extract exhibited non-interactive action with antibiotic against all tested strains (average FICI 2.0±0.0). Experimental data indicate that combination of plant extracts with macrolides in therapeutic regimens against MLS-resistant staphylococci is promising, particularly for the treatment of pyoderma. The introduction of combined chemotherapy in clinical practice can actually help to solve two problems of modern medicine - slow the process of microorganisms (such as staphylococcus) resistance to antibiotics acquiring and improve treatment of infections caused by resistant strains. Detection of bacteria antibiotic resistance modifiers in various plants stimulates to their intensive phytochemical study for isolation and identification of the active components. It will help to investigate the mechanisms of synergy on the molecular level. **Conclusion.** BAC of medicinal and aromatic plants potentiate antimicrobial activity of macrolides against skin isolates of staphylococci with inducible MLS-resistance. *Alnus incana L.* fruits ethanolic extract demonstrates the best direct antimicrobial activity and in combination with ERY synergistically inhibits the growth of *S. aureus* and *S. epidermidis* MLS-resistant strains. Ethanolic extracts of *Geranium pratense L.* rhizomes, *Arctostaphylos uva-ursi (L.)* Spreng. leaves, *Tamarix ramosissima Ledeb*. leaves and *Betula verrucosa L.* buds also exhibit synergistic effect with ERY against skin isolates of staphylococci with inducible MLS-resistance.
Keywords*:* plant extracts, erythromycin, staphylococci, MLS-resistance, synergistic effects.