2016 № 3

ЗМІСТ
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As a result of years' research of the many research groups around the world able to understand the reason why it will be impossible to create really effective vaccine for the prevention of tuberculosis infection in the near future. The main reason for the impossibility creating such vaccine is an intracellular nature of tuberculosis. In fact, TB is a pathology of the immune system. Mycobacterium tuberculosis persist within macrophages and thereby inhibit the process of phagocytosis completion and digesting the contents of phagosome. The destruction of the lysosomal membrane inside macrophages is blocked by changing the pH in lysosomes. For the presence of lytic activity for most lysosomal enzymes require need acidic environment. Mycobacteria are also getting into the lysosomes of macrophages start to rapidly hydrolysis for urea by urease to form ammonia. Wherein pH in the medium changes to alkaline, this inactivates enzymes and stabilizes lysosomal membrane. Thus mycobacterium prevent lysosome collapse at inactivated lysosomal enzymes and do not allow them to complete macrophage digestion phase by transition lysosomal to phagosomal stage. Stop phagocytolysis process leads to imbalance of the host immune system. Increasing the number of infected macrophages sensitized to Mycobacterium tuberculosis antigens, leading to constant hyperfunction of cellular immunity, particularly enhanced immune response to cell wall components of mycobacteria, induction high titers of interferon-gamma in response to a stimulus, a sharp jump IL-2 titers and TNF-α , IFN-γ specific activation CD8 + CTL. Need also focus attention on the main differences from the MBT and human BCG, that is well growth in the human body, persists along host life, but does not cause active TB (except in patients with HIV/AIDS). After MBT cell destruction in the environment gets some additional high allergenic antigens, such as 85B, ESAT6, Rv2660c, HyVaC 4 (Ag85B and TB10.4.). These antigens to provide high adhesion and allergenicity of human strains M. tuberculosis. Most allergens that cause obvious signs of active tuberculosis are the antigens ESAT6 and CFP10. Such protein antigens can be called endotoxins. Also to pathogenicity factors include cord-factor, it main component is a polysaccharide-mycolic complex from cell wall (Figure 2) containing ftiolic and mycolic acid - to ensure the stability of mycobacteria to lysosomal enzymes. Currently available diagnostic tools tuberculin preferably contain the above components of the cell wall and differences (from BCG) allergens ESAT6 and CFP10 []. Currently well established that the virulence of M. tuberculosis, mainly responsible genes encoding antigens ESAT-6 and CFP10. When comparing the genomic sequence of M. tuberculosis with attenuated *M. bovis* BCG was detected genomic deletion of the three sites in the vaccine strain (RD1, RD2, RD3). BCG vaccine strain genome stripped areas in the RD1, encoding mycobacterial antigens ESAT-6 and CFP-10 present in virulent strains of M. tuberculosis. Many researchers believe that mutations in genomic regions RD1, encoding mycobacterial antigens ESAT-6 and CFP-10, occurred in the process of creating a BCG strain. It remains not examine the question of whether all strains of M. bovis other than BCG have antigens ESAT-6 and CFP-10, and whether they depend on the degree of virulence of the mycobacteria strains. About a third of the population is infected with the MBT. Tuberculosis statistics show that out of every 100 man infected MTB, only 10 appear open clinical forms. In the remaining patients, positive skin test and/or gamma-interferon test, clinical symptoms of tuberculosis never does occur, and no signs of sensitization other than to MBT antigens and presence ESAT6 - antibodies in the blood. Thus, if the focus is not on the infection, but on the prevention of tuberculosis reactivation, can significantly reduce the number of cases with clinical manifestations. There have been recent publication comparing the immunity of patients with open clinical forms tuberculosis and without clinical symptoms, but ESAT6 - test-positive. One of the rational ways for helps to MTB - infected macrophages is the simultaneous use of urease inhibitors and simultaneously use selective activators of antibacterial complete phagocytosis. For the latter group, some authors include also histone deacetylase inhibitors (HDAi). The use of such inhibitors in the latter case will mass increase number reading frames in the macrophages genome and leads to stormy expression phagocytosis activators, that blocked by MBT. These inhibitors include valproic acid and trichostatin. Research in this area only started, and the expectations are very high. Another activator phagocytosis with very similar action mechanisms is the vitamin D3 - ergocalciferol. In a variety experiments shows that the soluble derivatives of vitamin D3 inoculation to the culture of MBT - infected macrophages leads to the completion phagocytosis and complete digestion of the MBT. The disadvantage of this method is the need to maintain a concentration of vitamin D3, which is quite toxic to the human body as a whole. Accordingly, a new form vitamin D3 is to be administered directly to the places where many infected macrophages, i.e. as an aerosol through the lungs. Also pay attention to the fact that, earlier for purpose combating tuberculosis the urease inhibitors have not been used, although quite a lot of well-known non-toxic compounds anti -urease activity. Thus, the most promising way to prevent tuberculosis reactivation in humans with positive test specimens and humans in remission following chemotherapy is to provide an aerosol preparation containing both urease inhibitor, activator phagocytosis vitamin D3 and histone deacetylase inhibitor. The use of such aerosol once a week will greatly reduce the number of macrophages with incomplete phagocytosis and prevent the background to tuberculosis with clinical open forms. This disease, like tuberculosis, prevention is better than cure, especially with the emergence of *M. tuberculosis* multiresistant strains.
Keywords: tuberculosis, immunity, phagocytosis, vaccines

PATHOGENESIS
Sviezhentseva I.O.

The presence of leukemic stem cells (LSC) in the bone marrow of patients with chronic myeloid leukemia (CML) is the cause of relapses as a result of the treatment with chemotherapeutic agents and target therapy drugs. This is due to the ability of LSC to attach itself to the microenvironment cells and to remain at rest for a long time. Vascular and osteoblasts niche play a very important role in this process. However, for being in G0 phase LSC have direct contact with the cellular elements of bone marrow microenvironment. So LSK contact with mesenchymal cells of bone marrow using the appendixes, connecting components invaginations and lint. The cadherins and integrins are important in the interaction of osteoblasts niche. They are able to activate intracellular signaling cascades that provide resting state of LSK. In addition, a bone marrow niche provides changes of LSC oxidative metabolism, which also plays an important role for cell entry into the G0 phase. Further, LSC also have certain physiological properties, which play an important role in the drug resistance formation, particularly drugs with targeted actions - tyrosine kinase inhibitors. LSK characterized by a high level of BCR-ABL expression and their population can have a lot of point mutations in the *bcr-abl* gene in the same patient. This leads to the fact that the taken medicines dose does not act against LSK, reducing the number of a whole leukemic cells clone. However, complete LSC elimination from the the patient's bone marrow need search the main differences between the LSC and normal HSC. After the literature analysis it was found that LSC have several significant differences such as the ability to cause leukemia during the transplantation to immunodeficient animals, this leukemia is morphologically and phenotypically similar to the original tumor, in addition the LSC can be transmitted from animal to animal. In addition, the LSC is also characterized by the mutations presence in the genes of kinase domains, transcription factors and tumor suppressors (genes of growth factors FLT3, C-KIT, genes K-RAS and N-RAS, mutations in genes STAT5A, TP53, AML1, RB1, MYC, p16 / NK4a, ENV1). Now the most important role in LSK biology research takes studying of signaling cascades involved in the processes of cell activity. This key molecule of cell signaling pathways can become targeted agents that may be used for the elimination of LSC from the patient bone marrow. However, it is necessary to distinguish the molecular cascades that are inherent to all bone marrow stem cells from LSC specific intracellular signal transmittors. Common to the LSC and HSC are the following signaling pathways: Wnt/β-catenin, Sonic Hedgehog and Noch signaling pathways. Moreover, there are signaling cascades that are specific only for LSC. They are charecterized by the exclusive expression of Alox5, AHI-1 and NFκB genes. In addition, the LSC are also characterized by the increased expression of AVSV-1 and ABCG-2 transporters, providing the evacuation of the cell chemotherapeutic drugs. LSC are characterized by the decreased expression of Oct-4, which ensures the supply of drugs to cells. The article also highlights the key therapeutic tactics that can be used to eliminate the recurrence of CML associated with the presence of LSC, which remain at rest for a long time, in the bone marrow of patients. The first tactic is elimination of LSC using the targeted drugs that operate solely on the target molecule in leukemic cells. The second approach is a direct administration of drugs to a patient that could promote a permanent state of rest for LSC in order to prevent relapses.

Keywords: leukemic stem cells, chronic myeloid leukemia, hematopoietic stem cell microenvironment.

СТРУКТУРНАЯ ОРГАНИЗАЦИЯ БАКТЕРИАЛЬНЫХ УРЕАЗ 22-30
Лисняк Ю. В., Мартынов А. В.

STRUCTURAL ORGANIZATION OF BACTERIAL UREASES
Lisnyak Yu. V., Martynov A. V.

This brief review concerns the basic principles of structural organization of multi-subunit bacterial ureases and formation of their quaternary structure. Urease is a nickel-containing enzyme (urea amidohydrolase, EC 3.5.1.5) that catalyses the hydrolysis of urea to get ammonia and carbamate which then decomposes with water to get ammonia and carbon dioxide. Urease is produced by bacteria, fungi, yeast and plants. On the basis of similarities in amino acid sequences, ureases assumed to have a similar structure and conservative catalytic mechanism. Within past two decades bacterial ureases have gained much attention in research field as a virulence factor in human and animal infections. The first crystal structure of urease has been determined for that from *Klebsiella aerogenes*. The native enzyme consists of three subunits, UreA (α-chain), UreB (β-chain) and UreC (γ-chain), and contains four structural domains: two in α-chain (α-domain 1 and α-domain-2), one in β- and one in γ-chain. These three chains form a T-shaped heterotrimer αβγ. Three αβγ heterotrimers form quaternary complex $(\alpha\beta\gamma)_3$. In case of *Helicobacter pilori*, the analogous trimers of corresponding dimeric subunits $(\alpha\beta)_3$ form tetrameric structure $((\alpha\beta)_3)_4$ in which four trimers are situated at the vertexes of the regular triangle pyramid. Active center is located in α-domain 1 and contains two atoms of nickel coordinated by residues His134, His136, carboxylated Lys217, His 246, His272 and Asp360, as well as residues involved in binding (His219) and catalysis (His320). Active site is capped by a flap that controls substrate ingress to and product egress from the dinickel center. Urease requires accessory proteins (UreD, UreF, UreG and UreE) for the correct assembly of their Ni-containing metallocenters. The accessory proteins UreD, UreF, and UreG sequentially bind to the apoprotein $(UreABC)_3$ to finally form $(UreABC\text{-}UreDFG)_3$ activation complex. UreE metallochaperone delivers nickel ions to this complex, UreE and $(UreDFG)_3$ are then released from the activated enzyme. An understanding of structural organization of bacterial ureases is the necessary factor in the studies of structure-function relationships of these enzymes, mechanisms of their enzyme and non-enzyme activity, in design of new safe and efficient enzyme inhibitors aimed to struggle with infectious diseases promoted by urease activity.

Key words: urease, sub-unit organization, quaternary structure, accessory proteins

ВЛИЯНИЕ ГЕРПЕСВИРУСНОЙ ИНФЕКЦИИ НА ИММУННУЮ СИСТЕМУ И ИММУННЫЙ ОТВЕТ ОРГАНИЗМА НА ВАКЦИНАЦИЮ 31-44
Волянский А. Ю., Кучма М. В., Колотова Т. Ю., Клыса Т. Л., Кучма И. Ю., Конорева Е. С., Смелянская М. В., Перемот С. Д., Кашпур Н. В., Давиденко М. Б.

THE IMPACT OF PERSISTENT HERPESVIRUS INFECTION ON IMMUNITY AND VACCINATION RESPONSE
Volyanskiy A. Yu., Kuchma M. V., Kolotova T. Yu., Klyisa T. L., Kuchma I. Yu., Konoreva E. S., Smelyanskaya M. V., Peremot S. D., Kashpur N. V., Davydenko M. B.

In this review we summarize current knowledge on the ability of latent herpesviruses to modulate the immunity and response to vaccination. Nearly all humans are latently infected with multiple herpesviruses but little is known about virus-host interactions. Meanwhile, the study of the immune response to Epshtein-Barr virus (EBV) and cytomegalovirus **(**CMV) has revealed significant regulatory effects on the immune system. During the primary infection a human cytomegalovirus is predominately found in peripheral blood monocytes and polymorphonuclear leukocytes. However, the virus can not be replicated in these cells. CMV induces the survival and differentiation of infected monocytes into long-lived macrophages capable of supporting viral replication and the release of virions, which infect CD34+ myeloid progenitor cells. CMV latently persists in myeloid progenitor cells and monocytes and reactivates during

their differentiation into macrophages. CMV-infected monocytes exhibit a unique reprogramming of their differentiation and secret both pro-inflammatory M1- and anti-inflammatory M2-associated cytokines. But cytomegalovirus induced macrophage phenotype skewed towards pro-inflammatory M1 type. MV has profound effects on the composition and function of both T cells and NK cells**.** CMV constantly reactivates during differentiation of monocytes into macrophages. Consequently, persons with latent CMV infection have substantially increased numbers and proportions of CD8+ T cells that lead to exhaustion and an early onset of immunosenescence. Also, it has been shown that the latent CMV virus infection markedly increases the proportion of NK cells expressing the activating NKG2C receptor. So, it has been proposed that CMV alters the composition of T cell and NK cell subsets and accelerates immune aging. Given the capacity of CMV to alter a macrophage, as well as NK and T cell responses it is reasonable to hypothesize that latent infection would alter the outcome of vaccination. The EBV virus remains in memory B cells throughout life. In healthy subjects the EBV remains latent in the latency phase 0 and EBV replication proceeds without production of infectious virions. But the virus can be reactivated in latency phases 1, 2 and 3. The virus reactivation can affect immunity and results in diseases. Chronic fatigue syndrome (CFS) is characterized by fatigue, exhaustion and flu-like symptoms. EBV latent reactivation in CFS patients is supported by certain data**.** In a subset of patients, CFS begins with infectious mononucleosis and enhanced EBV-specific antibody titers have been reported. Also, a profound deficiency in EBV-specific B and T cell memory response was observed in a majority of CFS patients. These data confirmed the EBV virus involvement in the CFS development. Cytokine dysregulation, decreased natural killer cell functioning, the presence of autoantibodies, and a reduced response of T cells to mitogens have been reported in CFS. But if immunity is disturbed in CFS patients, they might have an altered response to vaccination. Herpes viral reactivation has been documented in sepsis. Demonstration of the widespread reactivation of latent herpesviruses in sepsis provides strong evidence that sepsis results in functional immunosuppression. Reactivation of latent viruses may be associated not only with sepsis but with aging as well. Moreover, according to our data herpesvirus reactivation is common in recurrently infected children. These observations highlight the ability of herpesviruses to profoundly impact the host immune function. But the recent publications have shown that persistent herpesvirus infection can be beneficial for the host. The data obtained from the multiple mouse models demonstrate the potential for herpesvirus infection to enhance resistance against a secondary infection. It has been documented that during latent murine cytomegalovirus or murine gammaherpesvirus infection 68 mice are protected against lethal bacterial infections by prolonged macrophage activation and IFNγ secretion. Little is known about the potential benefits of herpesvirus latency in humans. The effect of CMV on vaccine responses is controversial. It is well known that vaccine responses is reduced in aging populations. CMV accelerates immune aging and may further reduce the response to vaccination. In fact, some studies show a negative effect of CMV while others showed no difference in CMV+ vs CMV− in older individuals. Conversely, in young individuals, a negative association between the CMV antibody titer and the response to the influenza vaccine has been found. Also, a negative association between the CMV antibody titer and the response to the influenza vaccine was found in young individuals. But, according to another results CMV enhances the immune responses of younger individuals to influenza vaccination. In summary, the hypothesis that CMV virus accelerates immunosenescence and decreases vaccination response is controversial. Moreover, CMV infection may enhance the immune responses in children and young individuals. Vaccinations can induce the aberrant immune response of CFS. But the available data do not support the CFS impact on the vaccination response. In conclusion, the host-persistent herpesviruses infection history is likely to play a significant role in the immune system response to vaccination.

Key words: latent herpesvirus infection, cytomegalovirus, virus Epshtein-Barr, chronic fatigue syndrome, T cells, NK cells, viremia, vaccination.

ЕКСПЕРИМЕНТАЛЬНІ РОБОТИ, МЕДИЦИНА
Experimental papers, medicine

ETIOLOGIC FACTOR IN THE DEVELOPMENT OF MYOCARDITIS IN THE KHARKIV REGION

Smelyanskaya M. V., Peremot S. D., Kashpur N. V.

Introduction. According to the research conducted in the last decade, there has been growth in the non-coronary disease infarction among all cardiovascular diseases. The prominent place among all non-coronary heart diseases is taken by myocarditis, which predominantly affects young people of working age (30-40 years old). According to the bibliography, the prevalence of myocarditis is 20% of the non-coronary heart lesions and by different authors 5 - 11% of the total amount of diseases of the cardiovascular system. To date, there are no clear criteria of infectious myocarditis. It is widely accepted that myocarditis is natural complication of infectious diseases in which any infectious agent may be the etiological factor. Until recently, Coxsackie virus of group A, B was considered the most cardiotropic. However, the role of enteroviruses has been recently reviewed in favor of persisting viruses and especially family Herpesviridae. Optimization of myocarditis diagnosis using noninvasive tests, will not only reveal the true extent of the disease but may also enable to examine viral myocarditis as a much more common pathology than it seems at present and will increase understanding of the significance of this pathology in the cardiovascular continuum. **Material & methods.** 87 people diagnosed with infectious myocarditis were examined. Blood and other biological fluids were subject to examination. Patients of the main group have also had their biopsy material, obtained in a result of endomyocardial biopsy, and pericardial fluid, derived as a result of diagnostic and therapeutic puncture under hydro pericarditis, examined. PCR was performed to determine the genomic sequence of enterovirus (HEV), adenovirus (HAdV), human cytomegalovirus (CMV), herpes simplex virus (HSV), Epstein-Barr virus (EBV), human herpes virus 6 (HHV6) and influenza A viruses and B. **Results & discussion.** Our research confirms the global trend of reducing the role of enteroviruses in infectious myocarditis. In the course of this investigation, herpesvirus markers in biological material from patients in the form of monoinfection or mixed infections were found in 67 patients with myocarditis, which amounted to 87%. While in the control group, similar markers have been found only in 9 patients or in 22.5% of cases. Herpesvirus detection rate is almost 7 times the allocation of other viruses in infectious myocarditis. Different types of herpesvirus with varying frequency were found in the main (SM and CM) and control groups. According to our data antigens of CMV, HHV6 were found in 60-70% of patients suffering from subacute myocarditis, and almost 40% of patients in this group had markers HSV1,2 and VZV. In patients with chronic myocarditis the percentage of CMV and HHV 6 markers identification reached 70-75%, HSV1,2 - exceeded 50%, EBV - 31,4%. While VZV markers did not much exceed the performance of the control group (16.2% and 12.5% respectively). Determining markers of enteroviruses in the study groups and the control group were not significantly different. Our data detection of mixed infections indicates a very significant (75%) share of the combination of five different herpesvirus in patients with myocarditis. It is predominantly a combination of HHV6 with CMV, HSV1,2, EBV and VZV. The data can be used not only to choose the etiotropic treatment of myocarditis, but also as a diagnostic criteria when combined with a history of clinical indicators. In comparison of herpesvirus DNA detection in patients with SM in blood and EMBS, it was revealed that markers of CMV and HHV6 were almost equally met both in blood and in heart biopsies. Viruses HSV1,2 and VZV were more often detected in the blood than in the EMBS (3 and 1.5 times respectively). This gives reason to believe that the identification of herpesvirus markers in the blood of patients with AM can be used for non-invasive etiological diagnosis of subacute viral myocarditis. Development and introduction into medical practice of new non-invasive methods of myocarditis diagnosis and their etiology specification will enable to examine viral myocarditis as a much more common pathology than it would

seem. **Conclusion.** During virological examination of biological material of myocarditis patients, a high proportion (87%) of antigens of Herpesviridae viruses were revealed. In patients with myocarditis, association of herpesvirus antigens of various types (in 75% of detection) dominate, while in healthy people of the control group - mono infection can be detected more often. Chronic myocarditis patients in 61% of cases are detected with three or four antigens of viruses of the family Herpesviridae, basically HHV6 with CMV, HSV1,2, EBV; with subacute myocarditis – HHV6 with CMV, HSV1,2 and VZV. The findings point to the need for mandatory examination for Herpesviridae virus of patients diagnosed with myocarditis.
Keywords: myocarditis, Herpesviridae virus, endomyocardial biopsy.

NEW FORMYL-PEPTIDES, AS STIMULATOR OF NON-SPECIFIC ORGANISM RESISTANCE AGAINST MYCOBACTERIA 49-52

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

Introduction. The key element in the formation of tuberculosis infection (TI) is the inability of alveolar macrophage to complete phagocytosis of mycobacteria absorbed by them, that caused by both features of the pathogens biology and the tissue macrophages. It is known, that M. tuberculosis is capable long-term persistence and proliferation in alveolar macrophage cytoplasm because of high stability of them cell walls to the lysosomal enzymes action. Mainly, the phenomenon of granulomatous reaction, inherent of tuberculosis (TB), reflects the inadequacy in elimination of tuberculosis pathogen of alveolar macrophages. Thus, the inclusion of agents that can activate completed phagocytosis of mycobacteria by alveolar macrophages, in the base of anti-TB therapy is a promising direction in the prevention of latent tuberculosis reactivation. **Materials and Method.** The ability of formyl-peptides activate the completeness of mycobacteria phagocytosis by alveolar macrophages absorbed by them were evaluated in vitro. For reaching the aim of the study we had used a broncho-alveolar lavage obtained by white laboratory male mice 2 months of age. As a comparison drug we used the officinal preparation "Liasten". To determine the lysosomal activity by the presence of peroxidase was treated with acridine orange causing selective staining red lysosomes. Acid phosphatase activity was studied using azocoupling reaction for staining granules in the cytoplasm blue or purple. Results are expressed as mean cytochemical coefficient (LZC). **Results and discussion.** Incubation of alveolar macrophages and formyl-peptides leads to a significant increase the index of mycobacteria phagocytosis completeness for vaccine strains - (1,70 ± 0,31) and (1,20 ± 0,22), respectively, ($p < 0.05$). The standard medication - Liasten - also increased the "killing" ability of tissue macrophages compared to control: (1,8 ± 0,25) and (1,20 ± 0,22), respectively, ($p < 0.05$). Lysosomal activity of alveolar macrophages exposed formyl-peptides significantly increased in comparison with the control - (97,80 ± 5,1) and (80,9 ± 4,3) acridine orange-positive cells, respectively, ($p < 0.05$). However, the effect of formyl-peptides on lysosomal activity of macrophages did not exceed the reference drug action - (97,80 ± 5,1) and (95,72 ± 5,3) acridine orange-positive cells, respectively, without significant differences. Incubation with formyl-peptides resulted in a significant increase of LZC of acid phosphatase in macrophages - (2,08 ± 0,20) and (1,59 ± 0,14), respectively, ($p < 0.05$). Significant differences between the content of this enzyme in macrophages when exposed formyl-peptides and the reference drug were not detected. **Conclusion.** As a result the co-incubation of alveolar macrophages and new formil-peptides activates BCG phagocytosis completeness. Also, there is the influence of the studied substances under significant lysosomal activity and increase the content of acid phosphatase in macrophages isolated from broncho-alveolar lavage in comparison with the control. The level of functional activity stimulation of phagocytes under the influence of formyl-peptide is the same, that we registered after the Liasten administration. It has indicating prospects of the medicinal preparation creation on the formyl-peptides basis, which stimulates the organism non-specific resistance.
Key words: Formil-peptides, non-specific resistance, lysosomal activity, in vitro.

ІНФІКОВАНІСТЬ HHV-6 ВІЙСЬКОВОСЛУЖБОВЦІВ ХВОРИХ НА НЕГОСПІТАЛЬНУ ПНЕВМОНІЮ 53-55

Бруснік С.В., Попова Н.Г., Попова Л.О.

INFECTION WITH HHV-6 OF MILITARY MEN AFFECTED BY COMMUNITY-ACQUIRED PNEUMONIA

Brusnik S. V., Popova N. G., Popova L. O.

Human herpesvirus, 6 type (HHV-6) was isolated at the end of the 20th century from the blood leukocytes of patients with lymphoproliferative diseases. Serological studies conducted in different countries, indicate ubiquitylation of the HHV-6 and the existence of two antigenic variants - HHV-6A and HHV-6B. Their high tropism is determined in vitro to lymphocytic, nervous and dendritic cells of the CNS. Virus replicates in many cell, primary and passaged cultures of different origins. The reproduction cycle of HHV-6 continues on average 4-5 days forming syncytiums and intracytoplasmic and intranuclear inclusions. Significant destruction and lysis almost 90 % of infected cells is reported after 5-10 day of monitoring. The utility of experimentation investigating the role of HHV-6 in the development of acute and chronic diseases in respiratory tract is caused by the fact that many patients, particularly those with chronic diseases, have complaints to chronic fatigue, decreased performance and low-grade temperature more than 3-6 months. Several studies demonstrate the presence of HHV-6 in saliva, salivary and bronchiolar glands, in swabs from pharyngonasal cavity and gorge. Tropism of HHV-6 to oropharyngeal epithelium with the possibility of finding the virus in the saliva and swabs from pharyngonasal cavity and gorge was found at the end of 20th century. This fact gave the basis for work determining the level of infection by this pathogen in patients with infectious and inflammatory pathology of the respiratory tract. **Materials and methods.** Serological studies were conducted with 38 soldiers affected by community-acquired pneumonia. Most of the surveyed patients were ranged in age from 20 to 45 years old, middle age (32,5±1,5) years. Patients were in stationary treatment in the Kharkov military hospital. The criteria for inclusion in the study on the infection of HHV-6 were soldiers affected by community-acquired pneumonia with atypical course of disease. Some patients have short-term (1-2 days), macular papulose eruption on hands and legs, prolonged low-grade fever, chronic fatigue. The control group included 18 apparently healthy persons. Enzyme immune analysis (EIA) using commercial diagnostic system "VektoHHV-6-IgG» was applied to determine the immunoglobulin class G (IgG) for HHV-6 in serum and saliva of the examined. Registration of the EIA results was performed using spectrophotometer StatFax 303 by determining the optical density (OD) in optical experimental values and control samples of blood serum and saliva. Result evaluation is carried out in accordance with requirements stated in the instructions to the test system with obligatory consideration of anamnestic and clinical data from the examined patients. **Results and discussion.** The above data show that among the examined patients' infection with HHV-6 was 26,3 % of apparently healthy persons in the control group, this figure was 2 times lower than 10,5%. In saliva of the CAP patients as well as in blood and serum was found IgG to HHV-6. One patient from IgG to HHV-6 control group have been identified in saliva and serum. The results of the research allowed establishing the level of seropositive individuals to HHV-6 and demonstrate the ability to diagnosis HHV-6 infection in infectious and inflammatory processes of the respiratory tract. **Conclusion.** The studies have shown the use of saliva as an object of study for the establishment markers of HHV-6 in patients affected by CAP with important advantage - non-invasive receiving of material from patients for laboratory diagnosis. It can be assumed that high level of infection for patients affected by CAP is

associated with ease droplet spread of herpesvirus through saliva.
Key words: human herpesvirus 6 type, community-acquired pneumonia, military man.

ЕКСПЕРИМЕНТАЛЬНІ РОБОТИ, ФАРМАЦИЯ
Experimental papers, pharmacy

QUALITY CONTROL OF HAWTHORN TINCTURE BY HPTLC METHOD

Khokhlova K.O.

Introduction. Hawthorn tincture is one of the most used herbal drugs at the domestic pharmaceutical market. According to the State register of drugs at the pharmaceutical market of Ukraine, there are 13 commercial offers of Hawthorn tincture from home-produced manufactures. The initial herbal raw materials for Hawthorn tincture are Hawthorn fruits, which are widespread at the territory of Ukraine. These are pharmacopoeial herbal raw material. Thus, 12 different species of Hawthorn fruits are included into monograph <Hawthorn fruits> of Ukrainian State Pharmacopoeia (SPhU) and State Pharmacopoeia of USSR XI ed. On the territory of Ukraine there are near 30 different species of Hawthorn, and the quantity of species is much arises due to its forms and hybrids. The 'natural variability' of bioactive substances of Hawthorn fruits of the same species and possibility of usage of many different species during manufacturing process of herbal drugs lead to the pitfalls in standardization of herbal drugs in general, and Hawthorn tincture particularly, and should be taken in mind while development of its quality control methods. For development of specific and reproducible identification method, it is necessary to ensure the number of parameters: usage of method and equipment that give reproducible results; big selections of different samples; rigorously observation of method's procedure of implementation. The modern, automated HPTLC method of analysis was chosen for identification purpose. If standardize procedure and suitable equipment are used, the reproducible results of the method have to be obtained. The **aim** of this paper was development of HPTLC method for identification of Hawthorn tincture, which could be appropriated for stability study and establishment of its expire date. **Materials and Methods.** In research 13 samples of Hawthorn tinctures from 8 manufactures from Ukraine and Russia were analyzed. These samples were manufactured in 2010, 2014, 2015 years. The research was conducted on the base of CAMAG laboratory, Muttenz, Switzerland. *Plates used*: HPTLC glass 20x10 cm, Si 60 F_{254}, Merck, Lot: 1.05642.0001. *Material used:* Automatic TLC Sampler 4, CAMAG; Twin Trough Chamber 20x10 cm, CAMAG; Chromatogram Immersion Device III, CAMAG; TLC Plate Heater III, CAMAG; Automatic Development Chamber ADC 2, CAMAG; Visualizer, CAMAG; TLC Scanner, CAMAG; Filter paper for chamber saturation, CAMAG; Centrifuge EBA21, Hettich; Ultrasonic Bath SW 3H, Sono Swiss; Analytical Balance MS 205 DU, Mettler-Toledo. *Chemicals used* were pharmacopoeial quality. *Reference substances used*: hyperoside, USP, batch: 33520F; rutin, EDQM, batch: A0299493; chlorogenic acid, EDQM, batch: A0290470. For identification of Hawthorn tincture by HPTLC the flavonoids were chosen as a group of bioactive substances. The method was developed using format and style of description, which are used for TLC Identification method for Crataegi fructus in European Pharmacopoeia and SPhU. For new identification method of Hawthorn tincture preparation of *test solution, reference solutions, system suitability solution (SST), intensity marker,* its *application, development* and *results* were proposed. For *specificity* study HPTLC-fingerprints of 13 samples of Hawthorn tinctures, which were produced by manufactures, were compared with HPTLC-fingerprints of laboratory sample of Hawthorn tincture, prepared from properly authentificated herbal raw material of *C. laevigata (C. oxyacantha) fructus* (solvent – 70% ethanol, ratio – 1:10) and HPTLC-fingerprints of *C. laevigata (C. oxyacantha) fructus* (solvent – methanol, ratio – 1:10). For *reproducible* results the research was conducted according to standardized procedure USP <203> High-performance thin-layer chromatography procedure for identification of articles of botanical origin. Visual evaluation of chromatogram of test solutions was conducted with respect to zone position and colour of reference solutions, intensity was evaluated respect to intensity marker of reference solution. **Results and Discussion.** According to the proposed identification method by HPTLC, the fingerprints of 13 different samples of Hawthorn tincture are quite consistent respect to zone position, color and intensity. All analyzed samples of tinctures are met the requirements of proposed HPTLC-test. Comparison of HPTLC-fingerprints of 13 samples of Hawthorn tincture with the test solutions of laboratory samples of Hawthorn tincture and Crataegi fructus has shown quite similar fingerprints respect to zones position and color. This, prove the specificity of the method. Description of results for developed HPTLC method was given. The tolerance range in fingerprints was specified. Despite of the storage period of Hawthorn tincture is 3 years, the old samples of tincture, which were manufactured in 2010 year are quite similar to new one and would passed proposed HPTLC test. This shows the chemical stability of marker substances of tincture during its storage period. Other investigation in this area is necessary. **Conclusion.** The specific and reproducible HPTLC identification method of Hawthorn tincture was developed. This could be used as optional/alternative method for conventional TLC. Image on chromatogram of Hawthorn tinctures could be used as a reference image of HPTLC method for Hawthorn tincture and to be included in Pharmacopoeia of Ukraine or any other HPTLC Atlas/Reference book. Developed HPTLC identification method of Hawthorn tincture shown the stability of marker group of bioactive substances (flavonoids) during the expiration period of tincture.
Key words: Hawthorn tincture, identification, HPTLC

FORENSIC AND PHARMACEUTICAL RESEARCH OF ORGANIZATION OF AVAILABILITY OF THE MEDICINES FOR PATIENTS WITH MALIGNANCIES IN THE UNITED STATES

Shapovalov V.V. (Jr.), Zbrozhek S.I., Shapovalova V.A., Shapovalov V.V., Kulykova O.V.

Introduction. The incidence of cancer in recent years has increased significantly. It is therefore particularly important today is the issue of provision for patients with malignancies with drugs. It is important to research the level of organization of availability of the anesthetic therapy to ensure the availability of pharmacotherapy for cancer patients worldwide. Material and methods. For the purpose of the study analyzed legislation, regulations of some states in the US that provide availability of narcotic analgesic drugs for patients with malignant neoplasms. The paper used the following methods: comparative, documentary, legal, medical, pharmaceutical and graphical analysis. Results and discussion. Noted the increase in expenditure on pharmaceutical provision for patients with malignancies on an outpatient basis in the US. During the study of the legislative and regulatory acts of the USA found that payments for treatment possible by insurance companies as part of the agreement, which in turn depend on the patient's age, number of family members, their total

income and so on. Coupons can be used to pay for the cost of medicines, but not all pharmacies accept coupons. There are charities with funds which are partially covered for the cost of chemotherapy and adjuvant therapy (analgesics, antiemetic, antipsychotics, drugs). Found, that in New York doctor may prescribe analgesic medication to the patient without limitation in the frequency and dose. It is not therapeutic use of analgesics, their improper accounting, drug addiction patient has contraindications to the prescription of narcotic analgesic drugs with malignant neoplasms. Reviewed examples from forensic and pharmaceutical practice, pharmaceutical violation of the US laws that regulate the accessibility of patients with malignancies to narcotic analgesic drugs. So, there have been cases of fake prescriptions for narcotic drugs, selling drugs, the shelf life has expired. Police of New Jersey the fact of abduction of injectable morphine and its falsification of employee hospital. Conclusion. Conducted a study of the availability of narcotic analgesic drugs for patients with malignancies in the US from a position of forensic pharmacy. Made an analysis of mortality from malignancies worldwide. Studied pharmaceutical legislation of Nevada, Texas, Georgia, New York in the pharmaceutical industry of providing patients with malignancies with narcotic analgesic drugs.