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Bacteriophage Therapy for Pan-Drug-Resistant *Pseudomonas aeruginosa* in Two Persons With Cystic Fibrosis

Andrea Hahn, MD, MS[1,2,3], Iman Sami, MD[1,3], Hollis Chaney, MD[1,3], Anastassios C. Koumbourlis, MD, MPH[1,3], Coralee Del Valle Mojica, MD, MPH[4], Claire Cochrane, BA[5], Benjamin K. Chan, PhD[5], and Jonathan L. Koff, MD[5]

Abstract

Cystic fibrosis (CF) is an important monogenic disease that affects more than 70 000 people worldwide. Defects of the CF transmembrane conductance regulator gene lead to dehydrated viscous secretions that result in chronic bacterial colonization. This leads to frequent recurrent lung infections called pulmonary exacerbations, lung inflammation, and resulting structural lung damage called bronchiectasis. *Pseudomonas aeruginosa* in particular is a common pathogen in persons with CF associated with increased pulmonary exacerbations, long-term lung function decline, and reduced survival. In addition, *P. aeruginosa* commonly develops antibiotic resistance and forms biofilms, making it difficult to treat. Here, we report the details of two patients with CF with pan-drug-resistant *P. aeruginosa* who were treated with a novel therapeutic strategy, bacteriophages. These cases highlight the need for further research and development of this treatment modality, including pediatric clinical trials.

Keywords

cystic fibrosis, *Pseudomonas* aeruginosa, drug resistance, microbial, bacteriophages, pediatrics

Introduction

Cystic fibrosis (CF) is an autosomal recessive disease caused by dysfunction of the CF transmembrane conductance regulator (CFTR) gene that affects more than 30 000 people in the United States.[1] CF transmembrane conductance regulator mutations cause a defect in chloride channel function that results in dehydrated secretions in the airways, which causes progressive lung damage that results in declines in lung function over time from recurrent infections and inflammation.[2,3] There is increased recognition that CFTR dysfunction negatively affects innate immune function, which leads to activation of proinflammatory signaling at baseline and exaggerated responses in the presence of bacteria.[4]

Pseudomonas aeruginosa is an important chronic bacterial pathogen in persons with CF which is associated with increased pulmonary exacerbations, long-term lung function decline, and reduced survival.[5-7] Antibiotic resistance is common,[8-10] with up to 17% of *P. aeruginosa* strains reported as multidrug resistant (MDR).[11] One mechanism for resistance is biofilms created by *P. aeruginosa* that protect this pathogen from host immune responses and antibiotics.[12] For example, the presence of mucoid strains is accompanied by a poor likelihood of antibiotic eradication during therapy, lung function decline, and increased lung damage.[7,13] New strategies are urgently needed to better treat *P. aeruginosa*, and MDR *P. aeruginosa*, infections in persons with CF.

Bacteriophage (phage) therapy, which uses lytic viruses as antimicrobials, has the potential to overcome the limitations of antibiotics to treat *P. aeruginosa* infections. Resistance mechanisms that limit the activity of antibiotics may not be as likely to limit phages, which includes bacteria within biofilms.[14-16] Over the past decade, adult cases from Western Europe and the United States have been reported, most following emergency-use authorization from

[1]Children's National Hospital, Washington, DC, USA
[2]Children's National Research Institute, Washington, DC, USA
[3]George Washington University School of Medicine & Health Sciences, Washington, DC, USA
[4]Children's Hospital of Philadelphia, Philadelphia, PA, USA
[5]Yale University, New Haven, CT, USA

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Corresponding Author:
Andrea Hahn, MD, MS, Division of Infectious Diseases, Children's National Hospital, 111 Michigan Avenue North West, Floor 3.5 Suite 100, Washington, DC 20010, USA.
Email: alhahn@childrensnational.org

the European Medicines Agency (EMA) or the Food and Drug Administration (FDA).[17] Multiple CF-associated infections treated with this approach were associated with a rapid decrease in sputum bacterial density and with short-term improvement in lung function for some individuals.[18,19] Here, we report two cases of patients with CF, one being a young adult and the other a school-aged child, with pan-resistant *P. aeruginosa* lung infection who were treated at our institution with personalized inhaled phage therapy.

Case Report

A 6-year-old African American female (Pt1) and 26-year-old Hispanic female (Pt2) patients, both with CF, respiratory cultures positive for MDR *P. aeruginosa*, and advanced lung disease, were treated with personalized inhaled phage therapy directed against each of their respective *P. aeruginosa* lung infections at Children's National Hospital (Washington, DC). Pt1 was not eligible for CFTR modulator therapy; Pt2 was started on elexacaftor-tezacaftor-ivacaftor but did not have a significant response to therapy. Pt1 had received more than 30 days of antibiotic therapy and remained in the pediatric intensive care unit (PICU) where she required continuous noninvasive ventilation and oxygen supplementation. Pt2 had a significant decline in clinical stability over the preceding 12 months, requiring 5 hospitalizations for treatment of pulmonary exacerbations in addition to multiple outpatient courses of intravenous (IV) and oral antibiotics.

Spontaneously expectorated sputum samples from both patients were collected and sent to the Center for Phage Biology & Therapy at Yale University (New Haven, CT). Multiple *P. aeruginosa* isolates were identified from these respiratory cultures and were tested against a well-characterized phage library with known lytic activity against *P. aeruginosa*. Three phages were identified for Pt1, and two phages were identified for Pt2. Based on prior experience,[19,20] a single sequential inhaled phage therapy protocol was reviewed and approved via single-patient investigational new drug (SPIND) by the FDA and our local institutional review board (IRB). Pt1 was treated in the PICU. She received phage INF (1×10^{10} plaque-forming units [PFU] in 3 mL phosphate-buffered saline [PBS] with 10 mM magnesium sulfate), inhaled over 5 minutes twice daily for 7 days. Phage was administered after airway clearance and pretreatment with albuterol. Phage treatment was also administered concurrently with IV meropenem and IV ciprofloxacin, immediately following a 4-week course of IV meropenem and IV colistin. Pt2's treatment was initiated in the hospital on the regular inpatient floor. She received the same dose of inhaled phage INF twice daily for 2 days, administered after airway clearance and pretreatment with albuterol. She was discharged home to complete a 7-day total course of phage INF inhaled once daily followed by a 7-day course of phage pB (1×10^{10} PFU in 3 mL of PBS with 10 mM magnesium sulfate), inhaled once daily over 5 minutes. This was given concurrently with IV meropenem immediately following a 2-week course of IV cefiderocol and IV tobramycin. All phages used are thought to target lipopolysaccharides (LPS) on *P. aeruginosa* and thus are predicted to improve airway inflammation following treatment as phage will exert evolutionary pressure on surviving bacteria to downregulate LPS production, similar to what has been observed using phage to target *P. aeruginosa* efflux pumps.[19,21]

Review of symptoms and respiratory support: Prior to phage administration, Pt1 was on continuous bilevel positive airway pressure (BiPAP) 18/9 with 40% FiO_2. Her percent predicted forced expiratory volume in 1 second (ppFEV1) was not known as she was too young for reliable spirometry testing. After phage administration, respiratory support was weaned to high-flow nasal cannula 10 to 12 L during the day and BiPAP (18/9 FiO_2 40%) continued at night. Sputum production decreased, and her energy level improved. Prior to phage administration, Pt2 was requiring 2 L of nasal cannula O_2 during the day, whereas at baseline, she only required 2 L of oxygen supplementation overnight. She also had significant dyspnea with exertion in the setting of severe lung disease (ppFEV1 19%). After 1 week of phage treatment, Pt2 reported improved energy, less dyspnea, and decreased productive cough. Her oxygen requirements had also returned to baseline levels. After 2 weeks of treatment, however, Pt2 reported continued dyspnea and productive cough. Repeat PFTs showed a ppFEV1 of 20%. Neither patient experienced bronchospasm while receiving the inhaled phage therapy.

Laboratory findings: At baseline, Pt1 had a white blood cell count (WBC) of 6.35 K/mL, hemoglobin (Hgb) of 10.5 g/dL, platelet (Plt) count of 274 K/mL, serum creatinine (Cr) of 0.2 mg/dL, alanine aminotransferase (ALT) of 50 U/L, aspartate aminotransferase (AST) of 34 U/L, and C-reactive protein (CRP) of 1.22 mg/dL. Her blood counts, liver function tests, and CRP remained stable throughout the treatment course through 2 months of follow-up. At 3 months after treatment, Pt1 had an increased AST at 134. At baseline, Pt2 had a WBC of 12.7 K/mL, Hgb of 10.2 g/dL, Plt of 530 K/mL, serum Cr of 0.49 mg/dL, ALT of 24 U/L, AST of 15 U/L, and CRP of 3.08 mg/dL. Her CRP improved to 2.20 mg/dL after phage treatment. Her blood counts and LFTs remained stable throughout the treatment course and for 3 months of follow-up.

Microbiology data: Prior to phage treatment, Pt1 had pan-resistant *P. aeruginosa* in sputum culture; this included resistance to the newer antibiotics such as ceftolozane-tazobactam, ceftazidime-avibactam, and meropenem-vaborbactam. A respiratory culture from day 4 of phage therapy grew 1+ normal respiratory flora (NRF), with no *P. aeruginosa* detected. A respiratory culture sent on day 7 of treatment grew 3+ NRF and 4+ *P. aeruginosa*, which was again pan-resistant. Pt2's *P. aeruginosa* from respiratory cultures were consistently pan-resistant, including to the newer antibiotics ceftolozane-tazobactam and ceftazidime-avibactam.

Respiratory cultures were obtained on days 4, 8, 11, and 14 of treatment. On day 4, the culture grew 2+ mucoid and 1+ rough *P. aeruginosa* and 3+ NRF with the rough strain being pan-resistant. On day 8, the culture grew 1+ mucoid *P. aeruginosa* and 1+ NRF, with the mucoid strain being susceptible to meropenem. On day 11, the culture grew 4+ mucoid and 4+ rough *P. aeruginosa*, with both strains being pan-resistant. On day 14, the culture grew 1+ mucoid and 4+ rough *P. aeruginosa* and 2+ NRF, with the *P. aeruginosa* mucoid strain showing susceptibility to several beta-lactams and the rough strain being pan-resistant.

Outcomes: Pt1 was transferred to another institution for evaluation for lung transplant after completion of the first phage therapy; it was deemed more important by the care team to facilitate this transfer than remain at our institution for completion of treatment with the two additional phages. While she was listed, a match was not found, and she passed away 6 months after phage therapy from respiratory failure. One week after completing the phage therapy, Pt2 was found to have a left apical pneumothorax and required chest tube placement and hospitalization. This was considered a grade 3 serious adverse event and was reported on the same day to the IRB and FDA. Pt2 was evaluated as an outpatient for a lung transplant at another institution. She was listed and received a lung transplant 5 months after the completion of the phage therapy.

Discussion

In these two cases of personalized inhaled phage therapy, both patients had symptomatic improvement while receiving phage INF. Inhaled phage was tolerated without bronchospasm or an increase in LFTs during the treatment course. However, both patients had quick regrowth of *P. aeruginosa* after the completion of treatment, and neither had a significant change in their disease trajectory. However, it is important to note that both patients had end-stage lung disease, and so it was unlikely 7 to 14 days of treatment with a targeted phage against an inflammatory virulence factor would have been able to lead to recovery from their condition. Importantly, Pt2 also had a grade 3 serious adverse event after the completion of therapy, but it is more likely this event was attributable to her underlying disease process as opposed to the phage received.

Because phage treatment is not yet approved by the US FDA and neither patient was eligible for participation in an ongoing clinical trial at their time of need, we pursued SPIND applications.[22] As Pt1 was critically ill, we applied for an emergency IND which meant the total process (including emergency IRB approval) took less than 3 weeks. For Pt2, as her problem was more chronic, the review process from the FDA and IRB was different; it took 4.5 months to set up the treatment. This process severely limits access to treatment albeit due to the lack of robust clinical trial data to support its safety and efficacy. However, it should be noted that there are limited published data that report adverse events associated with phage treatment, which is generally considered safe.[22] In addition, SPIND approval limits access to patients with the most severe disease (i.e., compassionate use),[23] which is likely outside the window of time where it might be more likely to impact a patient's eventual outcomes.

Over the past decade, numerous cases from Western Europe and the United States have been reported, most following emergency use authorization from the EMA or the FDA for single patient treatment.[17,22] However, the design of clinical trials for regulatory approval remains challenging. Phages use specific cell-surface receptors that lead to lysis of the targeted bacteria.[24] This mechanism of action inevitably leads to phage resistance in bacteria.[21] Thus, whether a clinical trial would be best designed by focusing on personalized phage therapy (i.e., selecting phages based on their susceptibility to that patient's bacterial isolate) or using a cocktail of predefined phages remains debated.[21,22,24,25] Nonetheless, well-designed prospective, controlled studies are necessary to determine the safety and efficacy of phage treatment before regulatory approval could be gained.[25] Such trials have been initiated in adults with CF, but similar studies in pediatric patients are lacking.

The potential significance of phage therapy in children with CF is remarkable. We have recently seen a decline in pulmonary exacerbations and hospitalizations with the introduction of CFTR modulators with elexacaftor-tezacaftor-ivacaftor being the most effective ones to date.[26,27] Based on earlier experience with the use of ivacaftor in persons with G551D mutations, there is an anticipated plateau to these effects, and 25% to 30% of persons with CF on modulators will still require hospitalizations for pulmonary exacerbations.[28] In addition, approximately 10% of persons with CF do not have a genotype that can be targeted by CFTR modulators. Furthermore, there are racial and ethnic differences in eligibility. While 92.4% of non-Hispanic whites are eligible based on CFTR mutations alone, this is significantly reduced for black (69.7%) and Hispanic (75.6%) persons with CF.[29] Thus, investigating the use of phage now will prepare for the need to have adjunctive and alternative therapies to treat those who continue to experience persistent and difficult-to-treat *P. aeruginosa* infections, which could also help to address racial and ethnic health disparities.

Conclusion

Phages have the potential to be used as direct therapy to fight *P. aeruginosa* lung infection, to augment antibiotic treatment of pulmonary exacerbations, or potentially eradicate initial pulmonary colonization in young children with CF. They could also be used to fight other hard-to-treat bacterial pathogens and in children and adults with non-CF bronchiectasis and chronic pulmonary infections caused by *P. aeruginosa*. Well-designed prospective, controlled studies are critically

needed to determine the safety and efficacy of phage treatment, especially in children with CF. Until that time, these therapies to treat multidrug-resistant infections will remain out of reach for many.

Author Contributions

Patient care and data collection were conducted by AH, IS, HC, ACK, and CDVM. Analysis of sputum samples to determine phage susceptibility was conducted by CC, BKC, and JLK. Phage was donated by the Center for Phage Biology & Therapy at Yale University (BKC and JLK). The original manuscript was written by AH. All authors reviewed and edited the manuscript and approved the final version as written.

Declaration of Conflicting Interests

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Ethics Approval

Our institution does not require ethical approval for reporting individual cases or case series. IRB approval was obtained at Children's National Hospital for administration of phage therapy and subsequent reporting to the FDA (Pro15390 and Pro16170).

Informed Consent

Parental permission and informed consent were obtained prior to administration of phage therapy. These consent documents included specific language granting permission for publication of de-identified data in scientific journals.

ORCID iD

Andrea Hahn https://orcid.org/0000-0002-5117-0080

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