

Phage in Therapy and Prophylaxis

History and Future Prospects



Bacteriophages are well known in the biopharmaceutical industry, mainly due to their destructive impact on productivity of bacterial-based fermentations necessary for obtaining the product¹. However, it seems that their positive role may soon dominate their rather grim reputation in the biopharmaceutical industry. In this short editorial the reasons for the failure to fulfill the high hopes that phages brought in the beginning of the 20th century is explained, and their re-birth described. Also, the author speculates on the future prospects of the use of phages in health protection.

Why was Phage Therapy Judged Ineffective in the Past?

Bacteriophages, also called phages, were discovered at the beginning of the 20th century. Initially, their viral nature was not recognised, however their ability to kill bacteria and to multiply became the basis for their use in the treatment of bacterial infections. Soon after their discovery, many companies producing phage preparations were founded, and their products were soon available on the market. Unfortunately, due to a lack of understanding of the nature of phages, they came to be used as a panacea for all types of diseases, including viral infections like herpes or even eczema and other allergies. Additionally, their preparation was often mishandled, e.g. instead of producing each phage from a phage cocktail separately, there were reported cases of adding all phages to one bacterial batch. This led to domination of the product by the phage which developed fastest in the conditions used, and thus the cocktail became virtually a preparation of one phage only. Historical studies reported cases of phage preparation preservation by boiling in order to kill the remaining bacteria. This treatment of course inactivates the vast majority of phages effective for human pathogenic

bacteria. Also, the preparations were often contaminated with enormous amounts of endotoxins LPS which after intravenous administration could be even more dangerous than the infection itself. A complete list of causes of the failure of early phage therapy attempts was described by Carlton in 1999².

All these mishandlings and misconceptions in phage use meant that phage therapy was judged as ineffective, and soon it became displaced by chemotherapy – first with sulfonamides, then with antibiotics. After that, work on phage therapy, which was considered ineffective, was discontinued. The only exception was the George Eliava Institute in Tbilisi, Georgia. This institute continuously worked on phage therapy in bacterial infections. The scientific approach to using phages as antimicrobial agents resulted in very effective therapy, which was able to replace antibiotics in many cases. The study, conducted by researchers from Eliava Institute, did not meet the requirements of Western medicine, but the results seem to be convincing and show the efficacy of the approach. They proved that bacteriophage-based medicines, when prepared and tested by specialists, can be very efficient in curing and prevention of certain bacterial diseases. The most prone to phage therapy seem to be infections of wounds, and the urinary and gastrointestinal tract; however, phages proved to be effective even in curing sepsis^{3,4}.

Phage Prevention of Gastrointestinal Infections

Preventive action of bacteriophages against gastrointestinal tract infections was already tested not only on animals (e.g. 5), but also on humans (reviewed in 6). In general, phages, when chosen properly, give excellent results, lowering the ratio of gastrointestinal infections even tenfold⁶. So far the majority of large-scale studies have been performed in the former Soviet Union

with phage and know-how provided by scientists from the Eliava Institute. Due to the Soviet Union decomposition and the fact that some of these studies were performed in the army, the knowledge gained during this process is partially lost or hidden. However, the known examples of successful use of this type of prevention e.g. one conducted in Tbilisi in the 1960s on 30,769 children, which showed a 3.8-fold reduction in dysentery occurrence, speak for themselves due to the results and the number of people involved in the study.

Another important trend in phage prevention of gastrointestinal infections is currently easily visible in veterinary work. The use of phage supplementing feedstock may help to prevent some diseases of domesticated animals. Bacteriophages may also be used as a prevention against the colonisation of the guts of these animals by bacteria, which are neutral for animals, but very dangerous for us, like e.g. various enterohemolytic *Escherichia coli* (EHEC). A recent outbreak in Germany caused by one of the EHEC strains shows how important this approach may be to take proper precautionary measures against prevention of outbreaks.

Another approach to prevention of gastrointestinal problems caused by bacteria is food preservation using phage. Currently there are several ongoing research projects of food preservation by phages and one ready product on the market. The addition of phage would in general prevent multiplication of certain bacteria during food storage and/or selective bactericidal action. The most interesting targets are these bacteria, which can multiply in conditions commonly used for food storage. *Listeria monocytogenes* is one of such targets which can be selectively eliminated by use of LISTEX – a commercial preparation of phage infecting this bacterium.

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Phage Treatment of Skin and Wound Infections

Another use of phage therapy is wound treatment. The attractiveness of using phages is in this case strongly augmented by the relative lack of effectiveness of antibiotics when their penetration to the wound is difficult. This happens when circulation nearby the wound is poor or the wound covers a large area of the body surface. It occurs especially often in some metabolic diseases, like diabetes. Due to poor microcirculation, almost any type of wound is hard to treat. The best example is diabetic foot, which may be extremely complicated to cure. Bacteriophages can be introduced to the wound as a liquid or a powder, or they can impregnate wound dressings. Also, special types of dressing made of phages embedded in biodegradable polymer are in use in Georgia. Spraying of phage preparations into wounds of soldiers injured on battlefield, including severe wounds like gunshots, increased recovery speed and decreased recovery time⁶.

Phage Treatment of Systemic Infections

Bacteriophages can also be used for treatment of systemic infections. Due to the severity of such infections, usually phages were introduced when antibiotic therapy failed. Despite the fact that this often caused patients to be in much worse condition when compared to the beginning of unsuccessful antibiotic therapy, the results obtained by researchers are very encouraging^{3,4}. In general, the ability of the phage to penetrate to different body compartments varies, and is usually relatively low when compared to small chemical molecules of antibiotic. However, when inflammation occurs, the permeability of tissues increases dramatically, and phages can penetrate easily to almost any tissue, including brain, despite the blood-brain barrier (reviewed in 6). The superiority of phage over antibiotic is that the phage will multiply directly in the infection site, unlike the antibiotics, which need repeated doses in order to maintain proper concentration, which would assure bactericidal (or bacteriostatic) effect.

Recently phage therapy against

chronic otitis was tested during a regular stage I and II clinical trial. Chronic otitis is one of these diseases when bacteria, usually *Pseudomonas aeruginosa*, due to its ability for biofilm formation, can effectively hide from the immune system, and due to biofilm characteristics, bacteria can easily avoid eradication by antibiotics. The results of the trial showed that phage therapy, even used just once in a relatively low dose, may be effective in treatment of such diseases⁷.

Phage as a Vaccine

Phage, after necessary modification, can be used as a carrier of DNA vaccines⁸, or it can contain antigens necessary for immunisation. Antigens from any pathogen can be introduced and exposed on the bacteriophage surface using phage display technology. It is also possible to combine both approaches – to expose antigens and introduce a DNA cassette responsible for the effectiveness of the DNA vaccine. The buildup of immunological response after the use of DNA vaccines differs considerably from antigen-based vaccine preparation. The latest one causes a fast build-up of immune response, contrary to DNA vaccines, which cause relatively slow, but much more effective, immunisation⁶. Therefore the idea to pack both types of vaccine in one phage seems to be very tempting, and relatively easy to perform.

Why Use Phage?

The superiority of phage over antibiotic is that phage will multiply exactly in the place, where it is needed – in the zone of bacterial infection. Antibiotics may not be able to penetrate to these spots, and even if they do penetrate, in order to maintain their concentration it is necessary to repeat the dose. In the case of phages, it is enough that just one virion penetrates to the infected area, and the multiplication of the phage should be able to reduce bacterial load considerably. The experiences gained during otitis treatment clinical trials show that the load of phages used in therapy does not need to be high – they used a dose of 10⁵ per phage in a cocktail of six phages (Wright et al. 2009). As the tailed bacteriophages in high density fermentation can easily grow to ~10¹¹ phages/ml, the effective treatment dose

is economically efficient. Even when phages are not administered directly to the infected site, the amount necessary for efficient therapy is not very high.

Another important aspect of phage therapy is that bacteriophages are a natural compound of our bacterial flora. They are present in our bodies in large quantities⁹ and in many varieties. Although some of them can increase virulence of bacterial hosts^{10,11}, lytic phages are considered safe, as they do not carry toxin, or immune evasion genes. This is due to the fact that contrary to temperate phages, they do not try to establish long-term cooperation with their bacterial host.

Why is Phage Therapy Research So Scarce?

The advent of phage research was easy to observe during the 1960s. The reason for that was a much lower level of complication of phage regulation cycles and a much simpler structure, including a much shorter genome when compared with bacteria or Eukarya. Such research was allowed to fund a strong basis for modern molecular biology, genetic engineering and the understanding of regulatory networks in live organisms. When a certain level of knowledge was gained, scientists rushed to investigate more complex organisms, and thus phages became the victims of their usefulness in founding the basis of knowledge. Relatively few groups of scientists all over the world decided to stay focused on bacteriophages, despite the fact that such research was considered not very interesting any more, and obtaining funds became a much more complicated task.

The result is that nowadays there are very few research groups experienced in phage research. The groups with the most experience in research on phage therapy are located in Georgia and Poland, thus in the region which was isolated from Western countries behind the Iron Curtain, from where knowledge and skills transfer was initiated recently.

The Future of Phage Use in Therapy – When is a Good Moment to Start?

At the moment only a few clinical trials with the use of phage have been conducted, with only one phage preparation undergoing a Phase II

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clinical trial⁹. On the other hand there is plenty of data on the efficacy of phages in therapeutic experiments, which were mainly conducted in Georgia and Poland (e.g. 3, 4, 6), which show a very high effectiveness of this form of therapy. Despite the fact that most authors consider them an alternative to antibiotics, it does not necessarily have to be true. Despite the fact that phages may be effective against bacterial infections without any supplements, the combination of antibiotics with phages may be extremely efficient in bacteria removal, since some groups of antibiotics increase phage replication in bacterial cells¹². This may be used for the construction of proper combined therapies. The ability of some bacteriophages to dissolve exopolysaccharides, which form extracellular skeletons of microbial biofilms, reducing the penetration of antibiotics and virtually blocking the action of the immune system, may make them, or enzymes isolated from them, to be important components of treatment of such diseases as respiratory tract infections accompanying cystic fibrosis or mucopolysaccharidosis, or wound and burns infections. The relatively low doses necessary for treatment make phages a very cost-effective medicine. Even in the case of vaccines, which require much higher doses, the cost of material preparation is not very high. Additionally, due to their lack of toxicity, their use in construction of new pharmaceuticals reduces considerably the costs of the initial stages of research, and thus shortens the way from idea to market.

Bacteriophages sooner or later will be present on the market in Western countries (in Georgia they are already marketed). Most probably they would not eliminate any drugs or vaccines present on the market, but they would provide an alternative, a possibility to augment some types of therapies and in the case of multi-drug-resistant infections or in some medical conditions, they will be the medicine of choice. At the moment, pharmaceutical phage research seems to be in its initial stages, but the situation is changing dramatically. Many (or even the majority of) groups of scientists performing phage research are associated with biopharmaceutical

companies, and thus their know-how is not always freely available. The best choice is to outsource such research due to fact that very specialised skills and knowledge are necessary for this kind of research. The alternative is to build one's own phage research group from scratch. However, there are a few companies which offer contract research in this subject (e.g. Phage Consultants), so pharmaceutical companies which are willing to enter this exciting field of phage exploration may use another company's expertise in development of the product ■

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