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THEORETICAL STUDY OF THE PRINCIPLES OF MODERN BACTERIOPHAGIC THERAPY

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Abstract. *Phagotherapy is an approach to treating bacterial infections using bacteriophages. Bacteria viruses can be used as an alternative to antibiotics in the widespread development of antibiotic resistance among microbial strains. This problem becomes more and more over time because of the misuse of antibiotic therapy. In the nature of bacteriophages have already laid specificity for certain microbes, while they do not harm the human body. Treatment using bacterium viruses provides an alternative to traditional methods in bacterial infections. It remains possible to develop bacterial resistance to bacteriophages, but such resistance is easier to overcome than antibiotic resistance. Because bacteriophages can evolve, like bacteria, causing resistance to disappear. Bacteriophages are specific and exhibit their effect on one or more species, (Maghsoodi, Chatterjee, Andricioaei, & Perkins, 2019) while traditional antibiotic therapy affects both harmful bacteria and the rest of the human body microflora. It is the peculiarity of bacteriophages to exhibit their therapeutic effect on a limited range of microbes make them safe for beneficial bacteria (Choudhury, Tanmoy, Maiti, Biswajit, Venugopal, Karunasagar, & Indrani, 2019). Also, a significant advantage is that lytic viruses of bacteria, unlike antibiotics, do not cause bacteriostat, but have only a bactericidal effect, which prevents a possible relapse of the infectious process for a longer period. Some scientists suggest the theory that administering one dose of bacteriophages is enough to cope with the causative agent of the disease, but the main criterion is the immune clearance of phages. Also, a significant advantage in favor of using of bacteriophage drugs is those bacteriophages are distributed throughout the globe, in addition, their composition and species diversity are huge. From this point there is one minus - in our time, the mechanism of patenting phages remains incomprehensible. For pharmaceutical companies, there are many questions about the biodiversity sharing, as it is necessary to amend various protocols. However, despite this, bacteriophages bred in the laboratory attract the attention of scientists in the field of intellectual property (Saha, & Mukherjee, 2019). The article contains a classification of bacteriophages, a description of the mechanism of action on bacteria and their recognition, a comparison of bacterium viruses with antibiotics. The purpose of this study was to describe the modern classification of bacteriophages, to substantiate the use of cellular phages, and to describe the principles of bacteriophages treatment of diseases associated with the respiratory system.*

Key words: antibiotics, bacteriophages, phagotherapy, pulmonology.

Introduction. The problem of bacterial antibiotic resistance deserves particular attention among scientists. Based on data provided by the World Alliance against Antibiotic Resistance (WAAR), the antibiotic class is within the limit of losing its efficacy. This situation occurs with a combination of factors:

- Self-treatment;
- Irrational purpose;
- Excessive use;

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The increase in demand for antibiotics at the moment are mainly due to a rapidly growing population, while the population is aging, which leads to an increase in the number of chronic diseases and infections, the number of people in need of medical care is growing. Overuse of antibiotic drugs leads to increase spread of resistance genes. The use of subtherapeutic doses also plays a significant part in the spread of bacterial resistance. (WAAAR: World Alliance Against Antibiotic Resistance)

The purpose of the work is to classify bacteriophages and theoretically study the principles of modern phage therapy.

Methods of work included description and generalization.

The main part. Bacteriophages (phage) is a safe natural effective strategy at fight against multi-drug resistant organisms (MDROs) and ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumon) (Haddad, Harb, Gebara, Stibich & Chemaly, 2018). Bacteriophage therapy was first used as a substitute for antibiotics in the 1900s (Maddocks, Petrovic, Lin, Zakour, Dugan, & Iredell, 2019).

Phages or bacteria viruses, this is one of the largest biological groups, because they are found around the globe, in all possible environments. Bacteriophages belong to intracellular parasites since they reproduce only inside the host bacteria (Prazak, Iten, Cameron, Save, Grandgirard, Resch & Haenggi, 2019). The degree of specificity of bacteriophages is different:

- Monovalent (one species of host bacteria);
- Typical (individual types of bacteria);
- Multivalent(various representatives of species and genera can act as host bacteria).

Bacteriophages are usually separated depending on the morphology of virions and their encapsulated nucleic acid. In total, scientists identify 13 families, 9 of which contain double-stranded DNA - Corticoviridae, Fuselloviridae, Lipothrixviridae, Myoviridae, Plasmaviridae, Podoviridae, Rudiviridae, Siphoviridae, Tectiviridae; Inoviridae, and Microviridae - are representatives from single-stranded DNA; Cystoviridae, and Leviviridae are representatives from double-stranded RNA. According to scientists, about 96% of all known bacteriophages are "tailed" bacteriophages from the order Caudovirales (Thung et al., 2018).

Mainly, lysogenic bacteriophages are used in the production of bacteriophage drugs. The use of lysogenic phages is mainly due to several factors: high bactericidal (as in combination with other drugs or as a full-fledged alternative to antibiotics), the possibility of automatic dosing, bacteriophages affect the biofilms of bacteria, they have a high level of specificity of action, along with this, bacteria do not develop resistance to phages.

Together with the proliferation of bacteriophages, bacteria are destroyed due to the lysogenic principle of interaction with a bacterial cell. Due to this, the number of bacteriophages increases at places of high concentration of pathogens where the process occurs again. Through their lysogenic cycle, bacteriophages can lysis cells and destroy biofilms without allowing biofouling. Due to its specificity, which is due to the presence of fibrils that recognize the receptors of a certain bacterium, and after the identification of the target, genetic material is transmitted inside the cell, bacteriophages act only on a certain species without destroying everything in their way, like antibiotics. Bacteria viruses have learned to circumvent microbial resistance through their own evolution, therefore, when using phage therapy, there is no such problem as in the case of antibiotics.

According to the World Health Organization (WHO), infectious diseases, in particular pneumonia, gonorrhea, tuberculosis, and salmonellosis, are becoming more difficult to treat every year due to a decrease in the effectiveness of antibiotic therapy. But scientists have an alternative approach to treating these diseases using bacteriophages. A prominent example is the treatment of Staphylococcus aureus-induced diseases using staphylococcal bacteriophage. This bacterium can cause about 120 diseases with different levels of severity of the infectious process. Bacteriophage is introduced into the focus of infection (locally in the form of plugging and lotions, under the skin, into the pleural, abdominal and articular cavities, orally, into the bladder through the catheter and rectally). In the treatment of pneumonia caused by this bacterium, the drug is introduced through capillary drainage into the pleural cavity. The course of treatment depends on the severity of the infectious process and is taken every other day in 3-4 doses.

When using bacteriophage preparations, the question arises about the criteria by which it is possible to characterize bacteriophage. During the I International Congress «Rational use of antibiotics. Antibiotic Resistance Stop!» Shyrobokov Volodymyr, Doctor of Medical Sciences, in his report noted that phage preparations should meet the following criteria:

- contain only virulent phages;
- bacteriophages should be reproduced in the host bacteria with a high yield of active viral lobes;
- bacteriophages should maintain lysogenic activity during long-term storage;
- bacteriophages should not adversely affect representatives of the normal human microbiota (Shyrobokov, 2018).

The mechanism of phage damage of target bacteria consists in the attachment of phages to the bacterial cell and the introduction of its genome into it. The viral genome is inserted and replaces the bacterial genome so that the bacterium and the infectious process cease to exist (Gordillo Altamirano, & Barr, 2019). After the viral genome enters the cell, the bacterium begins to produce bacteriophages instead of a continuation of the genus. In clinical practice, bacteriophage preparations are used:

- Enteral (oral);
- Parenteral (topical (for burns, for the treatment of infected wounds are also used in surgical interventions), the injection route of administration is not often used);
- Using inhalation.

Phagotherapy in pulmonology

In pneumonia, which was caused by bacteria, the functioning of the lungs suffers significantly due to the contact of the pathogen with the immunologically active pulmonary epithelium. In respiratory distress syndrome, which is caused by uncontrolled inflammation, the course of the disease can be complicated, due to which pneumonia presents high features of the patient's life. Due to the spread of antibiotic resistance among pathogens of bacterial infections in recent years, phage therapy has been of increasing interest to scientists. Due to the spread of antibiotic resistance among pathogenic strains of bacteria, there is an in-depth study of the possible effects of bacteriophages on the human body and on the passage of infectious inflammation.

In pulmonology, inhalation phagotherapy is often used. Preparations containing a solution of phages are easily sprayed into small droplets using most commercially available nebulizers. On the market, liquid phage suspensions can be found that should be stored in a refrigerator (2-8°C), the shelf life is usually 1-2 years (Chang at al., 2018).

Particular resistance of bacteria to antibiotic therapy in the treatment of pneumonia is caused by the pathogens Pseudomonas aeruginosa, Acinetobacter spp., Enterobacteriaceae (Wunderink, 2019). Also, the causative agent of pneumonia can be Streptococcus pneumoniae. Preparations of bacteriophagess can be used both independently and in combinations with various antibacterial agents. The use of bacteriophages is also appropriate and equivalent to the use of antibiotic drugs, in the case of allergies to the latter.

A group of researchers from the Georgia Institute of Technology (USA), introduced the latest method thanks to which it is possible to deliver a bacteriophage drug to the lower parts of the respiratory system. Scientists have found that lytic bacteriophages are effective in treating pulmonary infections, but their use is difficult due to difficulties in delivering active phages. The development looks like microparticles on which the bacteriophage is applied, and then driven into the lungs. Scientists have begun this development because of the ineffective use of nebulizers in the treatment of the lower parts of the lungs. But this fact excludes the possibility of further development of special inhalers. The significant advantages for the use of microparticles with bacteriophages applied to them are that the carrier is small enough to easily reach the lower parts of the lungs, while the development size is calculated so as not to be eliminated immediately after insertion but degraded for several days, also the bacteriophages applied to the nucleus do not lose their activity.

The mechanism for producing this preparation is quite easy to produce and looks as follows - the core is immersed in a solution with phages, after which it is dried. Testing for pneumonia patients in mice was successful so the phage drug began

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to be tested for the possibility of treating cystic fibrosis (this disease is most often caused by the antibiotic-resistant strain Pseudomonas aeruginosa). In the future, scientists plan to expand their experiments and switch to testing in large animals (Agarwal at al., 2018).

Discussion and conclusions. Phages having a lytic cycle of life lick the target bacterium and then lyse offspring are released, which are in a latent state until the target bacterium is identified and the entire cycle does not begin anew. Because of this peculiarity, phages can lick whole biofilms, whereas a conventional antibiotic is unable to do so. This is because an exopolysaccharide matrix is present in the biofilms and forms a protective layer, thereby preventing the antibiotic from fulfilling its purpose. Unlike other variants, bacteriophages can penetrate, due to the presence of depolymerase in them. Phages also have the ability to produce enzymes (for example, endopeptases) that destroy intercellular matter. These enzymes exhibit gram-positive and gram-negative activity. After joining the shell, they initiate a chain reaction, which first unfolds the outer layer of biofouling, after which the phages can further multiply in the middle until all the layers are "dissolved." The scientists concluded that in the treatment of infectious diseases that were caused by methicillin by a resistant strain of S. aureus, it was the use of combination treatment that produced more results than the use of one-by-one approaches (Tkhilaishvili, Lombardi, Klatt, Trampuz, & Di Luca. 2018).

Antibiotics should be maintained in therapeutic doses constantly in treatment, in contrast to the possibility of single-use management of bacteriophages, which at the same time can themselves regulate their numbers based on the number of target bacteria. When taking antibiotics for a long time, many side effects are possible (for example, dysbiosis caused by a violation of microflora in the patient's intestines). In the absence of target bacteria, bacteriophages are excreted from the body biologically without causing side effects. In many species infections, it is possible to use polyvalent bacteriophages or whole mixtures that contain several varieties of phages. The main and most significant disadvantage when using bacteriophages is their selectivity in acting on bacteria, due to the need to accurately determine the causative agent of the infectious process, which can take a long time in some cases.

Since the dosage is a very important aspect for each dosage form, at improperly selected doses of the antibiotic, our body can begin to resist treatment, which will further complicate its implementation. Bacteriophages in this case benefit, they have the ability to self-stabilize because of which it is possible to obtain a therapeutic effect from one dose of the drug, and after killing the target population of bacteria they are excreted from the body. (Gelman, Eisenkraft, Chanishvili, Dean, Glazer, & Hazan, 2018)

Results. With increasing awareness of the composition of the human microbiome, and its role in maintaining health, scientists' view of antibiotics is changing.

Generically, the advantages of using phage therapy include:

1. Their non-toxicity of the action to commensal bacteria;
2. May less toxicity than antibiotics;
3. Can be introduced into the body in various ways (as enterally and parenterally);
4. Manifest its action only in the presence of target bacteria, after destruction the microbe is excreted by biological route;
5. Flexible one-time reception;
6. It is possible to sow from the environment;
7. New bacteriophage can be constructed in laboratory conditions very quickly;
8. Do not cause allergies;
9. Use of bacteriophage cocktails for spectrum expansion;

The disadvantages of using these drugs include:

1. It is difficult to determine very accurately the causative agent of infection, due to the high specificity of the action of phages;
2. It remains unclear the mechanism of patenting new phages and the possibility of their use are not clear.

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WAAAR: World Alliance Against Antibiotic Resistance

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ТЕОРЕТИЧНЕ ДОСЛІДЖЕННЯ ПРИНЦИПІВ СУЧАСНОЇ ТЕРАПІЇ БАКТЕРІОФАГАМИ

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Анотація. Фаготерапія - це підхід до лікування бактеріальних інфекцій з використанням бактеріофагів. Віруси бактерій можуть бути використані як альтернатива антибіотиків при повсюдному розвитку антибіотикорезистентності серед мікробних штамів. Це проблема стає дедалі більшою з плином часу через неправильне застосування антибіотикотерапії. В природі бактеріофагів вже закладена специфічність до певних мікроб, при цьому вони не завдають шкоди організму людини. Лікування з використанням вірусів бактерій надає альтернативу традиційним методам при бактеріальних інфекціях. Залишається можливим варіант розвитку стійкості бактерій до бактеріофагів, але таку стійкість легше подолати, аніж антибіотикорезистентність. Тому що бактеріофаги можуть еволюціонувати, як і бактерії, внаслідок чого резистентність зникне. Бактеріофаги специфічні та проявляють свою дію на один або декілька видів, (Maghsoodi, Chatterjee, Andricioaei, & Perkins, 2019) в той час як традиційна антибіотикотерапія впливає як на шкідливі бактерії, так і на всю іншу мікрофлору людського організму. Саме особливість бактеріофагів проявляти свій терапевтичний ефект на обмежене коло мікробів робить їх безпечними для корисних бактерій (Choudhury, Tanmoy, Maiti, Biswajit, Venugopal, Karunasagar, & Indrani, 2019). Також вагомим плюсом є те, що літичні віруси бактерій, на відміну від антибіотиків, не викликають бактеріостаз, а володіють лише бактерицидною дією, чим попереджують можливий рецидив інфекційного процесу на більш тривалий термін. Деякими вченими припускається теорія, що введення однієї дози бактеріофагів достатньо аби впоратись із збудником захворювання, однак головним критерієм при цьому є імунний кліренс фагів. Також, вагомим плюсом у користь використання бактеріофагових препаратів є те, що бактеріофаги поширені по всій земній кулі, до того ж їх склад і видове різноманіття величезне. З цього пункту є один мінус - в наш час все ще залишається незрозумілим механізм патентування фагів. Для представництв фармацевтичних компаній залишається багато питань щодо спільного використання біорізноманіття, так як потрібно вносити правки до різних протоколів. Однак, не зважаючи на це, виведені в лабораторних умовах бактеріофаги привертають увагу науковців в галузі інтелектуальної власності (Saha, & Mukherjee, 2019). У статті присутня класифікація бактеріофагів, опис механізму дії на бактерії та їх розпізнавання, порівняння вірусів бактерій з антибіотиками. Метою цього дослідження було описати сучасну класифікацію бактеріофагів, обґрунтувати використання саме літичних фагів та описати принципи лікування бактеріофагами захворювань, що пов'язані з дихальною системою.

Ключові слова: антибіотики, бактеріофаги, пульмонологія, фаготерапія.