Colloids and Surfaces B: Biointerfaces 169 (2018) 411–417

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

In vitro evaluation of lysophosphatidic acid delivery via reverse perfluorocarbon emulsions to enhance alveolar epithelial repair

Diane L. Nelson[a,*], Yutong Zhao[b], Mario L. Fabiilli[c], Keith E. Cook[a]

[a] Department of Biomedical Engineering, Carnegie Mellon University, 5000 Forbes Avenue, Scott Hall 4th Floor, Pittsburgh, PA, 15213, USA
[b] Department of Medicine, University of Pittsburgh, Division of Pulmonary, Allergy and Critical Care Medicine, East 1200A Biomedical Science Tower, 200 Lothrop Street, Pittsburgh, PA, 15213, USA
[c] Department of Radiology, University of Michigan, 3226A Medical Sciences Building I, 1301 Catherine Street, Ann Arbor, MI, 48109, USA

ARTICLE INFO

Keywords:
Acute respiratory distress syndrome
Partial liquid ventilation
Perfluorocarbon
Lysophosphatidic acid

ABSTRACT

Background: Alveolar drug delivery is needed to enhance alveolar repair during acute respiratory distress syndrome. However, delivery of inhaled drugs is poor in this setting. Drug delivery via liquid perfluorocarbon emulsions could address this problem through better alveolar penetration and improved spatial distribution. Therefore, this study investigated the efficacy of the delivery of lysophosphatidic acid (LPA) growth factor to cultured alveolar epithelial cells via a perfluorocarbon emulsion.

Methods: Murine alveolar epithelial cells were treated for 2 h with varying concentrations (0–10 μM) of LPA delivered via aqueous solution or PFC emulsion. Cell migration was evaluated 18 h post-treatment using a scratch assay. Barrier function was evaluated 1 h post-treatment using a permeability assay. Proliferation was evaluated 72 h post-treatment using a viability assay.

Results: Partially due to emulsion creaming and stability, the effects of LPA were either diminished or completely hindered when delivered via emulsion versus aqueous. Migration increased significantly following treatment with the 10 μM emulsion ($p < 10^{-3}$), but required twice the concentration to achieve an increase similar to aqueous LPA. Both barrier function and proliferation increased following aqueous treatment, but neither were significantly affected by the emulsion.

Conclusions: The availability and thus the biological effect of LPA is significantly blunted during emulsified delivery *in vitro*, and this attenuation depends on the specific cellular function examined. Thus, the cellular level effects of drug delivery to the lungs via PFC emulsion are likely to vary based on the drug and the effect it is intended to create.

1. Introduction

Despite recent advances, improved treatments are needed for Acute Respiratory Distress Syndrome (ARDS). Acute Respiratory Distress Syndrome is characterized by severe inflammatory damage to the lung alveolar epithelium and capillary endothelium, resulting in the build-up of proteinaceous edema in the alveoli and the decrease in normal gas exchange [1]. Therefore, the ideal treatment for ARDS would both support the respiratory needs of the patient while also enhancing regeneration of the damaged alveolus and recovery of normal alveolar barrier function [2].

Low-volume, lung protective ventilation remains the primary beneficial advance in sustaining respiratory function and improving survival in ARDS by reducing lung damage [3,4]. To date, no pharmacological treatments, including delivery of intrapulmonary lung surfactant or systemic delivery of anti-inflammatory medications, have demonstrated significant reductions in mortality among adults [5]. However, potential therapeutic benefits might be achieved if drugs could be delivered directly to the injured alveoli where they are needed, achieving greater alveolar drug concentrations and lower systemic concentrations [6]. Unfortunately, inhaled drug delivery to alveoli is difficult, even in healthy adults, with only a small fraction of drug penetrating to the alveolar level [7]. ARDS further impedes inhaled delivery since the damaged, edematous regions of the lungs are poorly ventilated, making it even more difficult to deliver drugs to injured airways.

To remedy this, we propose delivering drugs to the alveolus during ARDS using a reverse water-in-perfluorocarbon (PFC) emulsion [8–12]. These emulsions contain a dispersed aqueous phase (< 2.5% by volume) that is emulsified within the liquid PFC. Any water-soluble drug can then be dissolved within the aqueous phase for delivery. The

* Corresponding author.
E-mail addresses: dlnelson@andrew.cmu.edu (D.L. Nelson), zhaoy3@upmc.edu (Y. Zhao), mfabiill@med.umich.edu (M.L. Fabiilli), keicook@andrew.cmu.edu (K.E. Cook).

https://doi.org/10.1016/j.colsurfb.2018.05.037
Received 29 August 2017; Received in revised form 15 May 2018; Accepted 16 May 2018
Available online 17 May 2018
0927-7765/

resulting emulsion is delivered to the alveolus by partially filling the lung with the emulsion and then ventilating with a standard gas ventilator over the emulsion. In a similar fashion to partial liquid ventilation [13–16], the perfluorocarbon would wash exudate from the alveoli and towards the conducting airways due to its low surface tension and high density [16–21] and enhance gas exchange. Unlike liquid ventilation, however, the emulsion would deliver drugs to actively enhance alveolar barrier function and repair.

The optimal drugs for this purpose are not yet known, but could potentially include epithelial and endothelial growth factors, anti-inflammatory drugs, antibiotics, and pulmonary surfactant. The purpose of this paper is to examine this concept through the delivery of one growth factor, lysophosphatidic acid, from PFC emulsion to lung epithelial cells. Lysophosphatidic acid (LPA), a serum-derived, phospholipid growth factor, induces epithelial cell migration and proliferation, and, most importantly, enhances barrier function [22,23]. After the inflammatory exudate has been washed up and suctioned out following treatment with PFC emulsions, an increase in barrier function would slow the influx of edema and neutrophils, allowing alveolar cells an opportunity to migrate into the wounded area and proliferate to re-establish functional tissue.

Delivery of antibiotics to biofilms via PFC emulsions has been shown to be effective in previous studies [24,25], but delivery of a growth factor to affect cellular repair and inflammation has never been attempted using a water-in-PFC single emulsion. In the current study, the effects of delivering LPA to alveolar epithelial cells via water-in-PFC emulsions were compared to that of aqueous LPA at similar concentrations using migration, proliferation, and barrier function assays in vitro.

2. Material and methods

2.1. Materials

1-oleoyl ($C_{18:1}$) LPA and fluorescein isothiocyanate–dextran (FITC-dextran; 4 kDa) were purchased from Sigma–Aldrich (Milwaukee, WI, USA). The alamar blue cell viability reagent was purchased from Life Technologies (Carlsbad, CA, USA). Bovine serum albumin (BSA) was purchased from New England Biolabs (Ipswich, MA, USA). Crystal violet (0.1% aqueous) was purchased from Ward's Science (Rochester, NY, USA). Luria-Bertani (LB) agar powder was purchased from ThermoFisher Scientific (Waltham, MA, USA). Similar to previous work with antibiotic-loaded, water-in-PFC emulsions [24,25], the PFC used was perfluorocycloether/perfluorooctane (FC-770) purchased from 3 M Inc. (St. Paul, MN, USA) and the fluorosurfactant used ("FSH-PEG") was perfluoropolyether (Krytox 157FSH oil: 7 kDa, n = 41)-polyethelene glycol (PEG: 1 kDa, m = 22)- Krytox 157FSH (i.e., FSH-PEG-FSH) triblock copolymers synthesized from Krytox 157FS oil purchased from Dupont (Wilmington, DE, USA) [26]. See Fig. 1 for the molecular structure of the FSH-PEG copolymer.

2.2. Cell culture

The murine lung epithelial cell line MLE-12 cells were purchased from A.T.C.C. (Manassas, VA, USA) and were maintained in HITES medium (Dulbecco's modified Eagle's medium/F-12 medium) complemented with 10% fetal bovine serum in a 37 °C incubator in the presence of 5% CO_2.

2.3. Preparation of aqueous solutions

Aqueous LPA solutions were prepared fresh in Dulbecco's serum-free medium with 1% BSA before experiments. Appropriate volumes of 5 mM LPA (in ethanol) were added directly into the serum-free medium + BSA to prepare 0, 1, 5, 10, 200, and 400 μM aqueous solutions. Uniform dispersion was ensured by vortexing for 2 min. A pH meter was used to ensure aqueous droplets would not be harmful to cells. Aqueous crystal violet solutions were either undiluted (i.e. 100%) or diluted to 2.5% v/v in sterile water.

2.4. PFC emulsion preparation

LPA-loaded, water-in-PFC emulsions were prepared as previously described in 5 mL batches [24]. Briefly, a mixture of PFC, aqueous LPA, and fluorosurfactant was emulsified via sonication (Model VCX 130, 20 kHz, 3.2 mm diameter microtip; Sonics & Materials, INC., Newtown, CT, USA) at 200 W cm^{-2} for 60 s. All emulsions had the same aqueous volume percent (2.5%) and aqueous concentration of fluorosurfactants (C_{fs} = 2 mg/mL of water). Aqueous LPA solutions of 0, 40, 200, and 400 μM were emulsified within the PFC to yield total concentrations of 0, 1, 5, and 10 μM LPA emulsions, respectively. Emulsion formulations are summarized in Table 1. The 0 μM emulsion, used to determine the effect of fluorosurfactant on cell behavior, was prepared the same way with a 0 μM LPA aqueous solution (serum-free media + BSA). Crystal violet-PFC emulsions were prepared by emulsifying 250 μL undiluted crystal violet in 9.75 mL of PFC and fluorosurfactant to yield a total concentration of 2.5% (v/v) emulsion.

2.5. Emulsion viscosity

The dynamic viscosities of pure PFC and the 0 μM emulsion were measured using a cone and plate rheometer (model DVII + Pro; Brookfield Engineering Laboratories, Middleboro, MA) at 37 °C as previously described [24]. Briefly, samples were interrogated at 200 s^{-1}. Three repeated measurements were taken.

2.6. Emulsion surface tension and aqueous interfacial tension

The surface and interfacial tension of pure PFC and the 0 μM emulsion were measured using a DuNouy ring tensiometer with a platinum-iridium ring (Cenco Model 70545; 6 cm circumference; CSC Scientific Company Inc., Fairfax, VA) as previously described [24]. Briefly, 0 μM emulsion and pure PFC were allowed to reach room temperature (22–25 °C) before measurements. Interfacial tension measurements, deionized, filtered water was introduced on top of the 0 μM emulsion or pure PFC. Three repeated measurements were taken for each condition.

2.7. Delivery of crystal violet to aqueous surface

To determine how emulsion creaming (separation of the less dense aqueous droplets to the top of the emulsion) affected delivery to an aqueous surface (cells, lung epithelium, etc.), LB agar (100 μL) was allowed to gel at the bottom of 2 mL microcentrifuge tubes and then exposed to 2 mL of either 2.5% aqueous or 2.5% emulsified crystal violet solutions for 2 h. The microcentrifuge tube was oriented such that the gel sat beneath, above, or adjacent to the media (see Fig. 2). After

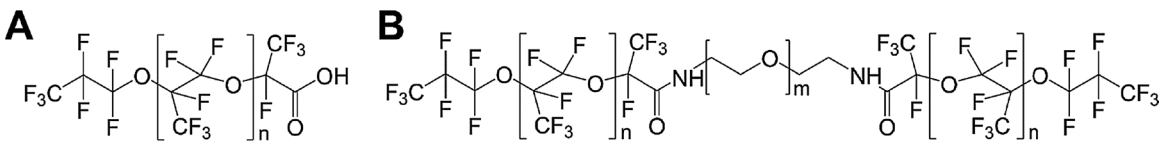

Fig. 1. A. Molecular structure of Krytox 157FS oil **B.** FSH-PEG-FSH triblock copolymer (n = 41, m = 22).