www.brn.cat

PERMANYER

BRN Rev. 2023;9(3):160-6

BRN Reviews

REVIEW ARTICLE

Use of Bacteriophages in Lung Transplantation

Manuel López-Meseguer, MD, PhD[1,2], Marta Zapata-Ortega, MD[1], and Cristina Berastegui-Garcia, MD, PhD[1,2,*]

[1]Department of Pulmonology, Lung Transplant Unit, Vall d'Hebron Hospital, Barcelona; [2]Consortium for Biomedical Research in Respiratory Diseases (CIBERES), Madrid, Spain

ABSTRACT

Bacteriophages (phages) are natural predators of bacteria and are becoming increasingly attractive due to the increase of multidrug-resistant organisms (MDROs), especially prevalent among transplant recipients. Antibiotic resistance is the biggest current threat to global health. An increasing number of infections is becoming harder or almost impossible to treat, carrying high morbidity, mortality and financial cost. The therapeutic use of bacteriophages, viruses that infect and kill bacteria, is well suited to be part of the strategies to combat antibiotic resistance. Infections, in particular those due to bacterial pathogens, are common among transplant candidates and recipients. For lung transplant (LTx) patients this is of paramount relevance, since some of the underlying diseases in LTx present recurrent infection and complex colonization (such as cystic fibrosis or bronchiectasis). Individual case reports and small case series suggest the possible efficacy of phage therapy for the treatment in pre- and posttransplant patients. Importantly, there have been no serious safety concerns in the reported cases, so it is reasonable to pursue phage therapy for difficult infections on a compassionate basis.

Keywords: Bacteriophages. Lung transplant. Multi-drug resistant organisms.

***Correspondence to:**
Cristina Berastegui-Garcia
Email: cristina.berastegui@vallhebron.cat

Received: 30-06-2023
Accepted: 21-08-2023
DOI: 10.23866/BRNRev:2023-M0096
www.brnreviews.com

INTRODUCTION

Phage therapy has been available for over a century and has had widespread clinical use in the pre-antibiotic era. It was effectively abandoned once penicillin was discovered, though there has been continued clinical use in Eastern Europe and Russia. The rapid spread of antimicrobial resistance is a major and increasing global health problem. In Europe, growing levels of antibiotic resistance are being reported, particularly in countries with existing high levels of multidrug-resistance, thereby limiting therapeutic options[1]. The possibility of developing therapeutic products that are alternatives to antibiotics could be a great help in the fight against antibiotic resistance. Since the first USA-based intravenous (iv) administration of phage for multidrug-resistant organisms (MDROs) for *Acinetobacter baumannii* infection in 2016, there have been numerous cases of compassionate phage use in the United States and globally[2].

Bacteriophages are viruses capable of infecting and replicating within bacterial cells. Phages are in every environment containing their bacterial hosts and play an important role in many biological processes; they are supposed to be the most abundant organisms on the planet. Phages replicate through two primary life cycles, the dynamics of which have important implications for their therapeutic application. Virulent or obligate lytic phages infect and quickly kill their bacterial host cell, whereas temperate or lysogenic phages may either stably integrate into the host's genome or enter into the lytic life cycle. Temperate phages are capable of protecting their host from phage reinfection and may change the bacterial phenotype through the expression of viral genes, a process known as lysogenic conversion[1]. Lysogeny and prophages can be beneficial to bacteria as they can encode genes for antibiotic resistance or other virulence factors[1]. For that reason, only lytic phages should be used for bacteriophage therapy. The main differences between the lytic and the lysogenic life cycle can be seen in figure 1.

Bacteriophage life cycles

Phage therapy is currently being used for multidrug-resistant and biofilm infections but also for non-infectious conditions potentially related to alterations in the microbiome such as inflammatory bowel disease and sclerosing cholangitis[3]. Phage therapy can be based on off-the-shelf combinations of phages designed to have sufficient breath to cover a high percentage of pathogenic strains for a given indication. A personalized phage cocktail can also be devised that is specific to a patient's bacterial isolates. Currently, in the USA, treatment authorization is usually sought under compassionate grounds via the Emergency Investigational New Drug mechanism at the FDA.

MULTIDRUG-RESISTANT INFECTIONS IN LUNG TRANSPLANT RECIPIENTS

Infections, in particular those due to bacterial pathogens, are common in transplant candidates and recipients. They have anatomic reservoirs and extensive experience with antibiotic treatment and tend to have MDROs. Prevalence and identity of MDROs are variable depending upon the transplant center and local epidemiology as well as the type of

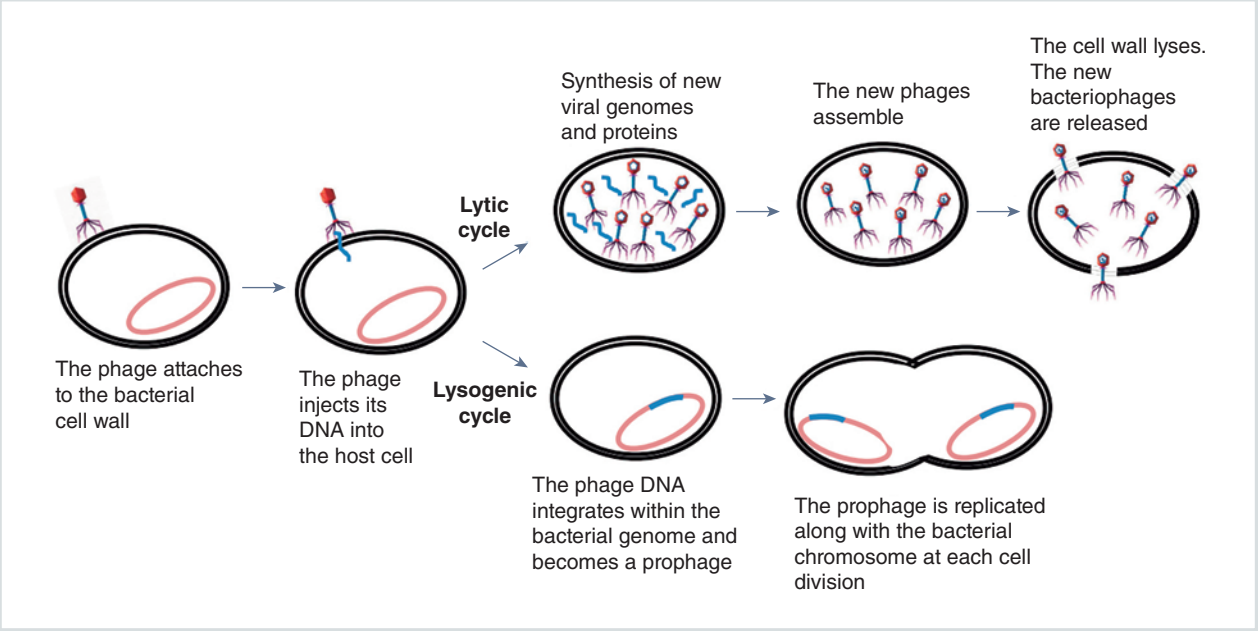

Figure 1. Overview of the main differences between the lytic and the lysogenic bacteriophage life cycle *(adapted from Kakasis and Panitsa, 2009)*[1].

organ dysfunction. Infection with carbapenem-resistant *Enterobacteriaceae* can range from 1 to 18% among solid organ transplant recipients and 16-24% in stem cell transplant recipients. Addressing MDROs among lung patients is especially important: cystic patients waiting for LTx present high rates of MDR *Pseudomonas* and *Mycobacterium abscessus*, while many centers list the presence of *Burkholderia cepacia complex* as a contraindication to lung transplant. Thus, the presence of MDROs before the LTx is associated with worse illness, and increased mortality and morbidity while awaiting transplant. Treating these infections can be very challenging, and recently, phages have been used to attempt to treat the most resistant and complex cases and, in some cases, allow the challenge of the LTx procedure to diminish and improve LTx outcomes.

APPROACH TO PHAGE THERAPY IN LUNG TRANSPLANT

Lung transplant patients are frequently colonized with MDROs, especially cystic fibrosis patients or those transplanted because of bronchiectasis. Besides, frequent lung infectious episodes are well-known risk factors for chronic lung allograft dysfunction. Many times, these episodes are treated with long antibiotic therapy and strategies for decreased immunosuppression. Non-tuberculous mycobacteria infections such as *Mycobacterium Abscessus* or *Burkholderia cepacia complex* are associated with high mortality. Phages therapy could represent a treatment alternative joined to antibiotic therapy. We find few cases in the literature of LTx patients where phage therapy has been treated. There are published few cases of LTx treated with phages for *Pseudomonas*

Table 1. Summary of recent phage therapy cases in LTx recipients and pretransplant patients

Patient	Organism	Clinical syndrome	Phage treatment	Ref
Lung transplant	*Pseudomonas aeruginosa*	Pneumonia	Phage cocktail (iv and nebulized) Outcome success AE: none	4 5,6
Lung transplant	*Pseudomonas aeruginosa*	Pneumonia	Phage cocktail iv Outcome success AE: none	5
Lung transplant	*Burkholderia dolosa*	Pneumonia	Phage single iv Outcome failed AE: none	5,6
Lung transplant	*Achromobacter xylooxidans*	Pneumonia	Phage cocktail nebulized Outcome success AE: none	7
Lung transplant	*Mycobacterium abscessus subsp massiliense*	Pneumonia/ disseminated	Phage cocktail with one engineered phage (iv and topical) Outcome success AE: none	8
Lung transplant	*Pseudomonas aeruginosa*	Wound infection	Phage cocktail Otucome success AE: none	9
Cystic fibrosis	*Achromobacter spp*	Pneumonia	Phage single Outcome Success AE: none	10
Cystic fibrosis	*Achromobacter xylosidans*	Pneumonia	Phage cocktail iv and po Outcome success AE: none mentioned	11
Pre-lung transplant	*Pseudomonas aeruginosa*	Pneumonia	Phage cocktail iv Outcome success (transplant) AE: none	12

AE: adverse events.

*aeruginos*a infection, *Achromobacter spp*, *Burkholderia dolora* and *Mycobacterium* infections or specifically *Mycobacterium abscessus*. Favorable responses were observed in more than half of the patients, including complete resolution of some infections, and successful lung transplantation after cultures negatives.

One case report described the development of phage resistance in the setting of recurrent *Pseudomonas* infections, and in this case, the subsequent course of phage therapy was adapted with new phage active against the new pathogen. In addition, in two lung transplant recipients with *P. aeruginosa* pneumonia, the use of phage therapy was associated with a change in antimicrobial resistance patterns of subsequent *P. aeruginosa* isolates. In these cases, subsequent bacterial isolates were susceptible to a wider selection of antibiotics that were not previously applicable[4-6]. Thus a major issue concerning the safety and efficacy of phage therapy is the immunological response towards bacteriophages, which comprises the adaptive and the innate immune responses[1]. No major life-threatening immune reactions have been described with this therapy; however, immune system reactions can neutralize

phages and thus reduce their antibacterial efficacy. On the other hand, phages can be relevant in the treatment of biofilms[5,13]. Indeed, some bacteria can create biofilms in which antibiotics are inefficacious, even against genetically sensitive targets. Phages have been reported to disrupt biofilms and to kill bacteria present inside the structure, as a result of active penetration into the bacterial biofilm[6]. Rubalsky et al.[14] described their experience treating a postoperative infection by *P. aeruginosa* of the sternal wound after LTx. A debridement, antibiotics and local phage application were performed in order to treat the patient. This resulted in a clinical and microbiological cure of the infection. In this case, fibrin scaffolds were capable of protecting the bacteriophages from degradation and gave a sustained release of high titre phage over 11 days. Such topical applications with prolonged release phage may offer hope for complex, non-healing surgical wounds.

The first report using engineered phage was published in 2019[8]. A case of *M. abscessus* with pulmonary and disseminated infection was treated with salvage phage therapy with concurrent antibiotics yielded a clinical improvement. Phage therapy for a mycobacterial disease is complicated by the prevalence of lysogenic phages and the slow growth rate of the mycobacteria. Recently, the same group has published their experience using phage therapy for *Mycobacterium* infections in 20 patients[15]. Two of them were LTx patients from the Vall d'Hebron hospital. No adverse reactions attributed to the therapy were seen in any patient regardless of the pathogen, phages administered or the route of delivery. Favorable clinical or microbiological responses were observed in 11 patients. In this paper, the authors identified neutralizing antibodies after initiation of phage delivery intravenously in eight patients, potentially contributing to the lack of treatment response in four cases but they were not consistently associated with unfavorable responses in others. No phage resistance was observed in any of these 11 patients.

PHAGE THERAPY SAFETY AND IMMUNE RESPONSE TO PHAGE THERAPY

Phages are generally considered safe, based on their abundant nature and our constant exposure to them in the environment, and because they have been used extensively in some parts of the world with no reports of harmful events. Despite this optimistic point of view, the safety of phage therapy must be verified by modern scientific experiments[16]. The therapeutic use of phages for treating drug-resistant bacterial infections has received recent attention but the types of infections and pathogens deemed suitable, routes, dosage and frequency of administration, interactions with antibiotics, and pharmacokinetics remain unclear. The safety concerns of phage therapy include the possible impact of bacteriophages on body tissues and non-target microbiota. Bacteriophages can modify their bacterial targets by expressing virulence genes or by transducing DNA between bacteria. Phages also may induce immunological reactions[17]. Thus another issue to be addressed is the immunological response towards bacteriophages, which comprises the adaptative and the innate immune responses[1]. Immune system reactions can neutralize phages and thus reduce their antibacterial efficacy. The production of

anti-phage antibodies in patients receiving phage therapy has resulted in phage inactivation[1], although a high rate of phage inactivation does not necessarily mean treatment failure. Another issue is the route of administration that seems to be important for the impact of antiphage activity of sera, where the local route of administration shows higher anti-phage activity than the oral[18]. Dedrick et al.[15], in 20 patients treated with phages, identified neutralizing antibodies in serum after initiation of phage delivery intravenously in eight of them, potentially contributing to lack of treatment response in four cases. This issue is of paramount importance among LTx patients. Immunocompromised patients may tolerate extended phage administration without antibody-mediated neutralization maybe due to immunosuppression. However, little is known about intracellular penetration or uptake of phages, particularly by macrophages, where most replicating mycobacteria are found.

Therefore phages, like other viruses, can be recognized by the immune system as invading elements and be rapidly eliminated from the circulatory system by the reticuloendothelial system or innate immunity before they reach infected tissues, thereby decreasing their effectiveness, especially in repeated and prolonged administrations. However, the development of this type of antibodies is not a significant problem in the initial phase of treatment of acute infections since the phage kinetics is much faster than the production of these antibodies. However, its presence can be an obstacle if subsequent administrations or chronic treatments are required. Thus, another issue to be addressed is how long the treatment should be maintained.

CONCLUSIONS

The potential of bacteriophage applications in the fight against bacterial pathogens can be expanded beyond their utilization as naturally occurring phages and recent studies have proved its safety. There is increasing evidence from case reports and case series that demonstrates the potential for the success of phage therapeutics in transplant candidates and recipients with MDROs or antibiotic recalcitrant infections. There are few ongoing clinical trials assessing the use of phage therapy in non-immunocompromised patients[2], though much work remains to be done.

Phages are already present in large amounts in our body forming the phageome[13], knowing the natural relationship between them and human cells shows that despite millions of years of intimate cohabitation, phages are not able to infect human cells. One of the interesting points in phage therapy is its limited cost. Phages are indeed easy to isolate and cheap and quick to produce. Furthermore, phages replicate only in the presence of their specific host, meaning that they proliferate according to the bacterial (infectious) load after administration.

In conclusion, it seems now that the success of phage therapy depends on part on the acceptance by the general public, as well as the knowledge of the professionals who see this profile of patients. Thus, the creation of adequate regulatory, oversight and safety protocols supervising their future utilization within the framework of carefully carried clinical trials shall help in this aim.

Despite the promising reports of phage therapy in certain parts of the world, more modern

randomized double-blind controlled clinical trials are needed to prove the safety and efficacy of phage therapy. Issues like bacteriophage choice, isolation, preparation, purification, storage and pharmacology should be addressed individually and researched in depth. Bacteriophages are potentially suitable alternatives for treatment of bacterial infections in the era of rising antimicrobial resistance.

REFERENCES

1. Kakasis A, Panitsa G. Bacteriophage therapy as an alternative treatment for human infections. A comprehensive review. Int J of Antimicrob Agents. 2009;53:16-21.
2. Nicholls P, Aslam S. Role of bacteriophage therapy for resistant infections in transplant recipients. Curr Opin Organ Transplant. 2022;27:546-53.
3. Suh GA, Lodise TP, Tamma PD et al. Considerations for the use of phage therapy in clinical practice. Antimicrob Agents Chemother. 2022;66:e0207121.
4. Aslam S, Lampley E, Wooten D et al. Lessons learned from the first 10 consecutive cases of intravenous bacteriophage therapy to treat multidrug-resistant bacterial infections at a single center in the United States. Open Forum Infect Dis. 2020;7:ofaa389.
5. Aslam S, Cortwright A, Koval C. Early clinical experience of bacteriophage therapy in 3 lung transplant recipients. Am J Transplant. 2019;19:2631-39.
6. Courtwright A, Koval C, Leheman S et al. Safety and efficacy of bacteriophage therapy in lung transplant candidates and recipients. J Heart Lung Transplant. 2019;38:S11-S12.
7. Lebeaux D, Merabishvili M, Caudron E, et al. A case of phage therapy against pandrug-resistant Achromobacter xylosoxidans in a 12-year-old lung transplanted cystic fibrosis patient. Viruses. 2021;13:60.
8. Dedrick R, Guerrero-Bustamente C, Garlena R. Engineered bacteriophages for treatment of a patient with a disseminated drug resistant Mycobacterium abscessus. Nat Med. 2019;25:730-3.
9. Rubalskii E, Ruemke S, Salmoukas C et al. Bacteriophage therapy for critical infections related to cardiothoracic surgery. Antibiotics (Basel). 2020;9:232.
10. Gainey AB, Burch AK, Brownstein MJ et al. Combining bacteriophages with cefiderocol and meropenem/vaborbactam to treat a pan-drug resistant Achromobacter species infection in a pediatric cystic fibrosis patient. Pediatr Pulmonol. 2020;55:2990-4.
11. Hoyle N, Zhvaniya P, Balarjishvili N et al. Phage therapy agasint Achromoctacter xylosoxidans lung infection in a patient with cystic fibrosis: a case report. Res Microbiol. 2018;169:540-2.
12. Clokie MR, Millard AD, Letarov AV, Heaphy S. Phages in nature. Bacteriophage. 2011;1:31-45.
13. Domingo-Calap P, Geroge P, Bahram S. Back to the future: bacteriophages as promising therapeutic tools. HLA. 2016;87:133-40.
14. Rubalskii E, Ruemke S, Salmoukas C et al. Fibrin glue as a local drug-delivery systema for bacteriophage PA5. Sci Rep. 2019;9:2091.
15. Dedrick R, Smith B, Cristinziano M et al. Phage therapy of Mycobacterium infections: Compassionate-use of Phages in Twenty Patients with Drug-Resistant Mycobacterial Disease. Clin Infect Dis. 2023;76:103-12.
16. Henein A. What are the limitations on the wider therapeutic use of phage?. Bacteriophage. 2013;3:e24872.
17. Abedon ST, Kuhl SJ, Blasdel BG et al. Phage treatment of human infections. Bacteriophage. 2011;1:127-9.
18. Lusiak-Szelachowska M, Zaczek M, Weber-Dabrowska B et al. Phage neutralization by sera of patients receiving phage therapy. Viral Immunol. 2014;27:295-304.