

Effects of Emulsion Composition on Pulmonary Tobramycin Delivery During Antibacterial Perfluorocarbon Ventilation

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Abstract

Background: The effectiveness of inhaled aerosolized antibiotics is limited by poor ventilation of infected airways. Pulmonary delivery of antibiotics emulsified within liquid perfluorocarbon [antibacterial perfluorocarbon ventilation (APV)] may solve this problem through better airway penetration and improved spatial uniformity. However, little work has been done to explore emulsion formulation and the corresponding effects on drug delivery during APV. This study investigated the effects of emulsion formulation on emulsion stability and the pharmacokinetics of antibiotic delivery via APV.

Methods: Gravity-driven phase separation was examined *in vitro* by measuring emulsion tobramycin concentrations at varying heights within a column of emulsion over 4 hours for varying values of fluorosurfactant concentration ($C_{fs} = 5\text{--}48\text{ mg/mL H}_2\text{O}$). Serum and pulmonary tobramycin concentrations in rats were then evaluated following pulmonary tobramycin delivery via aerosol or APV utilizing sufficiently stable emulsions of varying aqueous volume percentage ($V_{aq} = 1\%\text{--}5\%$), aqueous tobramycin concentration ($C_t = 20\text{--}100\text{ mg/mL}$), and C_{fs} (15 and 48 mg/mL H₂O).

Results: *In vitro* assessment showed sufficient spatial and temporal uniformity of tobramycin dispersion within emulsion for $C_{fs} \geq 15\text{ mg/mL H}_2\text{O}$, while lower C_{fs} values showed insufficient emulsification even immediately following preparation. APV with stable emulsion formulations resulted in 5–22 times greater pulmonary tobramycin concentrations at 4 hours post-delivery relative to aerosolized delivery. Concentrations increased with emulsion formulations utilizing increased V_{aq} (with decreased C_t) and, to a lesser extent, increased C_{fs} .

Conclusions: The emulsion stability necessary for effective delivery is retained at C_{fs} values as low as 15 mg/mL H₂O. Additionally, the pulmonary retention of antibiotic delivered via APV is significantly greater than that of aerosolized delivery and can be most effectively increased by increasing V_{aq} and decreasing C_t . APV has been further proven as an effective means of pulmonary drug delivery with the potential to significantly improve antibiotic therapy for lung disease patients.

Key words: emulsion, perfluorocarbon, respiratory infection, ventilation

Introduction

CHRONIC BACTERIAL RESPIRATORY INFECTIONS are the primary cause of morbidity and mortality in patients with cystic fibrosis (CF),^(1,2) as well as the main cause of exacerbation in chronic obstructive pulmonary disease (COPD).^(3,4) Conditions such as CF and COPD feature changes in mucus rheology and mucociliary clearance that can make bacterial elimination exceedingly difficult.^(5,6) Antibacterial perfluorocarbon ventilation (APV) has been

previously presented as a possible means of pulmonary antibiotic delivery in which the lungs are filled with aqueous antibiotics emulsified in perfluorocarbon (PFC) (i.e., water-in-PFC).⁽⁷⁾

During APV, the lungs are either partially filled with emulsion and ventilated with gas (i.e., partial APV) or fully filled and ventilated with emulsion (i.e., total APV). APV is proposed as a short-term (≤ 2 hours) adjunct therapy to systemic or inhaled antibiotics that could wash infected mucus from the lungs while delivering antibiotic to poorly ventilated

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regions. Mucus removal during ventilation with PFCs has been previously noted^(8,9) and occurs due to both buoyant forces and flow-induced shear stress acting on the mucus.

The water-in-PFC emulsions used during APV consist of small droplets of aqueous antibiotic (diameter previously shown to be 1–4 μm)⁽⁷⁾ suspended within a continuous PFC phase ($\geq 95\%$ by volume). Due to the immiscibility of water and PFC, a small amount of fluorosurfactant is used to stabilize the droplets and delay their coalescence and creaming by lowering the aqueous–PFC interfacial tension. Initial studies of APV have shown its ability to achieve increased pulmonary retention of delivered antibiotics while producing lower and delayed peak serum concentrations relative to aerosolized delivery.⁽⁷⁾ This presents the potential for not only increased efficacy in eradicating infection but also the opportunity for the administration of larger antibiotic doses without increased risk of systemic toxicity.

APV should also result in more spatially uniform drug delivery throughout the lung relative to aerosolized delivery. The effectiveness of aerosolized antibiotic delivery is limited by its inherent dependence on airflow within the lung. Poor ventilation during disease due to lung damage or mucus plugging can result in significantly decreased aerosolized delivery to the most burdened regions of the lung.^(10–12) Furthermore, the significant effects of aerosolized particle size on site of deposition often results in substantial deposition in the oropharynx and delivery device, typically resulting in 25–90% of delivered drug never reaching the lung.⁽¹³⁾

Delivery is further impaired for patients on a mechanical ventilator in which approximately 20% or less of the delivered drug reaches the lung with the majority deposited in the delivery device and ventilator tubing.^(14,15) PFC, on the other hand, has shown uniform spatial distribution in the injured lung during liquid ventilation.⁽¹⁶⁾ Additionally, the physical characteristics that determine this distribution (viscosity and surface tension) are retained, and possibly enhanced, following the emulsification process.⁽⁷⁾

During total APV, the lungs are completely filled with emulsion and thus the airway surfaces throughout the entire lung are in constant contact with the emulsion. During partial APV, the lung is filled to a lesser degree, typically to end-expiratory volume. In this setting, the entire lung would experience contact with the emulsion at the end of each expiration while some of the larger conducting airways would be gas filled during the rest of the respiratory cycle. Ultimately, by filling the lungs with a liquid and removing dependence on airflow, APV should result in more efficient delivery and spatially uniform drug distribution within the lung relative to currently used treatments.

Optimization of APV requires an understanding of the temporal patterns of drug delivery and removal from the lung. However, the pharmacokinetics of these emulsions are complicated and not yet fully understood. We hypothesize that the pharmacokinetics during APV are a function of the aqueous volume percentage (V_{aq}), aqueous antibiotic concentration (C_t), and fluorosurfactant concentration (C_{fs}). The V_{aq} and C_t are defined as the percentage of aqueous phase in the emulsion and the antibiotic concentration within that aqueous phase, respectively. Together they define the total amount of drug delivered to the lungs upon initiating APV.

The fluorosurfactant is responsible for maintaining emulsion stability and avoiding droplet coalescence and creaming.

C_{fs} , therefore, is likely to affect the transport of antibiotic to the lung parenchyma and pulmonary capillaries. Throughout this work, C_{fs} values are specified as a ratio of fluorosurfactant mass to aqueous volume (i.e., mg per mL H_2O). Previous studies indicate that *in vitro* bacterial killing of *Pseudomonas aeruginosa* biofilms is optimized by maximizing C_t with a far smaller increase in killing with increasing V_{aq} .⁷ However, the *in vivo* setting is a vastly different scenario with a more complex geometry and multiple transport processes continually affecting antibiotic presence and availability.

Therefore, this study sought to determine the effect of V_{aq} , C_t , and C_{fs} on antibiotic delivery to the lung. Because the fluorosurfactant enables the emulsification of the antibiotics within the PFC phase, there is a minimum C_{fs} value below which the dispersion of drug is not maintained following sonication and the potential for improved pulmonary distribution is lost. In order to establish an appropriate range of C_{fs} values to be examined *in vivo*, an additional *in vitro* study was performed to determine the minimum value of C_{fs} that exhibited sufficiently stable drug dispersion.

Materials and Methods

Tobramycin–PFC emulsion preparation

Tobramycin–PFC emulsions were prepared similar to methods described elsewhere using the same combination of fluorosurfactants.⁽⁷⁾ Briefly, Krytox 157 FSL (DuPont, Wilmington, DE, USA) and Krytox-PEG copolymer (synthesized as previously described)⁽¹⁷⁾ were first dissolved in PFC liquid. The PFC used in all experiments was per-fluorocycloether/perfluorooctane (FC-770; 3M Inc., St. Paul, MN, USA), although a PFC with a larger amount of documented *in vivo* safety would likely be used for eventual clinical translation. Next, tobramycin (X-Gen Pharmaceuticals Inc, Horseheads, NY, USA) dissolved in sterile water was added to the PFC solution. The mixture was then emulsified via sonication (Model S-450D, 3.2 mm diameter microtip; Branson Ultrasonics, Danbury, CT, USA) at 200 W/cm^2 for 60 seconds. If the emulsion was not used immediately following preparation, it was sonicated a final time at the same settings immediately prior to experimentation.

In vitro assessment of emulsion drug dispersion

The spatial distribution of tobramycin-containing aqueous droplets within a column of emulsion was evaluated over a period of 4 h following emulsion preparation in order to evaluate the effect of C_{fs} on emulsion stability. Emulsions with fixed V_{aq} (2.5%) and C_t (40 mg/mL) were examined with varying C_{fs} (5, 15, or 48 mg/mL H_2O). A graduated cylinder (polystyrene, 12.7 mm inner diameter) was filled with 30 mL of freshly sonicated emulsion to a height of approximately 24 cm. The graduated cylinder was modified to feature access ports with a needle (18 gauge) protruding through the cylinder wall into the center of the column lumen at heights of 4, 10, and 16 cm from the base. A schematic of the graduated cylinder can be seen in Figure 1.

At 0, 30, 120, and 240 min following emulsion preparation, a 200 μL sample of emulsion was drawn from each height. In order to quantify the presence of active tobramycin in each sample of emulsion, the samples were diluted with 4 mL of sterile water and re-sonicated at 200 W/cm^2 for 2 min,

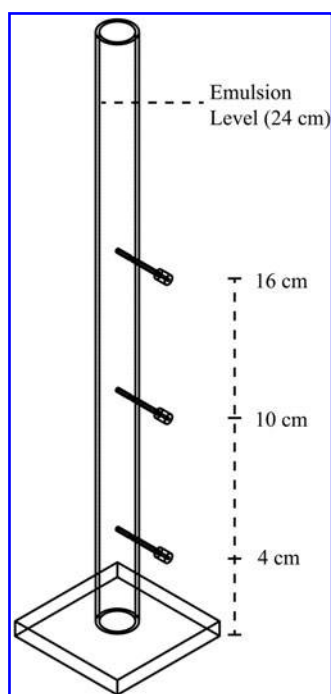


FIG. 1. A schematic of the graduated cylinder used during *in vitro* assessment of emulsion drug dispersion. Access ports to the cylinder lumen are featured at heights of 4, 10, and 16 cm from the base.

resulting in an inverted emulsion (continuous aqueous phase with dispersed PFC phase). The phase inversion was visually confirmed by the observation of PFC droplets accumulating at the bottom of the continuous aqueous phase. The inverted emulsion was then centrifuged at 3000 *g* for 20 min in order to separate the PFC and aqueous phase.

The presence of active tobramycin in the separated aqueous phase was then quantified via a microbiological assay similar to that described elsewhere.⁽¹⁸⁾ Briefly, the surface of LB agar plates was inoculated with 500 μL of mid-log growth *Pseudomonas aeruginosa* in tryptic soy broth. Aqueous samples were then diluted and loaded into wells (75 μL /well) within the inoculated agar along with standard solutions of known tobramycin concentration. The plates were then incubated at 37°C for 24 hours before circular inhibition zones were imaged and measured. The concentration of the unknown samples was then determined by interpolation from the inhibition zones of the standard solutions.

Each sample was measured in triplicate and the resulting values averaged to produce a single concentration. The lower limit of detection using these methods was 5 $\mu\text{g}/\text{mL}$, although all experimental values were well above this limit. Three separate trials were performed for each emulsion formulation. All emulsion formulations evaluated had a total emulsion tobramycin concentration of 1 mg/mL. In theory, if the tobramycin were homogeneously distributed throughout the emulsion and all tobramycin was recovered during the emulsion inversion process, the assayed aqueous phase would have a tobramycin concentration of 50 $\mu\text{g}/\text{mL}$. Experimental values from this study were normalized by this value and are thus reported as a percentage of the theoretical tobramycin content.

Pharmacokinetic evaluation

Pulmonary delivery of tobramycin (15 mg/kg) was achieved in specific pathogen-free, male Sprague Dawley rats [$n=40$, weight 416 ± 16 g (mean \pm standard deviation); Taconic, Hudson, NY, USA] via either intratracheal aerosolized delivery or partial APV with various emulsion formulations. All rats were initially anesthetized with a mixture of ketamine hydrochloride [50 mg/kg, intraperitoneal (IP); Hospira Inc., Lake Forest, IL, USA] and xylazine hydrochloride (5 mg/kg, IP; Lloyd Laboratories, Shenandoah, IA, USA).

Following initial anesthesia, intravenous (IV) access was acquired via the lateral tail vein, and a constant IV infusion of ketamine hydrochloride (1 mg/kg/min) was used to maintain sedation for the remainder of the experiment. Heart rate and arterial oxygen saturation were monitored via a pulse oximeter (VetOx Plus 4800; Hesa, Loveland, CO, USA). Rat body temperature was monitored and maintained with a homeothermic blanket system (Model 507220F; Harvard Apparatus, Holliston, MA, USA). Rats receiving aerosolized treatment were then orally intubated with a 16 gauge angiocatheter and placed in a supine position. Aerosolized delivery was accomplished via a Microsprayer Aerosolizer (Model IA-1B; Penn-Century, Wyndmoor, PA, USA).

Prior to intubation, the length of the angiocatheter was trimmed to ensure that the Microsprayer nozzle was sufficiently exposed when the Microsprayer was fully inserted into the angiocatheter. Following intubation with the trimmed angiocatheter, the Microsprayer was fully inserted into the angiocatheter and tobramycin (40 mg/mL tobramycin concentration, 15 mg/kg bodyweight) was delivered intratracheally. Although efforts were made to synchronize actuation of the Microsprayer to inspiration, the rats often became temporarily apneic following intubation and thus the aerosol was sometimes delivered in the absence of inspiratory or expiratory flow.

Following delivery, rats were extubated and provided supplemental oxygen via a nose cone until euthanasia. For rats receiving partial APV, the neck was shaved and cleaned with alcohol and a 1 cm midline incision made. The trachea was isolated and a tie positioned loosely around it. The rat was then orally intubated with a 16 gauge angiocatheter and connected to a ventilator (Model 683; Harvard Apparatus) in a supine position. Immediately after beginning ventilation the tie was tightened around the trachea, creating a seal on the angiocatheter already within the trachea.

Ventilation was carried out with a tidal volume of 9 mL/kg bodyweight, a respiratory rate of 50–80 breaths/min, a positive end-expiratory pressure of 3 cmH₂O, and a fraction of inspired oxygen of 1. Respiratory rate was adjusted to maintain peak inspiratory pressures less than 30 cmH₂O. Preoxygenated emulsion was instilled (15 mL/kg bodyweight) via a port connected to the angiocatheter, resulting in a delivered dose of 15 mg/kg bodyweight of tobramycin. The emulsion was prepared no more than 5 min before use and was instilled during gas ventilation in successive aliquots (2–3 mL/aliquot) with each aliquot instilled over a period of approximately 60 s. Gas ventilation was continued following delivery of the emulsion until euthanasia.

The delivery methods and emulsion formulations used are summarized in Table 1. In order to evaluate the isolated effect

TABLE 1. DELIVERY METHODS AND CORRESPONDING FORMULATIONS OF ALL TREATMENTS EVALUATED

Delivery method [-]	Aqueous volume percent, V_{aq} [%]	Aqueous tobramycin concentration, C_t [mg/mL]	Emulsion tobramycin concentration [mg/mL emulsion]	Ratio of each Krytox and Krytox-PEG mass to aqueous volume, C_{fs} [mg/mL H_2O]	Total concentration of each Krytox and Krytox-PEG [mg/mL emulsion]
APV	1.0	100	1	15	0.15
APV	2.5	40	1	15	0.375
APV	5.0	20	1	15	0.75
APV	2.5	40	1	48	1.2
Aerosol + PFC	2.5	40	N/A	0	0
Aerosol	100.0	40	N/A	N/A	N/A

of V_{aq} and C_t , emulsions with constant C_{fs} (15 mg/mL H_2O) and varying V_{aq} (1%, 2.5%, and 5%) were assessed. C_t was varied inversely with V_{aq} in order to maintain a constant delivered dose of tobramycin. Similarly, in order to evaluate the effect of C_{fs} , emulsions with constant V_{aq} (2.5%) and C_t (40 mg/mL) and varying C_{fs} (15 or 48 mg/mL H_2O) were assessed.

Additionally, tobramycin delivery via a combination of PFC and aqueous tobramycin without fluorosurfactant was evaluated using a modified partial APV procedure. Due to the immiscibility of the two phases in the absence of fluorosurfactant, the emulsion delivery procedure previously described was slightly modified in order to achieve accurate and consistent delivery. For this group, neat PFC was instilled in an identical fashion to that described for emulsion delivery during partial APV. Next, the angiocatheter was temporarily disconnected from the ventilator and the aqueous tobramycin phase intratracheally delivered via the Microsprayer. As with the aerosolized delivery group, the angiocatheters used in this treatment were appropriately trimmed in order to ensure uninhibited aerosol delivery. The rats were then reconnected to the ventilator and the remainder of the experiment carried out as described above.

In all experiments, blood samples were drawn via the lateral tail vein (opposite side of the tail relative to IV infusion) at 30, 60, 120, and 240 min following delivery of tobramycin. In treatment groups showing peak serum concentrations 30 min post-delivery, treatment was repeated with blood samples drawn at 10, 20, and 30 min post-delivery in order to better determine the time and magnitude of peak serum concentrations. A hematocrit measurement was taken at the time of each blood sampling and was used to normalize serum tobramycin measurements to account for hemodilution due to IV fluid delivery. Five rats were analyzed for each set of conditions evaluated.

Rats were euthanized with pentobarbital sodium (175 mg/kg, IP, Fatal Plus; Vortech Pharmaceuticals, Dearborn, MI, USA), the trachea immediately tied off with suture, and the lungs and trachea removed intact. The lungs were then thoroughly homogenized with sterile saline (25 mL/kg body weight). After centrifugation, supernatant from homogenized lung tissue samples was collected, serially diluted, and the presence of active tobramycin measured via the previously described microbiological assay. Any result below the lower limit of detection (5 μ g/mL) was assigned a value of 2.5 μ g/mL. Serum tobramycin concentration measurements were performed via an immunoassay by the Laboratory

Medicine Department within Allegheny General Hospital (Pittsburgh, PA).

Statistical analysis

SPSS 22 (IBM Corporation, Armonk, NY) was used to perform all statistical analysis. A one-way ANOVA and *post-hoc* analysis was performed on the peak serum tobramycin concentrations and lung tissue tobramycin concentrations. Due to unequal variances between groups, the Games-Howell method was used to determine significant differences. A mixed model analysis was performed to examine differences in the serum tobramycin concentrations with time post-delivery used as the repeated-measure variable. A similar analysis was also used in the *in vitro* stability studies to examine differences in tobramycin content, using time post-preparation and height as the repeated-measure variables.

Results

In vitro assessment of emulsion drug dispersion

The formation of a distinct aqueous layer of varying sizes was observed at the top of the emulsion column between 0 and 30 minutes post-preparation for all trials performed. Figure 2 shows tobramycin content for emulsions (V_{aq} = 2.5% and C_t = 40 mg/mL) with C_{fs} values of 5, 15, and 48 mg/mL H_2O . In Figure 2A, tobramycin content is shown as a function of height for each C_{fs} value and time point examined. Figure 2B shows the same data as a function of time for each C_{fs} value and height examined. Results showed a significant ($p < 0.01$) effect of C_{fs} on tobramycin content.

Tobramycin content resulting from a C_{fs} of 5 mg/mL H_2O was significantly ($p < 0.01$) lower than that for C_{fs} values of both 15 and 48 mg/mL H_2O . Emulsion utilizing a C_{fs} value of 5 mg/mL H_2O showed insufficient dispersion of antibiotic, even immediately following preparation and was thus not evaluated during the *in vivo* pharmacokinetic experiments. Tobramycin content for C_{fs} of 15 and 48 mg/mL H_2O did not significantly differ from each other. No significant effect was shown for time post-preparation or height on tobramycin content.

Pharmacokinetic evaluation

Lung tissue homogenate tobramycin concentrations at 4 hours post-delivery for all treatments evaluated are shown in Figure 3. Two of the five aerosolized delivery trials and all five of the aerosolized delivery in combination with PFC

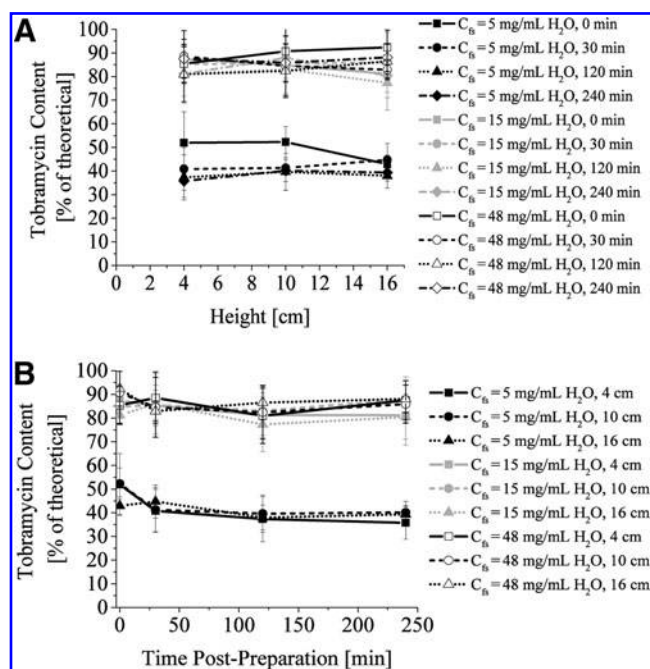


FIG. 2. Tobramycin content within the emulsion (normalized by theoretical value) as a function of height from the base of the column for varying C_{fs} values and times post-preparation (A) and as a function of time post-preparation for varying C_{fs} values and heights from the base of the column (B). Error bars represent standard deviations and $n = 3$ for each condition evaluated.

trials resulted in concentrations below the lower limit of detection ($5 \mu\text{g}/\text{mL}$). All APV treatments resulted in significantly (either $p < 0.01$ or $p < 0.05$, see Fig. 3 for distinction) greater pulmonary tobramycin concentrations relative to aerosolized delivery at 4 hours post-delivery. As shown in Figure 3A, pulmonary tobramycin concentrations increased with increasing V_{aq} (decreasing C_t), with the largest V_{aq} (5%) resulting in 22 times greater concentration than aerosolized delivery. As shown in Figure 3B, increased C_{fs} also resulted in larger pulmonary tobramycin concentrations, although the difference between the two C_{fs} values examined was not significant ($p = 0.14$).

Serum tobramycin concentrations for all treatments are shown in Figure 4. A V_{aq} of 5% resulted in significantly ($p < 0.01$) lower serum tobramycin concentrations relative to all other groups shown in Figure 4A. Similarly, the largest value of C_{fs} (48 mg/mL H₂O) had significantly ($p < 0.01$) lower serum tobramycin concentrations than all other groups shown in Figure 4B. The time and magnitude of peak serum concentrations for all treatments evaluated are shown in Table 2. Peak serum concentrations in all APV groups occurred at a later time point than both aerosolized delivery alone and in combination with PFC (60 min post-delivery for APV groups vs. 10 min post-delivery for aerosol groups).

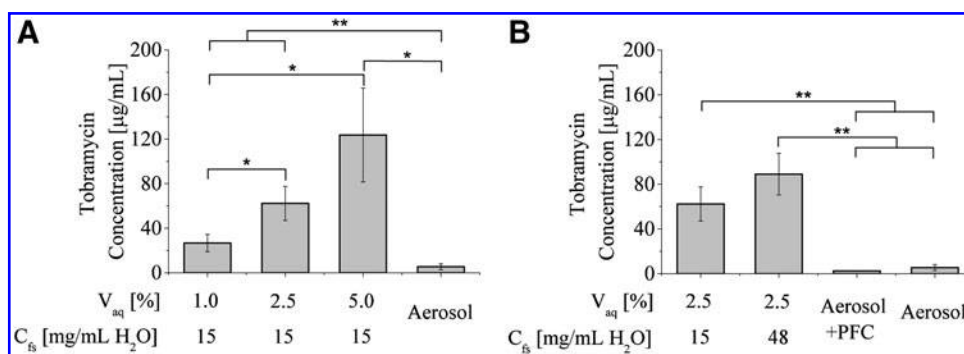


FIG. 3. (A) Tobramycin concentration of lung tissue homogenate at 4 hours following tobramycin delivery via aerosolized delivery and partial APV with varying values of V_{aq} . (B) Tobramycin concentration of lung tissue homogenate at 4 hours following tobramycin delivery via aerosolized delivery, aerosolized delivery in combination with PFC, and partial APV with varying C_{fs} values. Error bars represent standard deviations, $n = 5$ for each condition evaluated, and statistically significant differences between groups are denoted by an asterisk ($*p < 0.05$, $**p < 0.01$).

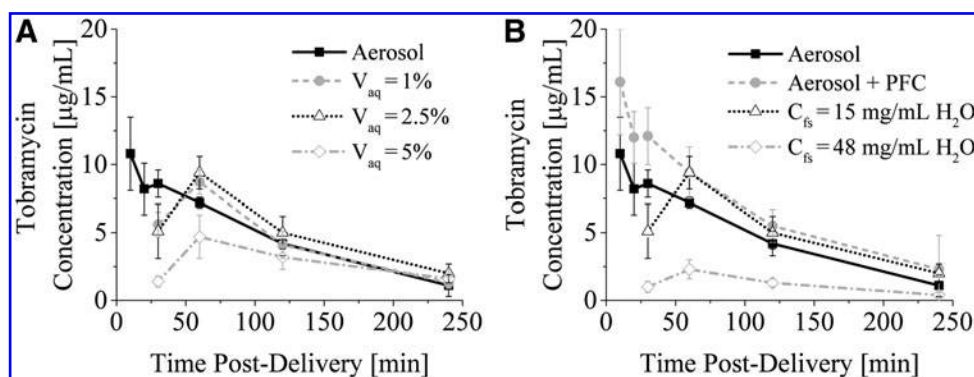


FIG. 4. (A) Serum tobramycin concentration out to 240 min following tobramycin delivery via aerosolized delivery and partial APV with $C_{\text{fs}} = 15 \text{ mg/mL H}_2\text{O}$ and varying V_{aq} values. (B) Serum tobramycin concentration out to 240 min following tobramycin delivery via aerosolized delivery, aerosolized delivery in combination with PFC, and partial APV with $V_{\text{aq}} = 2.5\%$ and varying C_{fs} values. Error bars represent standard deviations, and $n = 5$ for each condition evaluated.

Peak serum concentrations resulting from aerosolized delivery or aerosolized delivery in combination with PFC were greater than all those produced by APV, although not significantly different in all cases.

At the same C_{fs} value (15 mg/mL H_2O), a V_{aq} of 5% produced significantly ($p < 0.01$) lower peak serum concentrations than the 1% and 2.5% V_{aq} groups and aerosolized delivery. Similarly, a C_{fs} of 48 mg/mL H_2O produced significantly ($p < 0.01$) lower peak serum concentrations relative to a C_{fs} of 15 mg/mL H_2O , aerosolized delivery, and aerosolized delivery in combination with PFC.

Discussion

In order to achieve effective antibiotic therapy, the antimicrobial agent must reach the site of infection and remain in the vicinity for an adequate length of time.⁽¹⁹⁾ In the case of pulmonary treatment in the setting of lung disease, delivery to the site of infection is challenging. Inhaled aerosolized treatment is currently the most widely used and successful means of achieving such delivery. However, lung disease can result in poor ventilation in many of the airways, rendering a delivery method relying on airflow less than ideal. APV provides a means of pulmonary drug delivery with no dependence on airflow and thus increased ability to achieve effective delivery throughout the lung.

Previous work has proven APV to be a viable means of pulmonary antibiotic delivery, as well as shown the bactericidal ability of emulsions against CF-derived *Pseudomonas aeruginosa* biofilms.⁽⁷⁾ However, the effects of emulsion formulation on the resulting antibiotic delivery have yet to be fully investigated. Understanding these effects is a vital step in optimizing the emulsion formulation for safe and effective treatment. In this study we sought to evaluate the effects of emulsion formulation (V_{aq} , C_{t} and C_{fs}) on emulsion stability as well as the pharmacokinetics of antibiotic delivery via APV.

As the long-term biocompatibility of the fluorosurfactants used (or rather any available fluorosurfactants) has not been evaluated, there is warranted caution regarding their use in the lung. This only highlights the importance of understanding the effects of the amount of fluorosurfactant used in order to determine the degree to which it can be minimized without impairing effective drug delivery. Results from this work suggest that a C_{fs} value as low as 15 mg/mL H_2O (a nearly 70% reduction from previous APV *in vivo* trials)⁽⁷⁾ still exhibits uniform drug dispersion up to 4 hours following preparation.

As noted in previous work with APV, the emulsion preparation is straightforward and could be performed immediately prior to administration, similar to processes for drug-laden emulsions used in chemoembolization.⁽²⁰⁾ Thus,

TABLE 2. PEAK SERUM CONCENTRATIONS AFTER TOBRAMYCIN DELIVERY

Delivery method [-]	Aqueous volume percent, V_{aq} [%]	Ratio of each Krytox and Krytox-PEG mass to aqueous volume, C_{fs} [mg/mL H_2O]	Time to peak serum concentration [min]	Peak serum concentration [$\mu\text{g/mL}$]
APV	1.0	15	60	8.7 ± 0.9
APV	2.5	15	60	9.4 ± 1.2
APV	5.0	15	60	4.7 ± 1.6
APV	2.5	48	60	2.3 ± 0.7
Aerosol + PFC	2.5	0	10	16.1 ± 3.9
Aerosol	100.0	N/A	10	10.8 ± 2.7

Concentration values are reported as mean ± standard deviation.

even the reduced value of C_{fs} (15 mg/mL H₂O) evaluated should be able to maintain uniform drug dispersion for the entirety of the intended treatment duration (≤ 2 hours). This reduction of C_{fs} also caused a slight decrease in pulmonary antibiotic retention, but the effect of this retention on the anti-biofilm activity of the emulsion is not yet known.

The presence of antibiotics within the lung following treatment was evaluated by measuring the tobramycin concentration of homogenized lung tissue via a microbiological assay. A significant advantage of this detection method is the assurance that the measured tobramycin content is still active and able to exert its intended bactericidal effect. The most clinically relevant measure of antibiotic presence in the lung is likely the aqueous concentration at the airway surfaces, however, such a measurement is difficult to perform in rats.

Due to the complex environment present within the airways during APV, it is difficult to speculate how the tobramycin concentrations of lung tissue homogenate relate to the concentrations encountered at the aqueous surfaces of the lung. A greater understanding of the physical transfer of drug from the emulsion to the aqueous surfaces which it encounters would likely best be accomplished in a more controlled *in vitro* setting and should be a primary aim of future efforts towards advancing APV.

When interpreting the pharmacokinetic results of this work, it is also important to note that emulsion was not suctioned from the lungs prior to euthanasia and subsequent tissue tobramycin measurements. This differs from the clinical scenario in which some portion of the emulsion, along with dislodged mucus and biofilm, would be suctioned from the lungs within 2 hours of delivery.

From the authors' previous experience performing APV in rats, suctioning emulsion from the airways of a rat has proven to be technically difficult and highly inconsistent. Thus, during APV treatment in this work, the emulsion was allowed to remain in the lungs in order to reduce treatment variability. The volume of emulsion remaining in the lungs at the time of euthanasia was not measured in this work. However, a previous study evaluating liquid ventilation with neat PFC in similarly sized rats has shown an evaporative loss rate of 1.6 mL/hour.⁽²¹⁾ The average amount of emulsion instilled during this study was 6.2 mL. Although the evaporative loss rate likely decreases with time, one would expect very little, if any, emulsion to remain in the lungs after 4 hours of ventilation at the time of euthanasia and tissue tobramycin measurement.

Similar to a previous study,⁽⁷⁾ increased pulmonary antibiotic concentrations were measured following APV (all emulsion formulations) relative to aerosolized delivery. Aerosolized delivery in combination with PFC showed low retention similar to aerosolized delivery alone. During such delivery, the aerosol droplets likely initially deposited on the epithelium or PFC surface. Any further dispersion of the aqueous drug would have relied solely on the mixing induced by tidal gas flow once ventilation was resumed. These results suggest that the increased retention of delivered drug observed during APV is likely a result of the initial dispersion of the aqueous drug throughout the PFC and the presence of fluorosurfactant. Although ventilation with PFC has been shown to alter the distribution of pulmonary perfusion,⁽²²⁾ the similar lack of retention between aerosolized delivery alone and in the presence of PFC would suggest that PFC-induced

differences in pulmonary blood flow are likely not the primary cause of the retention associated with APV.

Regarding effects of emulsion formulation, increasing either V_{aq} or C_{fs} was shown to increase pulmonary retention, although the effect of V_{aq} was much stronger. A five-fold increase in V_{aq} (and corresponding five-fold decrease in C_t) resulted in a nearly identical increase in pulmonary tobramycin content 4 hours post-delivery. In the case of C_{fs} , an approximately three-fold increase resulted in a less than 50% increase in pulmonary retention. These results indicate that increased V_{aq} in combination with decreased C_t slows diffusion of tobramycin from the airways into the pulmonary circulation.

Antibiotics are believed to move between the bronchial space and blood at a rate dependent on the concentration gradient between the two compartments.^(2,3) During APV, as aqueous droplets come in to contact and combine with the aqueous lining of the airways, the local antibiotic concentration near the epithelium increases thus increasing diffusion into the blood. The rate at which this occurs is determined by both the frequency of droplet deposition as well as the antibiotic concentration within each droplet (i.e., C_t). An increase in V_{aq} increases the number of droplets and as such could increase the frequency of droplet deposition. However, these results suggest that increased V_{aq} does not increase droplet deposition enough to compensate for the decreased antibiotic mass within each droplet, leading to an overall slowed diffusion for increased V_{aq} and decreased C_t .

Although not as profound as with V_{aq} , increases in C_{fs} also showed increased pulmonary antibiotic retention at 4 hours post-delivery. Additionally, serum tobramycin concentrations resulting from the largest C_{fs} value (48 mg/mL H₂O) were significantly lower than those at 15 mg/mL H₂O, further suggesting delayed antibiotic absorption into the pulmonary circulation for increased C_{fs} . The cause of this diminished absorption at increased values of C_{fs} is not yet fully understood.

In vitro assessment of emulsion stability showed no difference between C_{fs} values of 15 and 48 mg/mL H₂O (both emulsions exhibited uniform antibiotic distribution) and thus suggests that the observed pharmacokinetic effect of C_{fs} is likely not due to gravity-driven phase separation. However, it is possible that coalescence and creaming is altered for emulsion in the airways at physiological temperatures under constant ventilation as compared to our *in vitro* experiment. Increased phase separation within emulsion in the airways for the 15 mg/mL H₂O group could enhance and accelerate contact between tobramycin within the bulk of the emulsion and epithelial surfaces through which it may ultimately leave the lungs.

Alternatively, the diminished absorption effects could be due to the accumulation of fluorosurfactant at the interface between the emulsion and airway aqueous lining. The fluorosurfactant molecules possess both hydrophilic and fluorophilic moieties and thus aggregate at aqueous-PFC interfaces. It is possible that some amount of fluorosurfactant accumulates at the boundary of the lung aqueous lining and impairs either the deposition of droplets or the diffusion of tobramycin following deposition. Further in-depth studies are needed to fully understand the effects of fluorosurfactant on drug transport from the emulsion in a physiological setting.

Considering the results of this study, the optimal emulsion formulation for APV may utilize a significantly reduced

C_{fs} value as compared to previous work (C_{fs} = 48 mg/mL H_2O in previous studies).⁽⁷⁾ Additionally, pulmonary retention of the delivered antibiotic can be most effectively increased by increasing V_{aq} and decreasing C_t to achieve the desired delivery dose. However, it is unknown whether the rate of tobramycin delivery from the airway to the tissue has a significant effect on anti-biofilm activity and thus treatment efficacy. Studies comparing rapid, nearly complete delivery of the antibiotic must be compared to slower, controlled delivery. Whatever the outcome, the ability to reliably control such kinetics represents an attractive quality of pulmonary drug delivery via PFC emulsions.

There are multiple factors to consider when choosing an optimal V_{aq} and C_t for treatment. As C_t is decreased, a scenario could be approached in which the aqueous concentration of antibiotics in the lung during APV is less than that required for bacterial killing. A previous study with APV has shown that emulsions utilizing C_t values as low as 4 mg/mL (five-fold smaller than lowest C_t value used in present study) were still able to reduce proliferation of biofilm-derived bacteria following exposure.⁽⁷⁾

Additionally, as V_{aq} is increased, larger aqueous volumes will be delivered to the lung during APV. Therefore caution should be used when determining V_{aq} as to not increase this value to the point of inhibiting normal respiratory function. If the V_{aq} values evaluated in this study (1%–5%) were to be used clinically during partial APV with a 15 mL/kg dose of emulsion (dose used in previous clinical trials of partial liquid ventilation), a 70-kg patient would receive approximately 10–50 mL of aqueous antibiotic delivered to the lung. However, at the conclusion of treatment, some portion of the emulsion would be suctioned from the lung. Conservatively, estimating that only one-third of the emulsion volume would be removed from the lung, 7–35 mL of aqueous antibiotic would ultimately remain in the lung. During currently used nebulized tobramycin treatment for CF patients, approximately 10 mL of aqueous antibiotics are delivered to the lung on a daily basis.⁽²⁴⁾ Considering that such treatment is generally well tolerated without supplemental oxygen or ventilatory support, the range of V_{aq} values used in this work are likely within reason for a patient on mechanical ventilation.

The current study has confirmed the use of APV as a viable and effective means of pulmonary antibiotic delivery and taken critical steps in further developing this emerging technology. APV utilizing an optimized emulsion formulation should result in increased pulmonary retention, decreased and delayed peak serum concentrations, as well as improved dispersion of delivered antibiotic relative to aerosolized delivery. Future studies should evaluate the efficacy of an optimized emulsion during APV in an animal model of lower respiratory infection, as well as the biocompatibility of the used fluorosurfactants and any others available.

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