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Toward Modern Inhalational Bacteriophage Therapy: Nebulization of Bacteriophages of *Burkholderia cepacia* Complex

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Abstract

Antibiotic-resistant bacterial infections have renewed interest in finding substitute methods of treatment. The purpose of the present *in vitro* study was to investigate the possibility of respiratory delivery of a *Burkholderia cepacia* complex (BCC) bacteriophage by nebulized aerosol administration. Bacteriophages in isotonic saline were aerosolized with Pari LC star and eFlow nebulizers, at titers with mean value (standard deviation) of 2.15×10^8 (1.63×10^8) plaque-forming unit (PFU)/mL in 2.5-mL nebulizer fills. The breathing pattern of an adult was simulated using a pulmonary waveform generator. During breath simulation, the size distributions of the nebulized aerosol were measured using phase doppler anemometry (PDA). Efficiency of nebulizer delivery was subsequently determined by collection of aerosol on low resistance filters and measurement of bacteriophage titers. These filter titers were used as input data to a mathematical lung deposition model to predict regional deposition of bacteriophages in the lung and initial bacteriophage titers in the liquid surface layer of each conducting airway generation. The results suggest that BCC bacteriophages can be nebulized successfully within a reasonable delivery time and predicted titers in the lung indicate that this method may hold potential for treatment of bacterial lung infections common among cystic fibrosis patients.

Key words: bacteriophage therapy, cystic fibrosis, lung deposition simulation, aerosol, nebulizer

Introduction

Cystic fibrosis (CF) is the most common fatal genetic disease among the Caucasian population. Inhaled microbes pose a significant threat to these patients as they exhibit impaired pulmonary mucociliary clearance. This deficiency makes CF patients susceptible to repeated and prolonged infections with a relatively narrow spectrum of opportunistic bacterial pathogens.[1] Chronic microbial colonization leading to debilitating pulmonary infection is the major cause of morbidity and mortality in CF patients.[1] The longest average life span of a CF patient is in Denmark, and is approximately 50 years of age. In North America, the average life span of a CF patient is shorter, being in the mid to late thirties.[2] The *Burkholderia cepacia* complex (BCC) is a group of opportunistic pathogens that have considerable impact on the quality of life and mortality of CF patients. Pulmonary infections with the BCC can result in variable clinical outcomes, one of which is "cepacia syndrome": a rapidly progressive necrotizing pneumonia and sepsis that occurs in up to 20% of CF patients at particular CF clinics.[3] Both direct transmission (e.g., interpersonal contact) and indirect transmission (via shared equipment or by third parties) play a potential role in spreading crossinfection among CF patients.[4] Evidence shows that BCC bacteria can be transmitted through social contact among siblings[5] and summer educational camps,[6] leading epidemiologists to employ segregation as a means to control possible outbreaks.[4] Furthermore, BCC bacteria are highly resistant to antibiotics, and even aggressive antibiotic therapy often does not result in improved clinical prognosis or a reduction in bacterial numbers.[1,7] Increasing resistance to common antibiotic treatments is an escalating concern, and opportunities for developing new effective chemical antibiotic treatments in the future may be limited.[8]

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As an alternative strategy toward finding substitute methods of treatment for antibiotic resistant bacteria such as the BCC, bacteriophages (or phages) have been suggested.[9] In several recent examples, the use of bacteriophages has shown therapeutic promise in controlling bacterial infections.[9,10] Bacteriophages—meaning "bacteria eaters"—are viruses that specifically attack and kill different types of bacteria. Because bacteria are their "natural" target cells, efforts have been made to enlist bacteriophages to fight bacterial infections of higher organisms.[11] Bacteriophages are harmless to mammalian cells and even to other nontargeted bacteria, and thus, they are more specific than broad-spectrum antibiotics that will kill beneficial bacteria in the intestinal tract along with the infectious bacteria.[12] One of the advantages of treatment with phages is their ability to exponentially increase in number over time, provided there are sufficient host cells in which to multiply.[13] In addition, based on population numbers, the phage mutation frequency is significantly higher than the mutation frequency of bacteria, allowing a ready response to phage resistant bacteria.[9] Finally, phage therapy is also expected to be relatively cost efficient, which, in addition to the aforementioned advantages, makes phage therapy an excellent candidate strategy among novel alternatives to antibiotics.

It should be noted that there are also a number of disadvantages attributed to bacteriophages. The specificity of bacteriophages requires production of a specific phage or phage cocktail for each type of bacterium. The broad range of pathogenic bacterial species, each needing different phages, makes the mass application of phage therapy a challenge. Therefore, it is reasonable to start with and focus on phage therapy for cases where alternative methods of treatment are most urgently required.[14] Moreover, because phages are host specific, identification of bacteria should be done to perform a suitable phage therapy. On the contrary, antibiotics are often used to treat unidentified bacterial infections. Storing and handling of phages can also be an issue, depending on the type of phages.[15] While some phages are very stable and can be stored in nearly any conditions, others are fragile and need special attention throughout their handling.[14,15]

Despite the disadvantages of bacteriophages, many examples exist where this method of therapy has been applied to human beings using nonaerosol delivery routes. Numerous research studies were published on the therapeutic use of phages in the 1930s and early 1940s.[16] However, phage biology was not well understood at the time, resulting in inconsistent data. Moreover, phages were improperly tested against bacteria insensitive to those particular phages or even against diseases that were not caused by bacteria; such missteps, along with the discovery and mass production of antibiotics, led to a reduced interest in bacteriophage therapy.[11] However, modern concerns regarding the increasing prevalence of antibiotic resistance have renewed interest in phage therapy. For example, an *in vivo* study in 2003 demonstrated that bacteriophages can serve as an efficient treatment for antibiotic resistant septicemia in humans.[17] Still, there is a need for more carefully controlled trials in defined clinical settings to document the efficacy of phage therapy in the treatment of bacterial infections.[18] In this regard, determining the best route of administration is a first step in developing such trials.

For bacteriophages, different routes of administration have been tested based on the origin and severity of infections.[18] Inhalation therapy has been considered to be favorable for certain respiratory infections because the aerosol is delivered directly to the site of infection, which accelerates the action of the drug. Moreover, less drug substance is needed and possible side effects are reduced.[19,20] Treatment costs and patient quality of life could also be considered as important reasons for the usage of nebulizers for drug delivery.[21] Aerosolized antibiotic agents are commonly used for patients chronically infected with *Pseudomonas aeruginosa*.[22,23] Twelve trials of nebulized antibacterials for CF patients showed that such treatment reduces the number of hospital admissions while also improving lung function.[24] It has been shown that the penetration of an aerosolized solution in the lung is more efficient when aerosolization is preceded by physiotherapy and a bronchodilator.[25] Furthermore, nebulization should be optimized by controlling the size of aerosols so that they reach the smaller bronchioles, which are commonly the sites of pulmonary infection in CF patients.[24] In addition to particle size, which is usually in the range of 1–5 μm for effective CF treatment, osmolarity is important for several reasons. First, the aerodynamics of hygroscopic therapeutic aerosols is affected by exchange of water vapor in the humid environment of the lung.[26] Second, bronchial secretions are iso-osmolar. As a result, adding large amounts of hypotonic or hypertonic solutions causes mucosal irritation.[27] Even after optimization, nebulized antibiotics are effective only in the short term, and CF patients may begin to show resistance to antibiotics over longer courses of treatment.

As an alternative to antibiotic therapy, many phage therapy trials have been performed, mostly with traditional nonaerosol delivery routes. However, a number of these have used poorly characterized commercially produced phages that did not contain viable phages or contained phages active against only a few of the targeted pathogens, leading to uncertainty regarding the effectiveness of phage therapy and perhaps less enthusiasm towards its adoption.[11,16] Contributing to the low prevalence of bacteriophage therapy has been the lack of technology to produce large quantities of purified stable phages. However, large bacteriophage preparations can now be produced endotoxin free, which reduces concerns about possible circulatory shock due to large quantities of endotoxin.[13] Finally, the isolation of bacteriophages of the BCC and confirmation of their lytic activity has recently been accomplished.[28]

There have been previous attempts to nebulize bacteriophages for veterinary medicine, human treatment, and agricultural applications. However, there are no quantitative data regarding the titer used, the inhaled number of bacteriophages, the nebulizer type, or aerosol properties.[29–31] Phages were delivered to chickens by an aerosol spray instead of providing them in drinking water, and it was concluded that the former method was a promising way of administration of the phages.[32,33] Inhalational treatment of phages has been performed on children and adults,[29,30] involving antibiotic-resistant streptocci and staphylococci bacterial infections, and deemed successful with a large percentage of patients showing recovery. With the substantial advances that have occurred in aerosol delivery since these latter two human studies were done, the goal of the present

study was to investigate *in vitro* aerosol administration of BCC endotoxin-free bacteriophages with two representative modern nebulizers: the LC star and eFlow. Such work is the first step toward consideration of aerosol phage therapy as an alternative to nebulized antibiotic treatment of BCC respiratory infections.

Materials and Methods

Bacteriophage preparation

Phage KS4-M was propagated on BCC strain K56-2[34] using standard liquid propagation techniques. Bacteria were grown at 30°C in $\frac{1}{2}$ strength Luria-Bertani medium. Prior to aerosolization, phage preparations were filter sterilized (pore size 0.22 μm) and passed through a Detoxi-Gel affinity pak™ Prepacked column (Pierce Biotechnology, Rockford, IL) to remove endotoxin from solution. Following aerosolization suspension media (SM) (50 mM Tris/HCl pH 7.5, 100 mM NaCl, 10 mM $MgSO_4$, and 0.01% gelatin solution) was used to collect phage from the filter. The phage were then quantified by plating serial dilutions with BCC strain K56-2 using the soft overlay agar method for the detection plaques.

Nebulization and inhaled fraction measurement

Nebulization of BCC lytic bacteriophages was performed with two types of nebulizers each in triplicate, with a total of six tests for each bacteriophage titer. Pari LC star jet nebulizers were used with a Proneb Turbo Compressor model 38B0201 at a flow of 3.6 L/min[35] (Pari Pharma GmbH, Starnberg, Germany). This type of nebulizer is commonly used in CF therapy and has demonstrated good performance in previous nebulization studies.[36–40] The second tested nebulizer in this study was the recently developed eFlow electronic nebulizer (Pari Pharma GmbH). The latter nebulizer has a shorter nebulization time and its gentle aerosol generation has demonstrated the potential to exert less shear on the fluid in the aerosolization of large molecules.[41]

For each test, the nebulizer was filled with 2.5 mL of isotonic (osmolarity of 282–290 mOsm) bacteriophage suspension at an ambient temperature of 21°C. The bacteriophage titer had a mean value (standard deviation) of 2.15×10^8 (1.63×10^8) PFU/mL. To obtain an isotonic suspension of phages, the derived column eluates were supplemented with 5.25 mg of sodium chloride/ml. In addition, to examine the effect of osmolarity, the nebulizer was filled once with a hypotonic (105 mOsm) suspension of bacteriophages with titer of 10^8 PFU/mL. The run time was measured for each test. Nebulization was stopped when there was a pause of more than 15 sec without aerosol production.

The size distribution of nebulizer aerosols can depend on the patient's breathing pattern;[35] thus, to simulate the breathing pattern, a computer controlled piston-type breathing machine (Pulmonary Waveform Generator, model: PWG S/N904, MH Custom Design & Mfg. LC, Midvale, UT) was employed. A tidal volume of 800 mL, with 14 breaths per min, and a duty cycle of 0.5 (i.e., equal inhalation and exhalation with no inspiratory pause) was selected as a breathing pattern for an adult according to previous studies.[42] The size distribution of the nebulizers was characterized by an in-line Phase Doppler Anemometer (PDA) (Dantec Electronics Inc., Mahwah, NJ), while sampling 10 sec at the beginning of each minute of the run time. For both nebulizers, size measurements were made using the procedure proposed by Finlay et al.,[35] in which the aerosol size distribution is measured at the mouthpiece of the nebulizer connected to the breathing simulator with optical sizing window in place. The refractive index of bacteriophage suspension was measured using a refractometer (Fisher Scientific Economy Refractometers, 13-947 series, Dubuque, IA) and found equal to that of pure water, so that the refractive index of water was used for the droplet size measurements by PDA. Also, the solution density of the suspension of bacteriophages (which is clear to the naked eye) is near that of water (1.002 g/cm^3). The mass median diameters (MMDs) and geometric standard deviations (GSDs) from the PDA were averaged for each nebulizer and inserted into a mathematical model for deposition calculations.

The total output of the nebulizer was captured on a low resistance filter (Respirgard™ II, Vital Signs Colorado Inc., Totowa, NJ) in line with the breathing machine. The number of bacteriophages collected on the filter was termed the "inhaled count." The filter collection was performed directly at the exit of the nebulizer mouthpiece, without the sizing region in place, to prevent any loss in the sizing region.

Numerical predictions

Regional lung deposition of the inhaled bacteriophages was estimated using a numerical lung deposition model similar to that described previously,[35,39,40] which has shown good agreement with *in vivo* scintigraphic measurements on normal subjects.[43,44] The model is based on a one-dimensional Lagrangian approach. The lung of a healthy adult has been assumed to branch symmetrically in this model based on the data presented by Phillips and colleagues[45] for conducting airways (generations 0–14, trachea being as generation 0) and Haefeli-Bleuer and Weibel for the alveolar region (generations 15–23).[46] A functional residual capacity (FRC) of 3000 mL has been used for an adult in the model. The length and diameter of each generation have been given in the literature.[39] In the model, the equations of Chan and Lippmann[47] have been used for inertial impaction, while for sedimentation, those of Pich[48] and Heyder and Gebhart[49] have been used. The equations proposed by Gormley and Kennedy[50] have been used for diffusion. Mouth–throat deposition has been predicted using the equations of Rudolf et al.[51]

Hygroscopic effects were neglected in this study because of the high aerosol mass fraction generated by the used nebulizers. In fact, the value of the parameter γ, which determines the importance of hygroscopic effects,[52] was within the range that allows neglect of such effects ($\gamma \geq 3$). The parameter γ is defined as the ratio of the mass of droplets per unit volume divided by the mass of vapor per unit volume that needs to be exchanged between the droplets and the surrounding air in order to reach equilibrium.[53]

To predict the titer of bacteriophages deposited in the airway surface liquid (ASL), the regional deposition data was combined with a model of generational distribution of ASL in the tracheobronchial region, as explained by Lange et al.[40,54] This model distinguishes the mucus and the watery underlying layer of perciliary liquid (PCL) as the compo-

nents of the ASL in the upper airways.[55] The PCL thickness is approximated by the length of the cilia.[56] The data presented by Serafini and Michaelson[57] has been interpolated in this model by applying an exponential function to the data, and has been used in all tracheobronchial generations as the average thickness of the PCL layer in each generation. Using the layer thickness and the morphometric dimensions, the volume of PCL has been calculated for each generation and used for the calculation of the PCL concentration. Because the thickness of the mucus layer is not known from direct measurement, it has been approximated by applying mass conservation in addition to the available models of mucus velocity and production rate for each generation as an input to the model.[40] A set of mucus velocities have been used as reference values to match the *in vivo* clearance rates data.[58] The mucus velocities were considered constant in each generation. More details can be found in the literature.[39] The aforementioned method gives a series of mucus velocities with a maximum at the trachea and a minimum of about one-thousandth of the tracheal velocity in the most distal region.[40]

Various combinations of mucus velocity and production rate can be used in the mucus model. With reasoning similar to Lange et al,[40] production rate and mucus velocity of 5 mL/day and 15 mm/min were selected, respectively, to estimate the maximum bacteriophage titer and 40 mL/day together with 5 mm/min were chosen for the prediction of minimum bacteriophage titer. The total ASL (PCL plus mucus layer) concentration was estimated by the model by assuming a uniform bacteriophage deposition in each generation and homogenous dispersion in the ASL volume. The concentrations were calculated for the condition immediately following nebulization and prior to significant clearance.

Student *t*-tests were used to examine statistical signficance.

Results

Average values of inhaled counts of bacteriophages, nebulization time, aerosol mass median diameter (MMD), and geometric standard deviation (GSD) of the two types of nebulizers (LC star and eFlow) are presented in Table 1. It is observed from the table that average nebulization time was longer for the LC star jet nebulizer compared to the eFlow electronic nebulizer ($p < 0.01$). Average mass median diameter of the eFlow was slightly larger than that of the LC star nebulizer ($p > 0.01$). The polydispersity was similar for the two types of nebulizers, with geometric standard deviation not significantly affected by the type of nebulizer ($p > 0.01$).

Average values of the inhaled counts of bacteriophages on the filters are also given in Table 1 for each type of nebulizer. The type of nebulizer did not significantly change the "inhaled count" and regional deposition ($p > 0.01$).

Table 1 also shows the number of bacteriophages that mathematically were predicted to deposit in different regions of the lung. It is worth mentioning that the number of phages quoted in plaque forming units (PFU) represents the number of phages that survived the nebulization. The extrathoracic deposition obtained with the eFlow is slightly higher, which could be attributed to the larger MMD of that nebulizer. Larger aerosols tend to impact on extrathoracic surfaces. In contrast, the alveolar deposition obtained by the LC star was higher compared to that of the eFlow, which could be explained by a related argument. The tracheobronchial deposition of LC star and eFlow were predicted to be 2.14×10^7 (2.5×10^6) and 2.58×10^7 (3.3×10^6) PFU, respectively.

To assess the effect of osmolarity, one set of experiments was performed with both types of nebulizers filled with 2.5 mL hypotonic suspension (105 mOsm) with titer of 10^8 PFU/mL (2.5×10^8 PFU). The "inhaled count" using LC star and eFlow were 1.75×10^7 (0.02×10^7) and 1.8×10^7 (0.02×10^7), respectively. These results revealed that inhaled count was much less than the inhaled count obtained from an isotonic formulation (Table 1). The reduced inhaled count with hypotonic solution is likely due to osmotic pressure sensitivity of bacteriophages during nebulization.[16] This part of the experiment was a preliminary trial and we abandoned this formulation due to concerns that hypotonic formulations can cause patient cough, which makes the drug delivery impractical. Therefore, detailed data for this set of experiments are not presented.

Predicted bacteriophage titers in ASL in different generations of the tracheobronchial region are given in Figure 1 for

Table 1. Average Values of Mass Median Diameter (MMD), Geometrical Standard Deviation (GSD), Delivery Time, and Regional Deposition Data Obtained by Application of LC Star and eFlow Nebulizers Filled with 2.5 ml of Isotonic Formulations (282–290 mOsm), at Mean Titer of 2.15×10^8 (1.63×10^8) pfu/mL for the Breathing Pattern of 800 mL Tidal Volume, 14 Breaths per Minute, and Duty Cycle of 0.5

	Nebulizer type		
Data	*LC star* *Mean ± SD*[a]	*eFlow* *Mean ± SD*	p-*Value*[b]
MMD, μm	4.98 ± 0.06	5.83 ± 0.43	0.086
GSD, μm	1.48 ± 0.01	1.44 ± 0.07	0.437
Nebulization time, min	7.56 ± 0.59	3.09 ± 0.25	5.64×10^{-7}
Inhaled bacteriophages, PFU	$1.06 \times 10^8 \pm 0.12 \times 10^8$	$1.15 \times 10^8 \pm 0.14 \times 10^8$	0.242
Extrathoracic deposition, PFU	$2.04 \times 10^7 \pm 0.25 \times 10^7$	$2.92 \times 10^7 \pm 0.66 \times 10^7$	0.023
Tracheobronchial deposition, PFU	$2.14 \times 10^7 \pm 0.25 \times 10^7$	$2.58 \times 10^7 \pm 0.33 \times 10^7$	0.030
Alveolar deposition, PFU	$3.02 \times 10^7 \pm 0.35 \times 10^7$	$2.96 \times 10^7 \pm 0.29 \times 10^7$	0.747

[a]Mean value and standard deviation are given based on six replicates of experiments for each nebulizer (three repeats at two titers).
[b]Two-tail *p*-value comparing LC star to eFlow using a Student *t*-test.

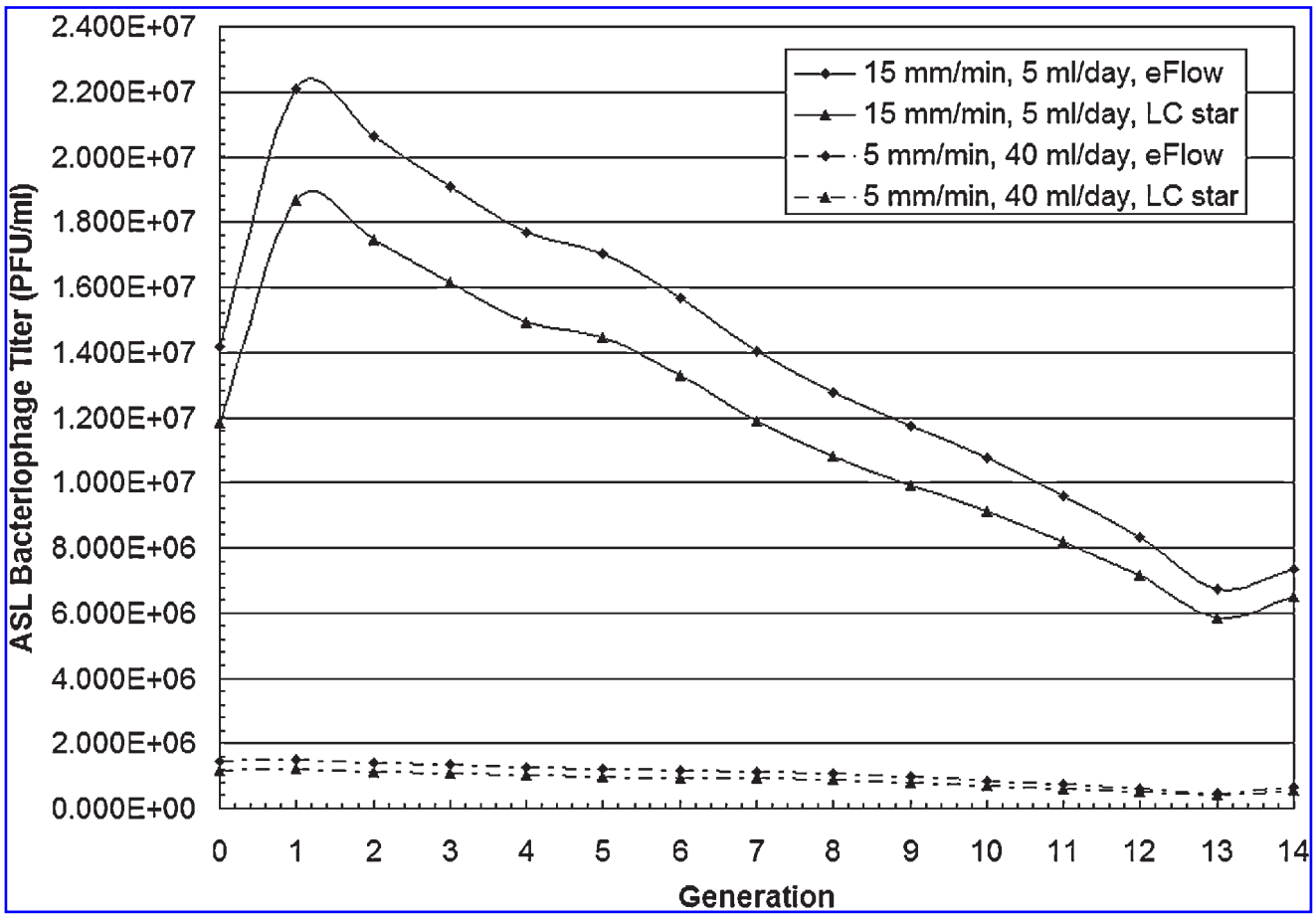

FIG. 1. Estimated generational bacteriophage titers in the ASL immediately after completion of nebulization with LC star and eFlow for adults at two combinations of mucus production rate and mucus velocity.

two combinations of tracheal mucus velocity–mucus production rate of 5 mm/min:40 mL/day and 15 mm/min:5 mL/day for both LC star and eFlow nebulizers. Nebulizing with eFlow resulted in higher ASL concentration in all tracheobronchial generations for both combinations of mucus velocity and mucus production rate. As expected, the largest ASL concentration was obtained with the largest mucus velocity (15 mm/min) and lowest production rate (5 mL/day), and the lowest ASL concentration was obtained with the lowest mucus velocity (5 mm/min) and highest production rate (40 mL/day). The nebulization time in our study (7.56 and 3.09 min for LC star and eFlow, respectively) is measured in minutes, while mucociliary clearance occurs on a time scale measured in hours. Therefore, considering a static state for concentration distribution in ASL is a reasonable approximation. This static state leads to a minimum titer for the low mucus velocity and high production rate because of the higher thickness of ASL for a given inhaled number of phages.

Discussion

To our knowledge, there is no example available to date on the bacteriophage treatment of respiratory infections caused by the BCC in CF patients. We have presented the results of an effort to nebulize BCC bacteriophages and to provide quantitative data describing the nebulization results. Because the lung deposition and clinical response to aerosolized antibiotics among CF patients have been shown to be affected by the nebulizer type,[59–62] we have compared two types of nebulizers.

Our experiments show that BCC bacteriophage survive nebulization both in LC Star and eFlow nebulizers, and result in good inhaled and deposition titers. Because patients prefer the shortest inhalation time, the eFlow is favored in this sense. However, the eFlow is currently more expensive, and may be less durable, with the average cycle life of an ultrasonic nebulizer being 600 to 1000 uses, which is reached within a year by a CF patient receiving multiple medications.[63] It is worth noting that imaging lung deposition studies using scintigraphic aerosol have shown good performance of vented jet nebulizers.[62] LC star and eFlow both have relatively similar MMD, and our mathematical model predicts similar distribution of phages in the lungs. The above suggests that the LCstar and eFlow both appear suitable for BCC bacteriophage therapy.

Realizing that bacteriophages are self-replicating, it is not known what initial bacteriophage titer is sufficient for successful treatment. Payne and Jansen[64] have mentioned that in addition to types of phages and bacteria involved, the success of active phage therapy (in which phages replicate themselves) is determined by the actual bacterial density over the

time of exposure. To estimate required doses, animal studies can be considered, with one of the most widely known series of phage studies for veterinary medicine having been performed by Smith and his coworkers,[65–68] although they do not use the aerosol delivery route. They found that a single dose of specific *Escherichia coli* phage decreased, by many orders of magnitude, the number of target bacteria among calves, lambs, and piglets infected by a certain type of *E. coli* strain. All animals receiving phage treatment survived the bacterial infection.[65–68] It has also been indicated that in mice, phage therapy failed completely for doses less than 3×10^4 particles for intramuscular injection and less than 3×10^3 particles for intravenous delivery.[64] Moreover, a pseudomonas phage with a PD50 of 1.2×10^7 particles protected mice against 5LD50 (10^8) of a strain of *P. aeruginosa*. It has been shown that no risk of using higher doses of bacteriophages has been observed among mice during acute toxicity studies, even after using a dose approximately 3500 times higher than the human dose estimated by body weight.[69] In the present study, we obtained values of inhaled counts of 1.06×10^8 and 1.15×10^8 PFU for the LC star and eFlow, respectively, which is higher than the minimum required effective dose for mice therapy (1.2×10^7 PFU). However, the required threshold titer for effective human treatment with the tested BCC phage is currently unknown.

Rather than pure bacteriophage therapy, combined treatment of antibiotic and bacteriophage therapy can also be considered. A combination of enrofloxacin antibiotic and intramuscularly administered bacteriophage has been evaluated *in vivo* and provided total protection of birds.[70] This finding may be interpreted as favorable to consideration of a combined treatment; however, addition of antibiotics in parallel with phages may also diminish phage efficacy.[10] It has been mentioned that parallel application of antibiotic and phages in a human clinical trial[71] reduced the efficacy of phage therapy from 95.2% to 84.9%.[72] *In vivo* studies are needed to evaluate the efficacy of BCC phage therapy in combination with antibiotic treatment for CF patients to test the synergy of the two methods.

Despite the obstacles that prevent predicting a successful dosage *a priori*, it should be noted that a single bacteriophage can be sufficient as long as it invades the bacterium and replicates itself. If the initial number of phages is high enough, then the first round of lysis can overcome the bacteria and this is termed "passive phage therapy."[64] There is instead the possibility that therapy be based on secondary infection (infection of bacteria by phage that has been released as a result of lysis of infected cells); in this case, the number of phage increases via self-replication and this mode of treatment is called "active phage therapy." Predicting the kinetic phenomena of active phage therapy for dose calculation is difficult. When correlating *in vitro* to *in vivo* data, it is worth noting that *in vitro* growth data for a phage often cannot be directly applied to the *in vivo* situation and the in vivo data for one phage cannot in general be transferred to another phage.[10] In this aspect, one of the critical parameters that affects phage therapy is the clearance rate of the phage particles from the body fluids by the reticuloendothelial system. Timing of phage treatment is critical and phage administered too early may be cleared from the body before it reaches the replication threshold.[10]

The prediction of actual adsorbed titer in airway surface liquid (ASL) is beyond the capabilities of our model, and we cannot be precise in the interpretation of these predictions at this point. It should be noted that the mathematical analysis for prediction of bacteriophage titer in each generation was based on healthy human lung data and aerosol deposition in CF patients is less homogenous compared to healthy lungs.[62] The regional deposition in infected lungs depends on the distribution of disease. Chest radiograph (CXR) patterns in 109 adult CF patients showed that some patients demonstrate a predominant upper lobe (UL) pattern of disease, while others may have unilateral or even unilobar disease.[73] Moreover, in an individual, the distribution of disease changes with time at different stages of disease. The geometry of the respiratory tract, respiratory rate, and depth of respiration all influence deposition in the respiratory tract. Due to the limited knowledge of the detailed geometry of diseased lungs, modeling deposition in diseased lungs at a precise level is challenging. There have been several attempts to model deposition in CF lungs. Martonen et al.[74] have modeled the lung of a healthy adult by considering Weibel's morphology and modeled the obstructions that occur in CF patients by reducing airway diameters by 20% and 40%, which may not be realistic for all of the patients. Weibel's symmetric lung morphology was also used by Brown and Bennett,[75] who altered airway diameter in each generation based on measured lung volume and the assumed effect of disease on lumen reduction. The latter effect assumed that pulmonary impairment begins in the small airways and progresses proximally and the apex is more severely affected than the base of the lung.[76,77] Their model predicted enhanced particle deposition in large airways of the apical lung and to a lesser extent in the basal lung. *In vivo* studies using radio labeled monodiperse particles (5 μm MMAD) suggest that significant coarse particle deposition may occur in the TB airways of poorly ventilated lung regions in CF patients; conversely, particle deposition in the TB airways of healthy subjects was directly related to the level of ventilation.[78] To decrease variability in lung deposition for a given dose, using a controlled breathing pattern for maximum alveolar deposition reduces the differences in lung deposition between healthy subjects and CF patients with impaired lung function and airway obstruction.[79] Maximum alveolar deposition occurred when 2–3 μm particles were inhaled with airflow rates of 250–500 cm^3/sec. It was concluded that inertial deposition at obstructed regions can be prevented and total deposition in patients and healthy subjects is the same if the aerosol is inhaled slowly enough ($<$200 cm^3/sec).[78] Results of another study suggest that targeted drug delivery to the larger, central airways is improved by inhaling fine particles (1 μm MMAD) at approximately 38 L/min.[80] Despite the above uncertainties in predicting the success of nebulized bacteriophage therapy for targeting and treatment of respiratory BCC infections, our results suggest that *in vivo* work in this direction is warranted.

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Author Disclosure Statement

No conflicts of interest exists.

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