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but also knowledge in a number of broad areas: from quantum physics and chemistry to synthetic organic chemistry, in order to synthesize engineered substances. Despite changes in the concept of drug development: from banal screening (out of thousands of synthesized compounds, only one showed biological activity) to those obtained as a result of molecular modeling (another name is drug-design). (named as drug-design). The approach with the use of molecular modeling led to the intensification of research - to the synthesis of drugs based on simulated inhibitor profiles. This increased the yield of drugs - out of every hundreds of the synthesized substances, one showed the expected activity. The cost of pharmaceutical development software is currently quite high and can even reach tens of millions of dollars. But this is a reasonable amount, which makes it possible to obtain the required pharmaceutical preparations, at least for known target proteins. However, for the design of drugs of new generations at all stages of development - from building a model of a target protein to creating a drug profile and its synthesis, TRIZ has not been used systematically. Pharmaceutical industry is a huge area to be explored by TRIZ.

Keywords: TRIZ, theory of inventive problem solving, Altshuller, TRIZ in pharmaceutical industry and pharmacology, Laws of technical systems evolution, problem solving, Su-field analysis, drug-design, dynamic self-organizing, quasi live drugs, anti-cancer, antiviral, multidrug bacterial resistance, antibacterial, synergy.

Experimental works

THE MIMICRY ANTIGENS OF BRONCHOPULMONARY SYSTEM AS FACTORS OF AUTOIMMUNE PROCESS INITIATION IN CHILDHOOD BRONCHIAL ASTHMA 35-39

Chernuskiy V. G,. Popov N. N., Govalenkova O. L., Letyago A. V., Kashina-Yarmak V. L., Tolmachova S. R., Popova A. N.

Introduction. Microorganisms, isolated from the sputum of children with bronchial asthma (BA) in the exacerbation period, are able to acquire mimicry antigens of the trachea, bronchi and lung tissue, and have sensitizing effect on the organism of the child not only through the truly microbial (viral) antigens, but through the acquitted mimicry antigens of the cellular and tissue structures of the bronchopulmonary system, thus shifting the pathological process towards autoimmunity. **Materials & methods.** A microbiological study of the sputum obtained from the 135 examined children with BA aged 6 to 14 years in the exacerbation period. The disease diagnosis was established according to the protocol and directive of the Ministry of Health of Ukraine from 08.10.2013 № 868. It was established that 45 children had non – atopic asthma, 46 – mixed type asthma (MTBA) and 44 – atopic form of BA (ATBA). Microbiological studies of the sputum were carried out with the help of the commonly accepted methods: plating onto the solid and liquid culture mediums with the subsequent strains isolation, microscopy, biochemical and serological identification. Strains identification was carried out according to the taxonomic tests of the Berge microorganism index. In order to determine the presence of mimicry antigens in the examined strains we have prepared hyperimmune rabbit serums to the trachea, bronchi, and lung tissue antigens. Section samples obtained from the accidentally deceased children with the I(0) blood type 2-4 hours after the moment of death served as a antigenic material. **Results & discussion.** BA in children is characterized by complex etiological structure that combines Gram-positive, Gram-negative and Candida spp. fungi, and their associations. A comparative study of the quantitative composition of the microorganisms isolated from the sputum of the 135 examined children aged 5 to 14 years in the exacerbation period was carried out. It was established that the following microorganisms were isolated from the sputum of the children with ATBA with the lowest frequency: S. pyogenes - 3 (6,8 ± 2,1%), S. aureus - 4 (9,1 ± 2,5%), and E. coli — 5 (11,4 ± 2,3%); among associations - S. aureus + S. pyogenes - 2 (4,5 ± 1,3%), S. aureus + P. mirabilis - 2 (4,5 ±1,3%). The most frequent microorganisms were: C. albicans - 8 (18,2 ± 4,4%), P. aeruginosa - 7 (16,0 ± 4,2%), and among associations - S. aureus + P. aeruginosa - 4 (9,1 ± 2,5%), and S. aureus + E. coli-3(6,8 ±2,1%). In the children with NABA, the least frequent microorganisms were: C. albicans fungi - 2 (4,4 ± 1,4%), as well as associations: S. aureus + E. coli - 2 (4,4 ± 1,4%), and S. aureus + P. mirabilis - 3 (6,7 ± 1,7%), and the most frequent - S. aureus 7 (15,2 ± 3,1%), P. aeruginosa - 7 (15,2 ± 3,1%), as well as associations: S. aureus + S. pyogenes - 4 (8,7 ± 2,2 %) и S. aureus + P. aeruginosa - 4 (8,72 ± 2,2%). In children with MTBA the lowest frequency of isolation from the sputum was observed for: Pr. mirabilis - 3 (6,5±1,8 %) and Candida spp. fungi - 5 (10,9±4,1 %), among associations - S.aureus + E.coli- 2 (4,3±1,6%); S.aureus + P. mirabilis- 3 (6,5±1,8 %), the most frequent microorganisms were: S.aureus - 7 (15,2±3,1 %), P.aeraginosa - 7 (15,2±3,1 %), and among associations: S.aureus + S.pyogenes - 4 (8,7±2,2 %), and S.aureus + P. aeruginosa -4(8,7±2,2%). The participation of the microflora isolated from the sputum in the etiopathogenesis of the disease can be proven based on the determination in their structure of the mimicry antigens of the trachea, bronchi and lung tissue. It was experimentally proven in course of the study that in NABA the titers of the agglutination of the organ specific serums with Gram-positive microorganisms (Streptococcus and Staphylococcus) were 1:131 — 1:149, which points out their decisive role in the etiopathogenesis in this form of BA, while in the Gram-negative microorganisms, the background values of the titers were observed - 1:17 - 1:85. In MTBA, the agglutination titer of organ specific serums with Gram-positive microorganisms (Streptococcus and Staphylococcus) was in the range (1:128 - 1:213), in Gram-negative microorganisms (E. coli and P. aeruginosa) – (1:64 - 1:160), which points to the participation of the pyogenic and Gram-negative microflora in the etiopathogenesis of this form of BA. In ATBA, the results of agglutination reaction of organ specific serums with Gram-positive microorganisms and Gram-negative microorganisms were in the range of 1:18 - 1:44, the lowest range compared to the NABA and MTBA. It can be concluded that microorganisms, isolated from the children with BA, are able through inclusion into their structure the mimicry antigens of the trachea, bronchi and lung structure, not only to determine the induction of the pathological process, but also to shift it towards autoimmunity. **Conclusion.** 1. Independent of the BA form in children, the microbial factor has the leading role in its etiopathogenesis, and can lead to the increased severity of the disease course. 2. BA in children is characterized by complex etiological structure that combines Gram-positive, Gram-negative, Candida spp. fungi, and their associations. 3. Microorganisms isolated from the sputum of children with BA, through varying their antigenic potential, are able to include into their structure mimicry antigens of the cellular and tissue structure of the bronchopulmonary system. 4. Microorganisms, through inclusion into their structure the mimicry antigens of the trachea, bronchi and lung structure, not only determine the induction of the pathological process, but also shift it towards autoimmunity.

Keywords: autoimmunity, mimicry antigens, asthma, children

CHARACTERISTICS OF THE VIABILITY AND ACCORDANCE WITH THE TAXONOMIC STATUS OF THE LYOPHILIZED SAMPLES OF MUSEUM STRAINS OF ESCHERICHIA COLI ISOLATED IN 1946-1959 YEARS 40-42

Popov M. M., Peretyatko O. G., Yagnuk Yu. A., Cholodna T. V.

Effective microorganisms' conservation with the aim of long-term storage of the strains in the collection without changes in the morphological, physiological and genetical properties is provided by methods that allow the shift of the vegetative forms of bacterial cells into the anabiosis state. The most widespread among them is the lyophilization method. The museum of microorganisms of the State

Establishment “Mechnikov Institute of microbiology and immunology of the National Academy of Medical Sciences of Ukraine” has one of the oldest bacterial collections in Europe that consists of more than 4000 lyophilized samples of microorganisms strains. **The aim** of the study was the testing of viability and species specific properties of the museum strains of *E. coli*, that were preserved in lyophilized state long-term. **Materials and methods.** The objects of the study were the 30 lyophilized samples of the 22 strains of *E. coli.* Lyophilized cultures were restored through dilution of the ampule content in the 1,0 ml nutritive broth and seeding of the microbial suspension from the 100-times dilutions onto the agar-based media (blood agar, Endo medium); the viability was determined based on the quantity of the colony forming units (CFU/ml). Re-identification of the microbial cultures was carried out with the use of API system produced by «Bio-Merieux», France (ID 32 GN – for enterobacteria identification). The phenotypic intra-strains heterogeneity of the population was evaluated by dissociation index that reflects the ratio (%) of the certain colony forms (S-, R-, D-, M- forms) compared to the total count. The statistical analysis was carried out based on the parametric statistic methods with the use of Microsoft Excel 2007 and STATISTICA 6.0 computer programs. **Results and discussion.** There were (66,7±8,6) % of the total amount of *E. coli* strains participating in the experiment that were able to be restored, and therefore those samples were selected for further studies. It was established that the quantity of the colonies on the solid nutritive media in the restored strains varied from 10^4 to 10^9 CFU/ml, the average survivability parameter was (26,7±4,6) %. During the statistical analysis of the results no correlations between the CFU count and the storage term were established (r=0). It was established that the majority of the re-cultivated strains (90,0±3,8) % was characterized by dissociation into different colonial and morphological variants. The dissociation index (ID) values in the microbial populations of the studied Escherichia strains varied in the range of 10,0 % to 90,0 %. The statistical analysis of the data has established the presence of correlation between the dissociation index and term of storage of the sample in the lyophilized state (r>0,95). The established colonial polymorphism of the studied strains of *E. coli*, in our opinion, is caused by adaptation to the stressful conditions and leads to the increase of the survivability of the bacterial population in course of the long-term storage in the lyophilized state. According to the re-identification results, the majority (90,0±3,5) % of the samples corresponded to the data indicated in the strains passport, except two strains that did not correspond to their initial identification based on the total of biochemical tests. **Conclusions**. The restoring of the lyophilized cultures of *E. coli* from 1946-1959 yy. of isolation the majority of samples was found to be viable and the viability varied in the range of 0,001 % to 100,0 %. It was established that the populations (90,0±3,8) % of restored strains were characterized by dissociation into different colonial and morphological variants. Based on the re-identification results of *E. coli* the correct identification of strains was carried out and corrections were put in their passports. **Further studies perspectives.** It is planned to study the sensitivity to antibiotics of the collection strains of *E. coli*, isolated in the different periods of antibiotic use.
Key words: lyophilized samples, museum *E. Coli* strains, viability, taxomic status.

DETERMINATION OF VAGINAL GEL COMPOSITION ON THE BASIS OF BIOPHARMACEUTICAL AND RHELOGICAL RESEARCHES 43-47

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Aim. The aim of the work is to determine the content of gelling agent in the composition of the vaginal gel for the treatment of urogenital symptoms during the period of women's hormonal instability and to determine the concentration of a non-aqueous solvent propylene glycol in the gel to provide release of the active ingredient, resveratrol. **Materials and methods.** As objects of the study samples of gels with resveratrol and hyaluronic acid with varying concentrations of gelling agent aristoflex AVC and non-aqueous solvent propylene glycol have been taken. Biopharmaceutical studies by equilibrium dialysis through a semipermeable membrane for 6 hours were performed to select propylene glycol concentration. The optical density of the samples obtained was determined using Evolution 60-S spectrophotometer. The concentration of the gelling agent was determined by rheological performance using the Rheolab QC rheometer, by Anton Paar, Austria. **Results**. In the course of the study, a comparison was made between resveratrol release from samples with propylene glycol concentration of 10% and 15%. At 6th hour of the experiment, a larger amount of resveratrol, which has passed to the solution, was observed in a sample with propylene glycol concentration 15%. It has been established that an increase in the concentration of propylene glycol contributes to an increase in the rate of the active ingredient release from gel samples. Thus, for further studies on the development of vaginal gel composition, the concentration of non-aqueous solvent was chosen to be 15%. At the next stage, the choice of gel-former concentration in the gel was made. In the course of the study, rheological properties of samples with gel-former concentration of 1%, 1.5% and 2% have been compared. The obtained rheograms of gel samples indicate that all systems are coagulating with a pseudoplastic flow type and a certain degree of thixotropy. A sample with the concentration of 1% is a liquefied system that has practically no structure, as evidenced by the large difference between initial and final viscosity. An increase in the concentration of gelling agent to 2% leads to a strengthening of the structure. The sample with Aristoflex concentration 1,5% has optimal rheological parameters that can provide high biological availability of active substances. **Conclusions**. It has been established that the optimal concentration of PG, which contributes to maximal release of the active substance, is 15% of the total mass of the sample. According to the results of rheological research, the rational content of the gel-former in the gel is 1,5%.
Keywords. Vaginal gel, rheology, dialysis, resveratrol.

AGE-RELATED CHANGES OF SENSIBILIZATION PROFILES TO INDOOR INHALATION ALLERGENS IN THE PATIENTS OF THE SOUTHERN REGION OF UKRAINE 48-53

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Introduction: It is known that epidemiology of allergic diseases is affected by range of plants, animal and insect allergens, social and living conditions, nature of food habits of certain populations, sex and age of patients. Age-related changes in immunological sensitization profiles may also be geographically dependent. Thus, it is important to have epidemiological data that characterizes a set of primary importance allergens for different age groups for each particular geographic region. The purpose of the study: Identify profiles of sensitization to household allergens, which often cause respiratory manifestations of allergic diseases throughout the year in patients of different age groups in the Southern region of Ukraine. Materials and methods: Patients from Southern Region of Ukraine aged 1 to 84 years were included in the study. During 2015-2018 years, we examined 2197 patients living in the Southern region of Ukraine (Odessa and Mykolaiv regions) with suspected respiratory allergic diseases and/or proven allergic diagnosis (pollinosis, allergic rhinconjunctivitis and bronchial asthma) for presence of IgE antibodies to household respiratory allergens by immunoblotting method. Results & discussion: The highest percentage of positive reactions in whole pool of patients was shown to mould Alternaria alternata (40,7%±1,0%), storage mite Acarus siro (38,7%±1,3%) and domestic dust mite D.pteronyssinus (28,3%±0,9%). First rise of antibodies to Alternaria alternata was registered from age 0-5 to 5-10 (46,3%±2,0% to 54,2%±2,6% respectively, p<0,05) and then were gradually decreased up to age group 20-25 and stay at the same level during life. Antibodies to dust mites and cat epithelium were gradually decreased with age, in the same time antibodies to storage mites were

gradually increased and reached the highest percentage at the age 20-30 years (48,6%±4,2%). Conclusion: Our study showed that in the Southern region of Ukraine antibodies to mouse and rat epithelium was registered from early age in high levels: 37,1%±4,5% and 24,1±4,0% respectively. Level of antibodies to mould Alternaria alternata (m6) in the Southern region of Ukraine was unexpectedly high. It was the highest comparing to data from other countries. On the base of our data could be proposed main target-allergens for examination of allergic patients in different ages.
Keywords: allergy, indoor allergens, household allergens, epidemiology of allergic diseases, sensibilization profiles

DOCKING STUDY OF MOLECULAR MECHANISM BEHIND THE QUERCETIN INHIBITION OF *MYCOBACTERIUM TUBERCULOSIS* UREASE

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Introduction. *Mycobacterium 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, known urease inhibitors (phosphorodiamidates, hydroxamic acid derivatives and imidazoles) are toxic and/or unstable, that prevent their clinical use. Therefore, the development of novel efficient and safe MTU inhibitors is necessary. To achieve this goal, we have chosen flavonoid quercetin as a scaffold to develop new MTU inhibitors. M**ethods. *Homology modeling*.** The target amino acid sequence of *M. tuberculosis* H37Rv urease was taken from GenBank at NCBI. Homology model of *M. tuberculosis* urease was built as described earlier by using molecular modeling program YASARA Structure. Amongst the top-scoring templates, five high-resolution X-ray structures were selected For each template five stochastic alignments were created and for each alignment a three-dimensional model was built. Each model was energy minimized with explicit water molecules using Yasara2 force field, and the models were ranked by quality Z-score. From these 25 three-dimensional models obtained, there was selected a model based on the template X-ray high-resolution structure for *S. pasteurii* urease (5G4H) which contained the flap in open state and had the highest quality score amongst the nonamer structures (i.e. $(\alpha\beta\gamma)_3$ macromolecular ensembles). ***Search of inhibitor binding sites on the surface of MTU.*** The search of inhibitor binding sites on the surface of MTU was carried out by two steps. At first step, we used computational solvent mapping method FTSite to identify a ligand binding sites on MTU surface. At second step, docking of quercetin on MTU surface by AutoDock Vina implemented in YASARA Structure was carried out within the ligand binding sites revealed by FTSite. *Mapping of protein surface by FTSite method.* Computational solvent mapping method FTSite was used through the online server. FTSite server outputs the protein residues delineating the first three binding sites. *Molecular docking by AutoDock VINA*. Docking of quercetin on the surface of *M. tuberculosis* urease by AutoDock VINA was carried within the binding sites previously revealed by FTSite server. Docking was performead by using default parameters within a cubic 30 Å × 30 Å × 30 Å simulation cell centered on S atom of Cys 532 residue. The *M. tuberculosis* urease structure was kept rigid while the ligand structure was flexible. The best hit of 25 runs having the lowest binding energy was chosen as a final binding pose. An analysis of molecular interactions and a representation of the results by molecular graphics were done by YASARA Structure, LigPlot+ and PyMol. **Results and discussion.** In the best binding pose, quercetin molecule is situated deep in the MTU cavity which leads to the active site channel and near the active site flap. The circle B of quercetin is directed to the active center, while the circle A is directed towards the exit from the cavity. Binding energy and dissociation constant of quercetin complex with urease is 8.7 Kcal/mol and 0.4 μM, correspondingly. Ligand efficiency equals 0.4. The binding of quercetin is provided by tight van der Waals contacts with eleven residues two of which (Cys 532 and His 533) belong to the active site flap modulating transit of substrate and products of catalysis through the active site channel. The binding of quercetin is additionally stabilized by six hydrogen bonds with residues Glu 376, Lys 379, Thr 380, Gly 490 and Ala 576. These intermolecular interactions (through the tight contact with flap residues Cys 532 and, especially His 533) cause steric hindrance for the flap transition from open to closed conformation thus fixing it in the open state that blocks catalysis. Our model of quercetin binding to MTU corresponds to the results of Xiao Z.-P. et al. which showed by enzyme kinetics and molecular docking that quercetin is a noncompetitive inhibitor of *Helicobacter pylori* urease and it is positioned near the active site flap as well blocking it in the open conformation. As well, our model of quercetin binding to urease corresponds to the results of Macomber L. et al. which showed by docking that quercetin binds to the flap region of *Klebsiella aerogenes* urease. However, our model disagrees with the proposed general mechanism of urease inhibition by aromatic poly-hydroxylated inhibitors through the covalent binding with Cys residue of the flap covering the active site. It may be a consequence of the limitation of molecular docking methods used in our study that can explore only non-bound interactions. **Conclusions.** Because of the absence of experimental structure of *M. tuberculosis* urease its homology model was built and used in further studies of ligand-urease interactions. It was revealed that quercetin molecule is situated in the MTU cavity leading to the active site channel, near the active site flap. The binding of quercetin is provided by van der Waals contacts with eleven residues and by six hydrogen bonds with urease residues. Based on the analysis of peculiarities of quercetin binding with MTU, molecular mechanism of MTU inhibition by quercetin was proposed. The model of quercetin binding with MTU corresponds well to the results of docking studies on quercetin binding to *Helicobacter pylori* and *Klebsiella aerogenes* ureases. The results obtained expand the knowledge on the molecular mechanisms of urease inhibition and contribute to the development of new anti-tuberculosis immunomodulators.
Key words: *Mycobacterium tuberculosis* urease, urease inhibitors, quercetin, molecular docking.

Short communication

INFLUENCE OF THE ENHANCERS ON THE BACTERIOPHAGES ADAPTATION TO PSEUDOMONAS AERUGINOSA CLINICAL STRAINS

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Introduction. One of the areas of treatment of infectious patients can be considered bacteriophage therapy. It is known that phages have a high specificity, have a different level of lytic activity, and are able to pass into a state of prophage, or adapt to the circulating bacteria strain and acquire the ability to cause their lysis. The purpose of this work was to obtain highly virulent bacteriophages by adapting their moderately sensitive clones to clinical strains of *P. aeruginosa*. **Materials and methods.** The object of study was the culture of 15 strains of *P. aeruginosa* and 13 freshly isolated clinical strains). For experimental studies used commercial preparations of bacteriophages (Microgen, Perm, Russia)). As enhancers, were used: 1- (3,4-dimethoxybenzyl) -6,7-dimethoxyisoquinoline (0.5 ml), 2.2 ', 2' ', 2' '- (4,8-di (piperidin-1 -yl) pyrimido [5,4-d] pyrimidine-2,6-diyl) bis (azanetriyl) tetraethanol (4 ml), 2- (Phenylmethyl) -1H-benzimidazole (1 ml), which previously showed the ability to synergize to activate microbial growth. Determination of sensitivity to specific bacteriophages was performed by the drip method. The adaptation process included sequential bacteriophage passages in *P. aeruginosa* cultures, obtaining phage filtrate and release from culture by centrifugation at 5000 rpm. **Results and discussion**. The total number of strains that became sensitive to phages under the action of adapted phages was 47%, and with enhancers – 67%. In 20.0 % cases, there was a slight growth of secondary colonies (grade "+++") after the 10th passage, and 2 cultures remained resistant to phages, both to the original and adapted by both methods.

Conclusion. Thus, by adapting phages to clinical strains of *P. aeruginosa*, it was possible to increase their lytic activity by 3.5–5 times, which indicates the promise of using this method to obtain highly effective phage preparations and phagolytic vaccines.
Keywords: Pseudomonas aeruginosa, phages, stimulation of bacterial growth, enhancing of phages adaptation