LITERATURE REVIEW

Bacteriophage Therapy for Treating Infections: Hope or Hype?

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Abstract

Bacteriophage therapy employs the use of viruses to kill bacteria and has been noted to confer reversal of antimicrobial resistance. It was proposed around the same time as antibiotic therapy for combatting infections but lost the race for becoming the mainstay therapy. However, antibiotic resistance is increasingly resulting in morbidity and mortality. Bacteriophage therapy as an alternative approach for combatting infections has garnered speculation and interest of many scientists with hopes that it may become a management strategy for multi-drug resistant infections.

The aim of this review is to shed light on the developments in bacteriophage therapy, explain lytic cycles as the proposed functional mechanism and discuss the evidence base: preclinical, case-based and clinical trials.

There is preliminary evidence that alludes to an element of safety and efficacy in treating multidrug resistant infections. However, there is a paucity of high-quality evidence, which could bring this therapy into routine practice. This is further burdened by limitations such as the need for an individualised approach and our lack of understanding of the immune reactions to it. This therapy is quite promising, but much work is needed before it can be considered for routine clinical practice.

Keywords: Bacteriophage, Infection, Antibiotic, Antibiotic resistance, *Pseudomonas aeruginosa*

Introduction

Following discovery of Penicillin in 1945, Alexander Fleming warned us that "it is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them"[1]. Now, we rely very heavily on the use of antibiotics. We use them prophylactically pre-operation and post-transplant, pre-emptively, empirically, and definitively, making modern medicine without antibiotics unimaginable. Yet we are faced with the very threat we were warned about: antimicrobial resistance[2].

Resistance to antibiotics has become a crisis, especially in certain bacterial pathogens where there is a newfound paucity of therapeutic alternatives and a marked prevalence of pan-resistant strains[3]. Antimicrobial resistance is the cause for an estimated 700,000 deaths annually; projected to escalate to 10,000,000 by 2050 without action[4,5]. It is imperative that other non-antibiotic options are explored. One potential solution is bacteriophage therapy: the killing of bacteria using viruses.

Bacteriophage therapy seems very promising for two main reasons. Firstly, it has bactericidal effects specific to the bacterium being targeted. Secondly, there have been claims that it can potentially reverse antibiotic resistance, indicating a prospective role as a complement or adjunct to therapy with antibiotic therapy[6]. As such, the aim of this review is to shed light on the developments in bacteriophage therapy, explain lytic cycles as the proposed functional mechanism and discuss the evidence base: preclinical, case-based and clinical-trials.

Discovery and Use in the Last Century

Bacteriophages are viruses whose existence was first postulated in 1896 by an English bacteriologist, Ernest Hanbury Hankin. Félix d'Herelle, a French microbiologist, finally discovered this mystery entity in 1917 when he analysed stools from patients recovering from bacillary dysentery. He had isolated what he referred to as an "invisible microbe," a filterable virus which infects Shiga bacilli and is able to lyse them—"a virus parasitic on bacteria". This was named "bacteriophage" after presentation to the Académie des Sciences in September that year[7].

This area seemed welcome initially, given that infectious diseases were decimating populations. Eventually, it was not given attention unlike its antimicrobial counterpart, antibiotics, after their discovery in 1928[2]. Antibiotics had broader spectra and their ease of use was unmatched as compared to bacteriophages. Antibiotics thrived while the bacteriophage therapy was forgotten about in all parts of the world except some. They were adapted in the

former Union of Soviet Socialist Republics with the development of the Eliava Institute in Tbilisi, Georgia. This centre for bacteriophage therapy still exists in the modern day, and it is not the only one. There are centres in other countries too such as Poland and Belgium; with Belgium being the first country to make bacteriophages available in pharmacies based on their magistral phage regulatory network[7].

Bacteriophage Biology

Classified based on genomics and morphology, the diversity between bacteriophage species is remarkable.

They are known to exhibit two primary cycle types: lytic and lysogenic.

Lytic cycles result in destruction of bacterial cells. The mechanism involves entry into a bacterium, replication, protein synthesis, assembly, and colonisation. The bacteriophage injects its DNA into a bacterium, which gets replicated using the bacterial nucleotides. The bacteriophage DNA then uses bacterial cellular resources to synthesise proteins conducive to its cloning. After they have colonised the cell, they secrete hydrolytic enzymes—called endolysins—to cleave the host bacterium's cell wall and infect other bacteria. This is the primary mechanism by which the proposed therapy with bacteriophages can kill bacteria and clear infections.

Lysogenic cycles, on the other hand, involve the integration of the viral DNA into the host bacterial DNA. When integrated into the bacterial host's DNA, the bacteriophage is referred to as a "prophage". This is a period where they are inactive and replicate harmlessly coupled with the bacterial cell. This pathway also has the capacity to convert to the lytic cycle under certain stressful circumstances. If stressful cellular environments are present, the bacteriophage DNA will excise from bacterial host DNA and initiate bacterial destruction using the lytic cycle.

Lambda phages are a well-known example of temperate bacteriophages—species that can develop using both lytic or lysogenic pathways depending on cellular environment. They infect the species *Escherichia coli*, and a lot of our knowledge regarding the molecular mechanisms of lysogeny are based on the study of it[8-10].

Combatting Antibiotic Resistance

Resolution of the rapidly rising antimicrobial resistance potentially lies in the use of bacteriophages. This was demonstrated in an article by Chan et al., published in *Scientific Reports* in 2016[6].

Some bacteria can gain resistance to antibiotics via their multi-drug efflux (Mex) systems. Interestingly, bacteriophages can infect bacteria by entering via those Mex systems. As such, Chan et al. focused on the effect of bacteriophages on this efflux pump resistance mechanism against antibiotics[13]. It was hypothesised that a bacteriophage, which binds to the outer-membrane protein of Mex (OprM), would cause the host bacteria it colonises to evolve. This evolution would involve downregulating the expression of the Mex system, and though the bacteria may gain resistance to the bacteriophage in this manner, success would still be achieved. This is because expression of efflux proteins would downregulate the bacteria, and antibiotic sensitivity would return.

To test this, Chan et al. obtained samples of naturally occurring bacteriophages from locations such as sewage, soil, lakes, and rivers[13]. In this study, they identified 42 species that were able to infect the PA01 and PA14 strains of *Pseudomonas aeruginosa* (an opportunistic gram negative, rod-shaped bacterium, increasingly seen to be pandrug-resistant[11]). In addition, a unique lytic bacteriophage from the family *Myoviridae* was identified in a freshwater lake in Connecticut, USA and given the name OMKO1. This was the only bacteriophage that infected the multidrug resistant (MDR) bacteria by binding to the OprM protein of the efflux pump.

Chan et al. then observed something wonderful after further investigation: a genetic trade off. The bacteriophage-sensitive bacteria tend to efflux antibiotics but get killed by the virus. The phage-resistant mutants have impaired drug efflux ability which makes them vulnerable to antibiotics that are typically useless against this bacterium. The paper elucidated a mechanism by which bacteriophages can increase sensitivity to antibiotics[6].

Preclinical Evidence

A review article published by Melo et al. in February 2020 discussed the efficacy of bacteriophage therapy based on 10 years of preclinical studies, including mostly the studies on murine models[12]. The infections at the focus of this study were broad and were grouped into various categories: skin and soft tissue, eye and ear, respiratory tract, gastrointestinal and urinary tract. Regarding skin and soft tissue infections, efficacy was demonstrated against common pathogens when the phage-based concoctions were applied topically and to a lesser extent using some other routes of administration in this study. Clear evidence of efficacy was documented from application of the therapy across all the other groups as well; however, it was mentioned that many studies produced varying infection clearance between subjects[12]. This study was important in demonstrating that bacteriophages have the capacity to become our asset for infection clearance in the future. However, the demonstrated variation in efficacy between subjects receiving the same therapy is undesirable[12]. Trials in human subjects are required to confirm if these results can be replicated in clinical use.

Case Report–Based Appraisal

A 76-year-old man in 2012 documented by Chan et al. had an aortic aneurysm treated by aortic arch replacement surgery and a post-surgical complication was the infection of the graft and mediastinum by *P. aeruginosa*[13]. In this case report, practical algorithms in place and applied back then were the use of systemic antibiotics, debridement of infected tissue and graft excision. The patient in this report had numerous recurrences in the subsequent years, the management of which was aided by antibiotics. This led to increased resistance and there

was also biofilm formation noted, which decreases penetrance of antibiotics. A solution containing OMKO1 bacteriophages and ceftazidime were applied into the mediastinal fistula and the patient was discharged home and four weeks post-surgery, the 76-year-old patient suffered an aortic perforation. However, the only thing which the cultures revealed was *Candida* species, and it was confirmed that recurrence of *P. aeruginosa* infection had not occurred[13].

A separate case report documented by Schooley et al. illustrated outcomes from treatment of an MDR *Acinetobacter baumanii* infection using bacteriophages[14]. This case describes management of a 68-year-old man with necrotising pancreatitis complicated by MDR *Acinetobacter baumanii* infection on a background of diabetes. The 68-year-old patient was comatose, and the infection was resistant to last line antimicrobials such as colistin. Bacteriophage therapy was initially administered to the patient as a cocktail (ΦPC). 36 hours after the initial administration of the therapy, a new cocktail (called ΦIV) was administered intravenously alongside minocycline and repeated frequently over the next 48 hours. The patient then recovered from the coma after several weeks and improvement was seen on all fronts over the next three weeks, and the patient was discharged[14].

There have also been numerous other case studies demonstrating bacteriophage efficacy. Two of these were published towards the end of 2019. Maddocks et al. demonstrated efficacy of the therapy in treating pneumonia and empyema caused by *P. aeruginosa* in a 77-year-old lady with hypersensitivity reactions elicited on administration of numerous antibiotic types[15]. In this case study, the patient had exhausted antibiotic therapy extending to meropenem, which she had developed resistance to. AB-PA01 bacteriophage therapy was initiated as an adjunct to the gentamicin and ciprofloxacin. The patient's status improved rapidly from this treatment as she had improved oxygenation and cessation of sedation and, within a week, the patient was stepped down from the intensive care unit to the high dependency unit[15].

A study by Law et al. was published in the same year and demonstrated the use of bacteriophage therapy in a 26-year-old cystic fibrosis patient who developed MDR *P. aeruginosa* pneumonia with respiratory failure[16]. This 26-year-old patient had been placed on colistin previously, which led to renal failure, AB-PA01 bacteriophage therapy was initiated as an adjunct to ciprofloxacin and piperacillin-tazobactam. The patient became afebrile by the seventh day of the therapy and ambulatory by the eighth week and monitoring of the first 100 days post therapy revealed no infective recurrence[16].

Although these findings make bacteriophage therapy appear lucrative, it is salient to note that the positive effect evidence demonstrated here might be due to exceptional circumstances. There is an array of variables that could be at play, and it cannot be assumed that bacteriophage therapy caused these effects alone.

Clinical Trials

The first phase I safety trial for the use of bacteriophage therapy was published in the United States of America in 2009; this investigation was of the potential use of bacteriophage-based preparation in the treatment of venous leg ulcers in humans[17]. In this trial, 42 patients with chronic venous ulcers were tracked on initiation of this treatment and outcomes were measured. The study followed patients for a control and an experimental group for twenty-four weeks after therapy. The experimental group was treated topically with WPP-201, which is a bacteriophage formulation targeting *P. Aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*[17].

In this study by Rhoads et al., the proportion of healed ulcers was not significantly different between the treatment and control groups at either 12 or 24 weeks[17]. However, in this trial there was also no significant difference between them in the frequency of adverse events, in either quality or quantity. Potential limitations included the amount or type of bacteriophages used and the minute sample size, since the safety profile for the therapy did not cause any major adverse events, the study concluded that the efficacy of the product will need to be re-evaluated in a phase II study[17]. While further trials have not investigated venous leg ulcers in humans, phase I/II clinical trials have shown low to moderate efficacy in the use of this therapy for treatment of other conditions.

One such study, by Wrights et al. investigated single dose bacteriophage therapy in chronic otitis caused by MDR *P. aeruginosa*[18]. This study contained 24 patients with an illness duration of several years and were divided into a control and an experimental group. Outcome measures of clinical change such as erythema, discharge, etc., were quantified using a visual analogue scale (VAS)[18]. Bacterial levels were measured initially and at follow-up on days 7, 12 and 42 and clinical indicators improved for the phage-treated group relative to the placebo group in that study and *P. aeruginosa* counts decreased[18]. For the experimental group in this study, mean reduction of the total VAS scores at the final follow up was 50% and three patients had more than 80% reduction. In contrast, the placebo group in this study had a 20% mean reduction with no patients having more than 80% reduction. No significant changes were found in relation to audiometry and no adverse effects were reported, however, the sample size here was quite small[18]. Larger sample sizes are needed to confirm that these effects are genuine and to reduce the amount of possible errors[18].

Limitations

While the prospect of bacteriophage therapy appears lucrative, we are still a long way from bringing it into practice. Part of the reason for this is its long list of limitations, which range from our current lack of knowledge of its pharmacokinetics and pharmacodynamics to the very large variation and evolution in bacteriophages. One obstacle to employing bacteriophage therapy is antigenicity of the viruses and the immune response that follows it, which can dampen their clinical response and undermine their therapeutic value. Apart from this, we run the risk of development of bacterial strains that are resistant to bacteriophages, despite the hope that bacteriophages would evolve too

and counter this resistance. It would also be important to be vigilant of life-threatening syndromes like the reaction to endotoxin-like substances[19,20].

The literature is scant on studies with well-designed clinical trials which can evaluate the efficacy of the therapy in different patients. The studies currently published address remarkable cases where it was effective, and some indicate a safe profile for it. However, there is still little proof that this therapy can be replicated consistently in different people. It is also proving difficult to contain standardised formulations of the bacteriophages because unlike antibiotics, these are living organisms with a propensity to evolve.

Conclusion

While it is apparent that we have a long journey ahead of us for bringing bacteriophage therapy into routine practice, it is also clear that it is not just a hype. Phase 2 clinical trials are the strongest evidence so far for demonstrating the therapeutic implications of this therapy. The therapy is bactericidal when conducted accurately and there is some evidence that it can have the ability to counteract antibiotic resistance. It has also predominantly been shown to be safe and efficacious. Nonetheless, it features numerous limitations, which would have to be dealt with prior to the promise of carrying the therapy into practice. There is warranted hope that this therapy can be utilised for the clearance of infections in the future with preliminary evidence prompting further research into the area. ◄

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Declarations

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