The Potential of Bacteriophages in the Treatment of Burn Wounds

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Bacteriophages were identified in 1915 and have been used since 1919. Despite the prevalence of their application the first users did not understand the nature of bacteriophages, thus, the efficiency of phage therapy proved controversial. Phage therapy was replaced by antibiotics. Fortunately, some centers in Middle and Eastern Europe continued research on bacteriophages. The renaissance of phage therapy was an obvious consequence of the increased percentage of hospital infections caused by multi drug-resistant strains (1).

Burn wound infections are a major problem in the recovery process, considering patients with III degree burns, where survival is determined by the severity of the burn and burn-related infections. Microbial infections which appear during the initial 24 hours are drug-resistant and enhance the complexity of the therapeutic problem. The more and more common alternative to antibiotics, widespread by Polish and Georgian centers is the use of phage therapy (2).

Based on study results it is estimated that phage therapy is effective in 80% of Enterococcus infections, and even in 90% of Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Klebsiella pneumonia infections. Bacteriophages may be effectively used in the treatment of burn wounds, especially opportunistic pathogens such as Pseudomonas spp (2). One of the most important areas of phagotherapy interest are gram-negative bacteria, which show multi-drug resistance. Pseudomonas aeruginosa is the flagship member of the above-mentioned and at the same time an etiologic factor of infection with questionable therapeutic success of antibiotics. Simultaneously, Pseudomonas aeruginosa displays a number of properties, which make it a suitable target for phagotherapy, including the creation of a biofilm, which is hydrolized during the phage infection (1).

Studies in mice, infected with resistant to imipenem P. aeruginosa, who developed bacteremia and died within 24 hours, is evidence of the high efficacy of phage ØA392, which was represented by 100% of survival of mice. The phage remained in circulation for 48 hours, that is until complete bacterial eradication without resulting in an unspecific immunological response (3).

The biggest problem in the treatment of burn wounds is their infection, which often prevents skin grafting. Studies demonstrated that the use of phages, prior to grafting, hinders P. aeruginosa growth, but also has a positive effect on wound healing (1).

Based on clinical and laboratory study results with the use of bacteriophages, one may come to the conclusion that the above-mentioned lead to faster recovery from septic com-
plications, normalizing temperature and wound debridement during the course of reduced mortality. The bacteriological analysis of wound secretions showed that after phage therapy, staphylococcus and streptococcus strains were isolated twice less frequently, Proteus spp. 1.5 less frequently, while E. coli was not observed (4).

Studies of the immune system have shown a statistically significant normalization of resistance at the cellular level. The level of phagocytosis remained unchanged, while in the control group (without the use of bacteriophages) it was reduced. The antibody level increased less intensively, as compared to the control group. The study results confirmed the positive effect of phage therapy in case of patients with burn wounds (4).

Another pathogen that plays an important role in hospitalized burn patients is Acinetobacter baumannii. Many infections with the above-mentioned pathogen, both of blood and wound, are common to the intensive care unit and burn centers. The ability of the microorganism to acquire resistance to many antibiotics, disinfectants, and dehydration gives him the opportunity to survive in hospital conditions. The use of bacteriophages is a potential mean to control A. baumanii infections. The bacteriophage AP22 is a DNA phage exhibiting lytic properties against A. baumanii. It was isolated from the clinical material and categorized to the Myoviridae family. It shows the ability to rapid adsorption and stability in a wide pH range. The above-mentioned bacteriophage has the ability to selectively penetrate and lysis of 68% of genetically differentiated, drug-resistant A. baumanii variants, which leads to growth inhibition. It seems that the phage could be successfully used to control hospital infections (5).

Phage therapy assumes targeted infection and bacterial lysis with the release of virions capable of migration and bacterial infection throughout the organism. The mechanism of bacterial destruction, however, does not only consist in lysis, but in the use of cells for the production of progeny virions, as is the case during the initial minutes of T4 phage infections (6). Additionally, phages have the ability to interact with the cellular wall of the bacteria, disturbing its metabolism (7).

Lysins are phage enzymes which cleave the covalent bonds into peptidoglycans leading to rapid bacterial cell lysis. Phages and lysins have the ability to kill bacteria, resistant to antibiotics, having a narrow range of antibacterial activity, and no toxic effects in mammalian cells. Experimental data showed that both phages and lysins can be effective in the treatment of MRSA (8).

The uniqueness of bacteriophage therapy consists in the increase of the amount of healing substance (phages) in the presence of the pathogen, which therapy is aimed at. It is believed that the specificity of phages is outstanding, and the effect on other bacterial species and host tissues insignificant (6).

Although the natural hosts of bacteriophages are bacteria, there exist more and more data demonstrating that phages may interact with selected mammalian cell populations, especially immune system cells. These interactions include two main aspects. The first one is the immunogenicity of phages, that is the ability of phages to elicit a specific immunological response, especially the production of specific antibodies against phage antigens. The second aspect consists in the immunomodulating activity of phages, that is unspecific activity of phages on different functions of main immune system cell populations, both in case of congenital and acquired immunological response. These functions include phagocytosis, cytokin production, and production of antibodies against non-phage antigens. Bacteriophages are able to function in cooperation with mammalian phages. They may inhibit bacterial phagocytosis or themselves be subject to phagocytosis. The ability of bacteriophages to reduce the production of reactive oxygen species by means of leukocytes in the presence of bacteria or endotoxins has also been confirmed. Studies have demonstrated that the high immunogenicity of bacteriophages might also be applied in the treatment of neoplasms (9).

Available data suggest that bacteriophages might also be effective and safe in the management of patients with impaired immunity (10).

Some of the study results might indicate that phage therapy may have anti-inflammatory properties. A significant decrease in mean CRP values has been observed, evaluated between the 9-th and 32-nd day of therapy. Similar tendencies were observed in case of mean WBC values. The ESR level during therapy remained unchanged. It is suggested that the use of phages might probably reduce
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The inflammatory reaction, which accompanies bacterial infections (11).

Bacteriophages have long been used, both therapeutically and prophylactically in children. The administration of phages in children is safe, even by means of the intravenous method. Additionally, recent analyses demonstrated that phage therapy is economically favorable, as compared to antibiotics treatment, considering multi drug-resistant strains (12).

Bacteriophages are numerous and ubiquitous, which means that they are in constant contact with humans and animals. The occurrence of phages in the human organism, especially in the alimentary tract, raises the question of their potential role in physiology and pathology. Particularly important is the question whether phages can pass through the intestinal wall and migrate to lymph nodes, peripheral blood, and internal organs, and if so, what effects might be observed? Available literature data showed that phage translocation might be possible and may have certain immunomodulatory properties. Additionally, intestinal phages might play a protective role, inhibiting local immune reactions against intestinal flora antigens (13).

It is widely known that the administration of antibiotics might lead to excessive release of bacterial endotoxins and thus, complicate the clinical course in patients with bacterial infections. Bacterial endotoxins may activate neutrophils to produce reactive oxygen species (ROS), which play an important role in the pathogenesis of multiorgan failure during the course of sepsis. It is necessary to extend the therapeutic arsenal of available devices. Data suggest that bacteriophages mediate in the inhibition of ROS production, considering cells exposed to endotoxin activities (14).

In case of hospital specialists wanting to apply bacteriophages in the treatment of bacterial infections resistant to antibiotics, the current legal position in Europe in respect to medicinal products is serious handicap. Although it is believed that phage therapy could be a promising supplementation (or even alternative) to antibiotic therapy, unified legal documentation has not been established, rendering possible the introduction of bacteriophages into the canon of therapeutics. Decades of studies, the fruit of which are important clinical data concerning phagotherapy (Middle and Eastern Europe), not fulfilling the present EU legal requirements have been ignored. The issue of the clinical use of bacteriophages is once again considered as something new. In consequence, the applicants conducting standard clinical trials concerning the use of bacteriophages are forced to begin the clinical study from scratch (15).

Bacteriophages should not be classified as a classical therapeutic product, not only because this categorization is scientifically inappropriate, but also it would limit the admission of the product into everyday manufacturing (15).

The safety of bacteriophages has been much improved by the possibility of their purification from endotoxins. Efficient and scalable purification of bacteriophages from endotoxins has been achieved by subsequent ultrafiltration, followed by chromatography (7).

Bacteriophage therapy might be of particular importance in countries of the Third World, where the incidence of burn wounds and ensuing infections, due to lack of stringent health and safety regulations, and limited access to antibiotics is a particularly difficult problem (2).

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