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An in depth analysis of bacteriophage therapy in the medical field: advantages, limitations and future challenges

Abstract

Due to the aggravation of antimicrobial resistance, alternatives have been explored to treat infections by multi-resistant bacteria, including bacteriophages and endolysins. The main advantages of bacteriophage therapy are its high specificity, less damage to the microbiota, its self-replication capability, its synergy with the individual's immune system, the option of applying them in conjunction with antibiotics, the possibility of reaching organs that are difficult to access, activity against biofilms and no adverse effects of its use have been described. Their opponents point out to the ability of bacteria to develop resistance to phages, the possibility that they contain genes that encode virulence factors or mutations that confer resistance to antibiotics, the risk of development of an immune response that clears them or that causes anaphylactic reactions. Additionally, published clinical studies do not provide conclusive results, although there are case studies with encouraging results for individual patients.

Keywords: Phage therapy, anti-bacterial agents, bacteriophages, endolysin, bacterial drug resistance, bacteria

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Introduction

Currently there are over 700,000 deaths each year related to antimicrobial resistant infections. If this trend continues, by the year 2050 there will be 10 million deaths annually worldwide.[1] The development of new antibiotics has decreased, due to the disinterest of pharmaceutical companies, mainly for economic reasons.[2] Therefore, it is necessary to explore alternatives against bacterial infections such as the use of bacteriophages as therapeutic agents: viruses capable of infecting and replicating in bacterial cells. This is called bacteriophage therapy or phage therapy.[3,4]

Bacteriophages were discovered more than 100 years ago and their use declined in Western medicine throughout the 20th century. However, its use has become widespread in Eastern Europe and continues to date in Poland, Russia and Georgia.[5,6] The prerequisites for the medical use of bacteriophages, the advantages, disadvantages and challenges of phage therapy are discussed in this research article. In addition, an account of clinical trials in humans and endolysins are briefly described.

Prerequisites for bacteriophages medical use

Availabilty

It implies having access to phages that are biologically active for the bacteria responsible for the infection. They must meet the standards of purity, traceability and be characterized. They must have low immunogenicity, be strictly lytic, lack genes that produce toxins or that confer resistance to antibiotics, a wide host range against multiple strains of the target pathogen and the ability to restrict the emergence of phage-resistant mutants. Phages with poor adsorption, replication, and distribution are excluded.[7-9]

Production

Phages must be purified and any trace of endotoxins and bacterial contaminants must be removed. If they are to be considered as medicinal products, they must adhere to Good Manufacturing Practices (GMP), requiring procedures for their manufacture, biological and physicochemical tests and strict production facilities.[7,8,10] The stability of dissolved bacteriophages is limited and influenced by temperature, acidity and salinity, for which sugar and polyols must be added.[11,12]

Formulation and administration

For infected superficial wounds, they are applied topically in the form on creams, gels or bandages. Oral administration is easy and has no adverse effects, but gastric acidity can interfere with the action of phages, so it must be alkalized. The inhalation route is used in the case of the respiratory tract, by means of nebulization or by spray. By intravenous means, diffusion is rapid, but circulating phages can be sequestered by the reticuloendothelial system.[7,11]

Dose

Little has been studied, theoretically, in situ application does not require multiple doses, but in practice this is not fulfilled.[7]

Therapeutic evaluation

Randomized, controlled, and double-blind trials are required. Many studies evaluate through the clinical improvement of the patient, without documenting the activity of phages within the body. The pharmacokinetics, pharmacodynamics, possible immunological reactions to one or repeated applications of phages and their interaction with bacterial biofilms and microbiota needs be studied.[7,8]

Advantages and disadvantages

Bacteriophages act against Gram positive and Gram negative target bacteria with high specificity, which means the absence of collateral effects towards the microbiota as well as the reduction of superinfections, since, as a result of their self-replication, the concentration of phages increases at the infection site, which prevents the overgrowth of secondary pathogens. In addition, the adverse

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effects for the host appear to be null, since they are unlikely to enter eukaryotic cells or cause anaphylaxis with their use.[8,13,14]

Bacteriophages are ubiquitous and the most frequent place to find them is water, which facilitates their isolation and implies low production costs.[15,16] There are synergistic interactions between phage and the host's immune system. The lysis caused by the phages would decrease the bacterial densities, but it would not eliminate them completely, since they would have no way to replicate. With a low population of bacteria, the innate immune system would eliminate the remaining bacteria. Without phages, bacterial densities would increase so much that they would be outside the control range of immune cells.[13,17] In addition, there may be an impact on the inflammatory response towards infection. Decreases in C-reactive protein, erythrocyte sedimentation rate, and leukocyte count have been documented.[14]

Using phage therapy, bacteria may be more vulnerable to the use of antibiotics. Sub lethal concentrations of different classes of antibiotics have a positive effect on the size of phage plaques and on the efficiency of bacteriophage propagation; This phenomenon is known as phage-antibiotic synergy (PAS).[13,15] Phages can access organs where some antibiotics cannot, such as the prostate and the brain, preventing bacteria from eluding treatment. Likewise, they lack cross-resistance to antibiotics and mechanisms developed by bacteria to resist antibiotics, such as efflux pumps, since they can use them as receptors, which can affect the clearance of antibiotics.[13-16]

The use of phage cocktails makes it more likely that several phages can infect the same species and strains, as they can recognize different receptors on the bacterial cell surface, including the cell wall and teichoic acids. They are also used to combat phage-resistant mutants and are considered another alternative to treat antibiotic multi-resistant biofilms and to hydrolyze the exopolysaccharide capsules present in *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella pneumoniae.*[17-19]

Among the disadvantages is the possibility that bacteria develop resistance to phages and that these can carry genes for resistance to antibiotics or bacterial virulence factors such as toxins.[14,15,20,21] Another point is that the immune system could recognize them as invaders. Due to their ubiquity, patients could have low antibody titers against phages, which would increase and the phages would be neutralized by the immune system, or would cause unwanted immune responses. In addition, by lysing the bacteria, endotoxins and super antigens could be released, which may induce an inflammatory cascade and potential multi organ failure.[14,15,19]

There are no guidelines for their use, nor can a single phage be used to treat diversity within the same bacterium.[15] In addition, the process of exclusion by superinfection (the virus residing in a cell blocks the infection by additional viruses by eliminating the receptor, reducing the internalization of particles or blocking at the level of replication or transcription), could lead to a failure in the treatment if instead of entering a lytic virus, a lysogenic virus did so that would prevent the subsequent entry of the lytic virus[17]. Other problems are the ignorance about the function of many genes, the difficulty to extrapolate data obtained in vitro to in vivo, the necessity to identify the bacteria causing the infection given the specificity of phages, given that only lytic phages can be used, the time it takes to prepare a specific bacteriophage solution to each patient, as well as the fact that phage treatment is not covered by public health insurance and they are not yet considered as a pharmaceutical treatment.[14]

Challenges of bacteriophage therapy

Bacteria have generated resistance mechanisms towards bacteriophages, most commonly preventing the adsorption of phages by modifying cell surface structures (receptors) and the injection of restriction enzymes, CRISPR / Cas systems (regularly grouped, interspaced short palindromic repeats and associated with proteins, whose main function is to counteract phage infection), the BREX system (phage exclusion mechanism) and through the Abi system or abortive infection, where the bacteria inhibit phage development through the programmed death of a cell bacterial infection thus protecting the entire population.[9,17,22]

The emergence of resistant strains should be considered before initiating phage therapy, for example with the use of phage cocktails, targeting highly conserved structures that are essential for the survival of the bacteria.[19] Immunocompromised patients could have treatment failure, since in vitro a high density of phage-resistant mutants is observed, but in animal models the results are different, so it is thought that it is the immune system that eliminates them.[9] Lyophilized preparations are more stable but costly and phage activity may be lost.[12] One of the biggest challenges to phage therapy is incorporating genetic engineering. Currently, it has allowed the expansion of the host range, has added factors to improve therapeutic activity, degrade biofilms or express heterologous genes to increase the killing power of phages.[15]

Cocktails present complexity in pharmacodynamics; the combination of two or more phages can produce synergistic effects, facilitating infection by another phage, which competes for resources, so the phage with the highest adsorption speed, the shortest latency time and the largest burst size, will be the have a selective advantage and the others will be eliminated; causing it to be the same as only one phage is infecting but with a lower titer.[23] It is required to replicate in vitro results in vivo, including adsorption dynamics, biofilm formation and latency time distribution; as well as clinical trials that reflect the phenotypic and genotypic changes of bacteria and phages. In vivo studies need to be quantitative, to evaluate each of the components and thus statistical methods can be applied.[23] There is no regulation for formulations, so shorter development and approval times are needed to optimize processes and reduce costs, and thus facilitate the granting of pharmaceutical licenses.[8]

Clinical trials in human beings and case studies

In 2019, genetically modified phages were used for therapy in humans in a patient with a condition caused by multiresistant *Mycobacterium abscessus* of 15 years of evolution. For this, a cocktail of 3 genetically modified phages who had eliminated the genes Zoej and BP, which are repressors and made them obligate lithics.[15] Moelling et al. refer to the results of the study published by Jault et al. in 2018, whose objective was to test phages produced under GMP for the treatment of burns infected by *Pseudomonas aeruginosa*; phages applied topically managed to reduce the bacterial load, but more slowly than standard treatment and with fewer adverse effects. Jennes[24] published the case of a patient with peritonitis and abdominal sepsis due to *Enterobacter cloacae*, who developed necrotic pressure ulcers, colonized by multi-resistant *P. aeruginosa* only sensitive to colistin, which led to septicemia. Intravenous (IV) colistin therapy was started but it was discontinued when the patient developed acute renal failure. Phage therapy was started and the patient's condition improved by making blood cultures negative, reducing serum C-Reactive Protein levels, and eliminating fever; Additionally, the patient's renal function

recovered and there were no unexpected adverse effects, clinical abnormalities, or changes in laboratory tests. However, the patient died 4 months later, due to *K. pneumoniae* septicemia.

Ujmajuridze[18] carried out a two-phase prospective study, for which they recruited 130 patients who were scheduled for a retro-urethral transection of the prostate and underwent a urine culture for screening. Of these, 118 had positive urine cultures for defined uropathogens and counts greater than or equal to 104 CFU / mL. Subsequently, the isolates obtained were subjected to in vitro bacteriophage sensitivity tests of the Pyo bacteriophage solution and underwent adaptation cycles to increase the percentage of isolates sensitive to said cocktail. In the second phase, 9 patients whose isolates were sensitive to the cocktail received bacteriophage treatment, twice a day for 7 days and beginning the day after surgery. The urine culture was repeated 7 days after surgery or when any adverse effect occurred. After completion of treatment, there was a decrease in bacterial counts in 6 of 9 patients and 4 of 9 patients did not show significant bacterial growth.

Patey[7] conducted a count of case studies in which phage therapy was applied along with antibiotics as paleative therapy at the Villeneuve Saint Georges hospital, in France. The first case that received this treatment was a chronic suppurative otitis caused by *Staphylococcus aureus* in which the results were a complete cure of the infection. Another 14 patients, most of them suffering osteoarticular infections received this kind of therapy. It is worth noting that 12 of the 15 cases resulted in a complete recovery from the infection and that there were no adverse effects in the treated patients.

Rubalskii[25] reported 8 cases of patients who presented infections that were refractory or were caused by multi-resistant bacteria associated with implants or transplants, who received successful treatments with individualized phages or in combination with fibrin glue, which allowed a sustained release of phages at the sites of infection. In 7 cases, the bacteria were completely eradicated.

Doub[26] published the case of a patient with a chronic prosthetic joint infection by methicillin-resistant *S. aureus* (MRSA). After receiving unsuccessful treatment with vancomycin, daptomycin and doxycycline, he underwent an intra-articular (IA) and IV phage therapy using the phage SaGR51ϕ1 together with daptomycin. After the third dose of phage, the values of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increased, with no apparent explanation, for which phage therapy was suspended and the administration of daptomycin was continued; AST and ALT values returned to normal 10 days later. After stopping the antibiotic treatment, the patient underwent a new procedure during which another dose of phage was applied via IA and tissue samples were taken for culture with negative results. Then, he underwent another surgery to place a new prosthesis, where he received a dose of the phage via IA and intraoperative cultures were performed with negative results.

Endolysins

They are lytic, free and soluble enzymes produced by phages at the end of their replication cycle.[27] Once the phage has entered the bacterial cell, the endolysin is released into the periplasm and degrades the peptidoglycan layer from within, producing a sudden lysis of the bacterial wall and the dispersal of new mature virions. It is unknown whether bacteria have developed natural resistance to lytic enzymes and since endolysins do not replicate, they must be applied in a high dose to exert their bactericidal effect before being cleared by the body.[28] At the pharmacokinetic level, they resemble a traditional medicine, making it easier to determine the dose and adapt to the regulatory framework for medicinal products. In addition, they generally have a broader spectrum of action than phages, so they would offer more flexibility for the treatment of acute and chronic infections. They have also been shown to be effective against colonizing pathogens on mucosa or biofilm surfaces and their manufacturing process would not require the removal of endotoxins derived from the host bacteria.[28-30]

There is concern that endolysins stimulate the production of neutralizing antibodies that prevent their antibacterial action in subsequent administrations,[30] however, more research is needed in this regard. Endolysins can be modified by means of protein engineering, redirecting or broadening their specificity, improving their catalytic activity, increasing their stability in serum and their solubility. In addition, they can be fused with other particles such as peptides that permeabilize the outer membrane of Gram Negative bacteria, improving the bacteriolytic action (called artilysins) or add polycationic peptides that increase and accelerate the bactericidal effect of streptococcal endolysin. Two phase I trials and one phase I / II trial have been completed, in which its use has been shown to be safe in humans.[27,28]

Future prospects

Currently, the application of phage therapy for acute systemic infections such as septicemia or meningitis is complicated, since the treatment of these pathologies must be administered urgently and parenterally. This would require the availability of characterized and purified bacteriophages, which can be quickly approved.[7] Governments, academic institutions and private companies should support initiatives for the study of phage therapy around the world. An example is the German consortium Phage4Cure, whose objective is to evaluate the safety, tolerability and efficacy of a purified phage cocktail for inhaled therapy against chronic *P. aeruginosa* infections.

This cocktail will be manufactured following GMP standards throughout the production chain, in order to obtain a product that meets the requirements of Western regulatory agencies and is the first phage therapy approved for inhalational application in patients. with bronchiectasis in Germany and possibly becomes a model for the development of phages for therapy with other indications.[8]

The establishment of a greater number of phage banks is a priority, to increase their accessibility in order to apply them in clinical trials or palliative therapy[11] as well as to carry out studies regarding pharmacokinetics and pharmacodynamics, phage-antibiotic synergy, the development of possible immune responses, among others.

Conclusion

Based on current research, phage delivery is seen as a possible response to antibiotic resistance. Among the advantages of its use is its low cost, the little resistance that bacteria have towards these and that no adverse effects have been described. However, there are still unsolved problems that derive mainly from the lack of robust in vivo studies. There is a need to evaluate formulations, as well as to develop protocols for the use of bacteriophages, phage cocktails and endolysins.

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None.

Conflicts of interest

None.

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