

Characterization of a Reverse-Phase Perfluorocarbon Emulsion for the Pulmonary Delivery of Tobramycin

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Abstract

Background: Aerosolized delivery of antibiotics is hindered by poor penetration within distal and plugged airways. Antibacterial perfluorocarbon ventilation (APV) is a proposed solution in which the lungs are partially or totally filled with perfluorocarbon (PFC) containing emulsified antibiotics. The purpose of this study was to evaluate emulsion stability and rheological, antibacterial, and pharmacokinetic characteristics.

Methods: This study examined emulsion aqueous droplet diameter and number density over 24 hr and emulsion and neat PFC viscosity and surface tension. Additionally, *Pseudomonas aeruginosa* biofilm growth was measured after 2-hr exposure to emulsion with variable aqueous volume percentages (0.25, 1, and 2.5%) and aqueous tobramycin concentrations ($C_a=0.4, 4, \text{ and } 40 \text{ mg/mL}$). Lastly, the time course of serum and pulmonary tobramycin concentrations was evaluated following APV and conventional aerosolized delivery of tobramycin in rats.

Results: The initial aqueous droplet diameter averaged $1.9 \pm 0.2 \mu\text{m}$ with little change over time. Initial aqueous droplet number density averaged $3.5 \pm 1.7 \times 10^9$ droplets/mL with a significant ($p < 0.01$) decrease over time. Emulsion and PFC viscosity were not significantly different, averaging $1.22 \pm 0.03 \times 10^{-3} \text{ Pa}\cdot\text{sec}$. The surface tensions of PFC and emulsion were $15.0 \pm 0.1 \times 10^{-3}$ and $14.6 \pm 0.6 \times 10^{-3} \text{ N/m}$, respectively, and the aqueous interfacial tensions were $46.7 \pm 0.3 \times 10^{-3}$ and $26.9 \pm 11.0 \times 10^{-3} \text{ N/m}$ ($p < 0.01$), respectively. Biofilm growth decreased markedly with increasing C_a and, to a lesser extent, aqueous volume percentage. Tobramycin delivered via APV yielded 2.5 and 10 times larger pulmonary concentrations at 1 and 4 hr post delivery, respectively, and significantly ($p < 0.05$) lower serum concentrations compared with aerosolized delivery.

Conclusions: The emulsion is bactericidal, retains the rheology necessary for pulmonary delivery, is sufficiently stable for this application, and results in increased pulmonary retention of the antibiotic.

Key words: liquid ventilation, respiratory infection, pulmonary antibiotic delivery

Introduction

BACTERIAL BIOFILMS are a key pathologic feature of chronic airway infections and an important cause of morbidity.^(1–3) Limited diffusion from the epithelial lining fluid into the biofilm mass and enhanced resistance make biofilm-based infections refractory to the concentration of antibiotics typically achieved with systemic administration.⁽⁴⁾ Inhaled aerosolized antibiotics have shown the ability to achieve higher intrapulmonary antibiotic concentrations while limiting systemic toxicity and, as a result, have established an important role in treatment.^(4–6) However, there are

still many shortcomings associated with inhaled antibiotics in the treatment of bacterial respiratory infections, making aerosolized delivery less than ideal. High sputum production by the infected host and poor ventilation in lung regions with the largest infectious burden can cause ineffective delivery to these areas.^(6,7) In addition, the narrow range of aerosolized particle sizes resulting in effective penetration of the lower airways presents further difficulties.^(7,8) The nonuniform intrapulmonary distribution of delivered antibiotics resulting from these challenges can hamper infection clearance as well as promote the development of antibiotic resistance.⁽⁹⁾ For these reasons, a modified method of antibiotic delivery to the

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lungs resulting in more spatially uniform distribution and increased pulmonary residence time could significantly improve the treatment of chronic respiratory infections.^(6,10)

Antibacterial perfluorocarbon ventilation (APV) is thus proposed as an adjunct to traditional systemic or inhaled antibiotic therapy. During APV, the lung is filled with a liquid perfluorocarbon (PFC) containing emulsified antibiotic (*i.e.*, water-in-PFC) and ventilated for a few hours. This can be done by either partially filling the lung and then ventilating with gas (partial APV) or fully filling the lung and ventilating with liquid PFC (total APV). Although APV could be used in a variety of respiratory infection cases, it would be best suited for patients already on a ventilator due to the need for the patient to be intubated during the therapy. Respiratory bacterial infections in cystic fibrosis, chronic obstructive pulmonary disease, and bronchiectasis are common and often exacerbate the disease state, thus often requiring the patient to be mechanically ventilated. Such patient groups could greatly benefit from APV with very little added procedures or discomfort. If successful in this setting, the technique might also be attempted to treat persistent infections in these same patients. Here, prevention of recurrent exacerbations might offset the undesirable necessity of sedating the patient for APV.

In theory, APV could improve antibiotic therapy in several ways. First, antibiotic is delivered directly to the source of infection, yielding higher concentrations in the lung and lower systemic concentrations. Second, PFCs have anti-inflammatory properties that may promote lung healing and improve mucociliary clearance.^(11–15) Lastly, tidal PFC flow during ventilation can actively detach infected mucus from the airway walls due to fluid shear, reduced surface tension, and buoyancy. The mucus can then be transported from the lungs via suction (partial APV) or the ventilating emulsion (total APV). Although this article is confined to the delivery of antibiotics, PFC-based drug delivery in general may offer a treatment advantage for any condition in which improved drug penetration is desired.

Ventilation with PFC liquids has been previously used as a means of respiratory support. In this application, it has proven able to remove mucus and inflammatory exudates,^(16–18) to distribute uniformly within injured lungs, and to provide respiratory support in animal models^(19–22) and in humans with acute respiratory distress syndrome.^(17,23–25) PFC-based antibiotic delivery has been previously explored, primarily through the use of solid particle suspensions.^(26–28) Although ventilation with such suspensions has shown some promise, it has failed to demonstrate treatment benefit over systemic antibiotic delivery in conjunction with liquid ventilation.⁽²⁷⁾ In the current study, we examined the stability and rheological, anti-biofilm, and pharmacokinetic characteristics of an emulsion consisting of aqueous tobramycin in a PFC vehicle. To be effective in its intended application, the emulsion must (i) be effective against typical airway biofilm pathogens such as *Pseudomonas aeruginosa*, (ii) be stable over the intended 2-hr duration of treatment, and (iii) maintain surface tensions, aqueous interfacial tensions, and viscosity similar to those of neat PFC. Low surface tension and aqueous interfacial tension are necessary for the liquid to penetrate otherwise plugged airways and aid in mucus detachment.⁽²⁹⁾ The viscosity of PFCs has significant effects on our ability to adequately ventilate the lungs⁽³⁰⁾ and the shear stress-induced disruption of infected mucus lining the airways.

Materials and Methods

Tobramycin-PFC emulsion formulation

A perfluorinated polyether-polyethylene glycol (PEG) block copolymer surfactant (Fig. 1A), was synthesized as previously described.⁽³¹⁾ The emulsion was prepared by first dissolving 6 mg of Krytox 157 FSL (poly[oxy]trifluoro (trifluoromethyl)-1,2-ethanediyl], α -(1-carboxy-1,2,2,2-tetra fluoroethyl)- ω -[tetrafluoro(trifluoromethyl)ethoxy]-, CAS no. 51798-33-5; DuPont, Wilmington, DE) and 6 mg of Krytox-PEG copolymer in 0.6 mL of PFC liquid. The PFC used in all experiments was perfluorocycloether/perfluorooctane (FC-770; 3M Inc., St. Paul, MN). FC-770 has been used in previously published studies on partial liquid ventilation.^(32–34) For eventual clinical translation, a PFC with a higher level of purity and larger amount of documented *in vivo* safety data would be used. Next, 125 μ L of 0.4, 4, or 40 mg/mL tobramycin (Abraxis Pharmaceutical Products, Schaumburg, IL) in sterile saline was added. The mixture was emulsified via sonication (model 450, 20 kHz, 3.2 mm diameter; Branson, Danbury, CT) at 200 W/cm² for 60 sec in continuous mode. PFC was then added to the emulsion to a final volume of 5 mL and was sonicated for an additional 10 sec to disperse the aqueous droplets in the PFC phase. Additional PFC was then added to the emulsion to create the varying aqueous volume percentages examined.

Particle size and number

The number and size distribution of aqueous droplets in an emulsion containing 2.5% aqueous volume were acquired via photomicrography using a hemacytometer (Brightline, Hausser Scientific, Horsham, PA). Images were digitally acquired using Spot Advanced Software (Diagnostic Instruments Inc., Sterling Heights, MI) and processed in MATLAB (The MathWorks Inc., Natick, MA) to determine the number of droplets per volume and their size distribution. To determine emulsion stability, analysis was repeated 1, 2, and 24 hr after preparation. Five different optical fields of view (FOV), each containing a minimum of 500 droplets per FOV, were analyzed to determine the droplet number density and size distribution at each time point. This manual droplet counting/sizing method has been used with similarly sized emulsions and shown to produce results not statistically different from those obtained using a Coulter counter.^(31,35)

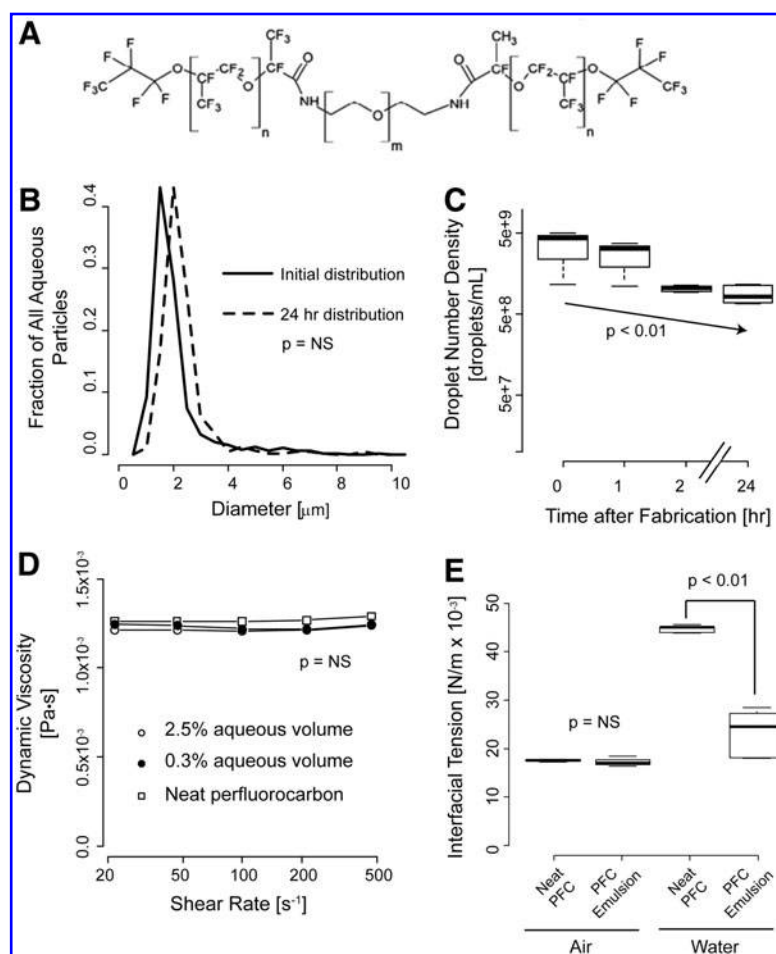
Emulsion viscosity

The dynamic viscosities of neat PFC and emulsions with aqueous volume percentages ranging from 0.3 to 2.5% were measured using a cone and plate rheometer (AR 1000; TA Instruments, New Castle, DE) at 37°C. Samples were interrogated over a range of shear rates from 20 to 500 sec⁻¹, capturing a range of values estimated to be achieved at the airway epithelium during total liquid ventilation. Three repeated measurements were taken for each set of conditions examined.

Emulsion surface tension and aqueous interfacial tension

The surface tension and aqueous-emulsion interfacial tension of an emulsion containing 2.5% aqueous volume, as well as the corresponding properties of neat PFC, were

FIG. 1. (A) Perfluorinated polyether-polyethylene glycol (PEG) block copolymer surfactant ($n=13$ and $m=75$) used to stabilize water:PFC interface. (B) Size distribution of aqueous droplets in reverse PFC emulsion initially and 24 hr after formulation. (C) Number density of aqueous droplets over time. (D) Viscosity over differing shear rates for neat PFC and emulsions with different aqueous volume percentages. (E) Surface tensions and aqueous interfacial tensions of neat PFC and emulsion.



measured using a DuNouy ring tensiometer with a platinum-iridium ring (6 cm circumference; Interfacial Tensiometer; CSC Scientific Company Inc., Fairfax, VA). Immediately after emulsion preparation, 10–15 mL of emulsion or neat PFC was placed in a petri dish and allowed to reach room temperature (22–25°C) before being used for measurements. For aqueous-emulsion or aqueous-PFC interfacial tension measurements, 10 mL of deionized, filtered water was introduced on top of the emulsion or neat PFC. Three repeated measurements were taken for each set of conditions examined.

Anti-biofilm activity

Pseudomonas aeruginosa strain ATCC 27853 was generously provided by David McLeod (Gilead Laboratories, Foster City, CA). This strain is a cystic fibrosis-derived strain selected for its biofilm-forming capacity. Biofilms were grown on solid-phase transfer pin lids as described elsewhere⁽³⁶⁾ and as summarized in Figure 2A. Bacteria were first grown in tryptic soy broth (TSB) with 1% glucose at 37°C on a gyratory shaker. A solution of approximately 10^8 colony forming units (CFU) per milliliter of mid-log growth *P. aeruginosa* in TSB with 1% glucose was then placed into wells of a 96-well plate (150 μL /well). A solid-phase transfer pin lid (Nunc-TSP 445497; Nunc, Rochester, NY) was then placed such that pins were immersed in the bacterial broth. Trays were incubated for 18 hr at 37°C

without shaking. Pin-adherent biofilms were then exposed to the emulsions, neat PFC, aqueous antibiotics, or growth medium by transferring the lid to a new 96-well plate. All emulsions in Table 1 were examined. After 2 hr of exposure, biofilms were centrifuged for 20 min at 840 g into fresh growth medium (TSB with 1% glucose). Because centrifugally released biofilm material could not be reliably separated into individual cells to perform CFU counting, bacterial viability from recovered biofilms was quantified as the change in broth turbidity over 6 hr. Luria-Bertani agar cultures were used to confirm complete biofilm killing in cases where no change in broth turbidity occurred over 6 hr of observation. Sixteen wells were used for each type of exposure, resulting in 16 repeated measurements for each set of conditions examined.

Pharmacokinetic characterization

Specific pathogen-free, male Sprague Dawley rats [$n=20$, weight 401 ± 14 (SD) g; Charles River Laboratories, Wilmington, MA] were divided into the following groups: partial APV treatment ($n=10$) and aerosolized treatment ($n=10$). All rats were initially anesthetized with a mixture of ketamine hydrochloride [50 mg/kg, intraperitoneal (IP); Hospira Inc., Lake Forest, IL] and xylazine hydrochloride (5 mg/kg, IP; Lloyd Laboratories, Shenandoah, IA). Rats receiving aerosolized treatment were then placed in a prone position and orally intubated with a 16-G angiocatheter.

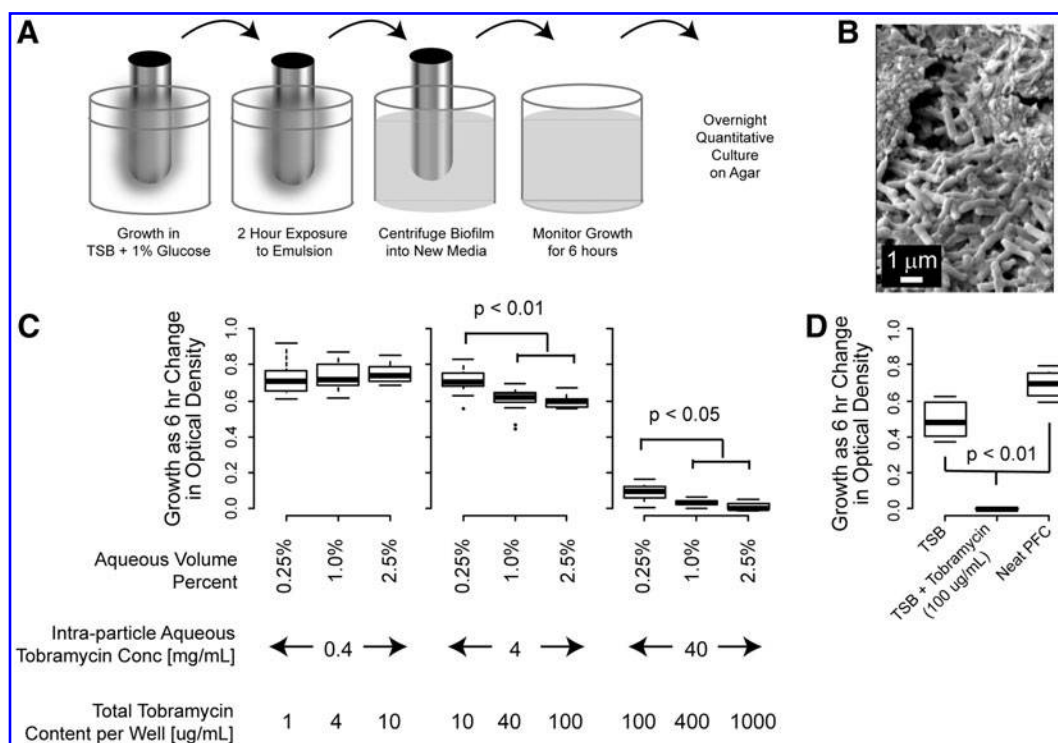


FIG. 2. (A) Sequence of steps in biofilm exposure experiment. (B) Scanning electron micrograph (10,000 \times) of typical biofilm. (C) Six-hour biofilm growth after 2-hr exposure to emulsions with different aqueous volume percentages and different aqueous particle tobramycin concentrations. (D) Six-hour biofilm growth after 2-hr exposure to TSB alone, tobramycin in TSB (100 $\mu\text{g/mL}$), or neat PFC.

Aerosolized delivery was accomplished via a Microsprayer Aerosolizer (Model IA-1B; Penn-Century, Wyndmoor, PA). 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 (15 mg/kg in 250 μL of sterile saline) was delivered intratracheally. Following delivery, rats were extubated and allowed to recover under supplemental oxygen provided via a nose cone. For rats

TABLE 1. EMULSION FORMULATIONS

Aqueous volume percent	Krytox and Krytox copolymer concentration (mg/mL emulsion)	Aqueous tobramycin concentration (mg/mL H ₂ O)	Total tobramycin concentration ($\mu\text{g/mL}$ emulsion)
0.25	0.12	0.4	1
1.0	0.48	0.4	4
2.5	1.2	0.4	10
0.25	0.12	4	10
1.0	0.48	4	40
2.5	1.2	4	100
0.25	0.12	40	100
1.0	0.48	40	400
2.5	1.2	40	1,000

Formulations of all emulsions were examined for anti-biofilm activity.

receiving partial APV, intravenous (IV) access was acquired via the lateral tail vein following initial anesthesia. Sedation was maintained with a constant IV infusion of ketamine hydrochloride (0.6 mg/kg/min). Heart rate and arterial oxygen saturation were monitored via a pulse oximeter (VetOx Plus 4800; Heska, Loveland, CO). Rats were orally intubated with a 16-G angiocatheter and connected to a ventilator (Model 683; Harvard Apparatus, Holliston, MA). Ventilation was carried out with a tidal volume of 9 mL/kg, a respiratory rate of 50–80 breaths/min, a positive end-expiratory pressure of 3 cm H₂O, and a fraction of inspired oxygen (FiO₂) of 1. Respiratory rate was adjusted to maintain peak inspiratory pressures less than 30 cm H₂O. Preoxygenated emulsion (15 mL/kg, 2.5% aqueous by volume, 40 mg/mL aqueous tobramycin concentration) was instilled via a port connected to the angiocatheter, resulting in a delivered dose of 15 mg/kg tobramycin. The emulsion was prepared no more than 20 min before use and was instilled slowly over a period of less than 3 min without interrupting gas ventilation. Gas ventilation was continued for 60 min following delivery of the emulsion, after which the rats were either euthanized or allowed to recover under supplemental oxygen.

Rats were euthanized with pentobarbital sodium (175 mg/kg, IP, Fatal Plus; Vortech Pharmaceuticals, Dearborn, MI) and the lungs removed aseptically at either 1 hr ($n=5$ for each treatment group) or 4 hr ($n=5$ for each treatment group) following the delivery of tobramycin. In the 4-hr groups, blood samples were also drawn via the lateral tail vein at 30, 60, 120, and 240 min following the delivery of tobramycin. Serum tobramycin concentration measurements were

performed via an immunoassay by the Department of Pathology within the University of Michigan Hospital. Lung tissue was homogenized with 10 mL of sterile saline, and the presence of active tobramycin in the homogenate was measured via a microbiological assay as previously described⁽³⁷⁾ with the following exception: seeded agar plates were prepared by inoculation of the surface of Luria-Bertani agar plates with 750 μ L of diluted culture. The lower limit of detection using these methods was 5 μ g/mL. Any result below this limit was assigned a value of 5 μ g/mL as a conservative estimate (see Results). In addition to the tobramycin-treated samples, lung tissue from untreated rats and rats receiving an emulsion containing only sterile saline in the aqueous phase was examined as a negative control.

Statistical analysis

SPSS (IBM Corporation, Armonk, NY) was used to perform one- or two-way ANOVA and *post-hoc t* tests on comparisons of interest. The Tukey-Kramer method was applied to differentiate significant differences between groups. Repeated-measures ANOVA was used to determine the effect of treatment mode on serum tobramycin concentration with time after delivery used as the independent, repeated variable. Differences were deemed significant for $p < 0.01$ unless otherwise specified. All subsequent reported values are given as means \pm SD unless otherwise specified.

Results

Emulsion physical characteristics

The aqueous droplet diameter, aqueous droplet number density, emulsion viscosity, emulsion surface tension, and aqueous-emulsion interfacial tension data are shown in Figure 1B–E. Emulsions containing 2.5% aqueous volume had an initial mean aqueous droplet diameter of $1.9 \pm 0.2 \mu\text{m}$ and droplet number density of $3.5 \pm 1.7 \times 10^9$ droplets/mL. Aqueous droplet diameter was shown to be stable, with no significant change between 0 and 24 hr. Aqueous droplet number density was shown to significantly change over time, exhibiting a nearly half-log decrease over 24 hr. The emulsions and neat PFC exhibited Newtonian behavior with constant viscosities over the range of shear rates examined

(20–500 sec^{-1}). No significant differences in viscosity were seen between neat PFC and emulsions. Over all of the shear rates tested, the ranges of average viscosities were $1.22\text{--}1.32 \times 10^{-3}$, $1.21\text{--}1.34 \times 10^{-3}$, and $1.26\text{--}1.37 \times 10^{-3}$ Pa \cdot sec for emulsions with 2.5, 0.3, and 0% (neat PFC) aqueous volume, respectively. Lastly, the mean surface tensions of neat PFC and emulsion (2.5% aqueous by volume) were $15.0 \pm 0.1 \times 10^{-3}$ and $14.6 \pm 0.6 \times 10^{-3}$ N/m, respectively. The mean aqueous-PFC and aqueous-emulsion interfacial tensions were $46.7 \pm 0.3 \times 10^{-3}$ and $26.9 \pm 11.0 \times 10^{-3}$ N/m ($p < 0.01$) for neat PFC and emulsion (2.5% aqueous by volume), respectively.

Anti-biofilm activity

A typical biofilm recovered from an immersed peg is shown in Figure 2B. As shown in Figure 2C and D, tobramycin delivered via emulsion was capable of significantly limiting or altogether preventing growth in biofilm-derived *P. aeruginosa* after a 2-hr exposure. Intraparticle aqueous tobramycin concentrations of at least 4 mg/mL were necessary to reduce the proliferation of biofilm-derived bacteria following exposure, and bactericidal effects were achieved only with aqueous tobramycin concentrations of 40 mg/mL at the highest aqueous volume percentage (2.5%). Under these conditions, the total well concentration of tobramycin that the biofilm was exposed to was 1,000 μ g/mL, and eight of 16 replicates had no growth on follow-up culture at 24 hr. It is important to note that a mean inhibitory concentration cannot be determined in a traditional way with this emulsion, as bacterial growth is a function of both the overall antibiotic concentration and that within an individual droplet.

Pharmacokinetic characterization

Lung tissue from an untreated rat and a rat receiving ventilation with an emulsion containing only saline in the aqueous phase produced no distinguishable zones of inhibition. As shown in Figure 3A, tobramycin delivered via partial APV resulted in significantly higher concentrations in the lung tissue at both 1 and 4 hr post delivery relative to aerosolized treatment. Mean lung tissue homogenate tobramycin concentrations at 1 hr post delivery were 197 ± 36 and

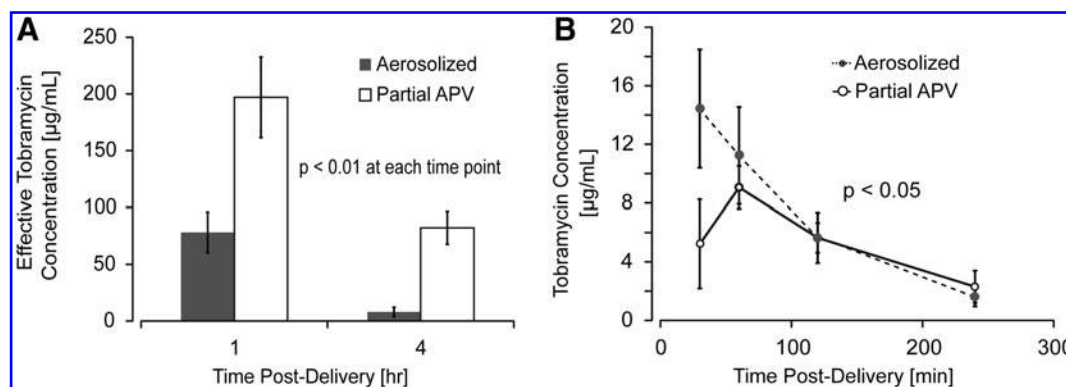


FIG. 3. (A) Effective tobramycin concentration of lung tissue homogenate (lung tissue + sterile saline) at 1 and 4 hr following tobramycin delivery via partial APV (white) and aerosolized delivery (shaded). (B) Tobramycin concentration of serum at 30, 60, 120, and 240 min following tobramycin delivery via partial APV (solid line) and aerosolized delivery (dotted line). Errors bars represent standard deviations.

$78 \pm 18 \mu\text{g/mL}$ for partial APV and aerosolized treatment, respectively. Mean lung tissue homogenate tobramycin concentrations at 4 hr post delivery were 82 ± 14 and $8 \pm 4 \mu\text{g/mL}$ for partial APV and aerosolized treatment, respectively. Furthermore, three of five measurements in the aerosolized group at 4 hr post delivery resulted in concentrations below the lower limit of detection ($5 \mu\text{g/mL}$). A two-way ANOVA showed a statistically significant effect of treatment mode (partial APV or aerosolized delivery; $p < 0.01$) and time post delivery (1 or 4 hr; $p < 0.01$) on lung tissue homogenate tobramycin concentrations. Serum tobramycin concentrations from rats receiving tobramycin delivered via partial APV or aerosolized treatment are shown in Figure 3B. A repeated-measures ANOVA showed that tobramycin delivery via partial APV produced significantly ($p < 0.05$) lower serum concentrations relative to aerosolized delivery. Maximum measured serum tobramycin concentrations for partial APV and aerosolized treatments were 9.1 ± 1.5 and $14.4 \pm 4.0 \mu\text{g/mL}$ and occurred at 60 and 30 min, respectively.

Discussion

In the current work, we demonstrated the feasibility of formulating a PFC emulsion containing tobramycin and its effectiveness as an antibiotic delivery vehicle. In order for this emulsion to be effective, it must be relatively stable over the likely application duration (< 2 hr), preserve PFC's viscosity and low interfacial tensions that allow for effective ventilation, and be bactericidal. Our emulsion has met these conditions. The sonication process for generating the emulsion is straightforward and could readily be performed just prior to administration, similar to the activation of ultrasound contrast agents [e.g., Definity (Lantheus Medical Imaging) or Optison (GE Healthcare)] or preparation of drug-laden emulsions used in chemoembolization.⁽³⁸⁾

Aqueous droplet size was sufficiently stable over a period of 24 hr, whereas a significant decrease in aqueous droplet number density was observed over the same period. Multiple destabilization phenomena, including Ostwald ripening and coalescence, could be responsible for these observations. Coalescence of aqueous droplets within the emulsion would directly increase mean droplet size as well as decrease droplet number density. During Ostwald ripening, larger droplets will tend to grow in size at the expense of smaller droplets, which decrease in size. Because the optical methods used to size the droplets cannot account for nanometer-sized droplets, it is possible that Ostwald ripening caused some small, but initially countable droplets to become undetectable at later time points. This would cause even a small increase in mean droplet size to correlate to a large decrease in the measurable droplet number density.

The viscosity of the emulsion compared with that of neat PFC was not substantially different. This is not surprising given the low volumetric fraction of aqueous phase in the emulsion (0.3–2.5%). This range of aqueous volume percentages examined was determined by considering the targeted mass of drug to be delivered relative to the lung fill volumes used in these trials (functional residual capacity). The surface tension of the emulsion did not significantly differ from that of neat PFC, whereas the aqueous-emulsion interfacial tension was shown to be considerably less than the aqueous-PFC interfacial tension. This behavior is likely

due to the addition of surfactant when forming the emulsion. Due to their hydrophilic and fluorophilic moieties, the surfactants likely accumulate at the emulsion–aqueous interface, causing a decrease in the interfacial tension. These results show that the emulsion exhibits the low surface tension of PFCs that allows them to penetrate small, branching airways and alveoli during liquid ventilation and an even lower aqueous interfacial tension (relative to neat PFC) that should enhance mucus removal.^(39,40) First, low aqueous-PFC interfacial tension allows the PFC to more effectively penetrate airways that are partially or fully plugged with mucus. Second, it reduces the adhesivity of the mucus during liquid ventilation, resulting in mucus detachment at lower flow rates and shear stresses.^(29,39)

As expected, the tobramycin concentration needed within the aqueous phase to achieve bacterial killing against biofilms was substantially higher than the measured planktonic mean bactericidal concentration against this strain ($25 \mu\text{g/mL}$; data not shown). The reasons are likely twofold. First, antibiotic activity against biofilms is known to be reduced. Second, the physical means of antibiotic delivery from the droplets to the biofilm is not yet understood and may be reducing antibiotic effectiveness.

It is worth noting that although the aqueous phase concentrations of tobramycin are quite high, the total dose administered to a patient using this technology is similar to currently used doses. If the highest concentration and aqueous volume percentage in our study were to be used clinically (40 mg/mL aqueous phase tobramycin concentration, 2.5% aqueous by volume), a 70-kg patient undergoing partial APV with a 15 mL/kg dose of the emulsion (the dose used in previous clinical trials of partial liquid ventilation) would receive approximately 1 g of tobramycin instilled into the lung. However, at the completion of therapy, a significant amount of the emulsion would be drained from the lung. Conservatively, estimating that 10 mL/kg is left behind, the delivered dose would be 700 mg, only slightly larger than the currently recommended 600 mg daily inhaled dose.⁽⁴¹⁾ However, the fraction of the administered dose actually delivered to the lungs may be higher during APV than aerosolized delivery due to aerosolized drug loss via exhaled drug as well as deposition in the oropharyngeal region and delivery device. It should be noted that the *in vivo* efficacy of different emulsion formulations has not yet been determined. The ideal aqueous volume percentage, aqueous phase tobramycin concentration, and fill volumes are all likely to change based on their *in vivo* efficacy.

Based on these results, delivery of the same tobramycin dose via emulsion may lead to lower toxicity than aerosolized delivery. Pulmonary retention of the delivered tobramycin was significantly greater for partial APV than aerosolized delivery (2.5 times larger at 1 hr post delivery and 10 times larger at 4 hr post delivery). As a result, peak serum concentrations resulting from partial APV were smaller and occurred at a later time point relative to aerosolized treatment, suggesting delayed absorption of drug into the pulmonary circulation. Clearance of tobramycin from the systemic circulation after approximately 60 min post delivery appears to be similar between the two modes of treatment. The difference in pharmacokinetics observed between the two routes of administration could also be due

in part to a difference in the mass of drug reaching the lungs. Aerosolized delivery in this study was performed with the Microsprayer nozzle positioned within the rat trachea. Although this technique likely results in much greater lung deposition than shown with clinical devices in humans, it may still result in some drug deposition in the trachea, thereby potentially reducing the fraction of drug reaching the lungs.

The physical means by which antibiotic is transferred from aqueous droplets within the emulsion to the aqueous surfaces of the lung (epithelial lining fluid or biofilm mass) is not yet understood and warrants further investigation. The bactericidal effects resulting from biofilm exposure to emulsions as well as the presence of tobramycin in the systemic circulation following APV treatment indicate that the antibiotic is available and delivery is achieved. A better understanding of this process would allow for easier optimization of the emulsion and therapy in order to achieve maximum treatment benefit.

Lastly, the long-term safety of the fluorinated surfactants used (Krytox 157 FSL and Krytox-PEG copolymer) was not assessed in this work. Although multiple *in vitro* studies have shown biocompatibility of nonionic Krytox-PEG copolymer surfactants with mammalian cells,^(42–44) the biocompatibility in an application such as APV has not been assessed. Accordingly, future work should examine the long-term, *in vivo* biocompatibility of these surfactants when used during APV, as well as the possible use of other suitable surfactants. In this study, excess surfactant was used to ensure stability. Future work will examine the degree to which this concentration can be reduced while still retaining emulsion stability and bactericidal effectiveness. This includes (i) finding the minimum concentration of surfactant needed to stabilize specific aqueous volume percentages and (ii) reducing total surfactant using more concentrated aqueous tobramycin at a smaller aqueous volume percentage.

In conclusion, the tobramycin-PFC reverse-phase emulsion exhibits a stable droplet size over a period of 24 hr, maintains the same viscosity as neat PFC, retains a low surface tension, creates an even lower aqueous interfacial tension than that of neat PFC, and is capable of significantly lowering bacterial growth. In addition, delivery of tobramycin via partial APV results in enhanced pulmonary retention as well as delayed absorption into the systemic circulation relative to aerosolized delivery. Therefore, this emulsion may provide a novel treatment for lower respiratory infections during APV through a two-pronged approach: biofilm and mucus removal and simultaneous antibiotic delivery. Future investigations are needed to evaluate this treatment in animal models of lower respiratory infections.

Acknowledgments

This work was supported by a research fellowship grant from the Society for Academic Emergency Medicine (C.I.B.), The Ford Foundation (R.A.O.), and NIH grants GM081702 (J.G.Y., L.P.) and R03AI096029.

Author Disclosure Statement

The authors declare that there are no conflicts of interest.

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Received on April 30, 2013
in final form, November 11, 2013

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