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Original Article

Pulmonary surfactant dysfunction in pediatric cystic fibrosis: Mechanisms and reversal with a lipid-sequestering drug☆,☆☆

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Abstract

Background: Airway surfactant is impaired in cystic fibrosis (CF) and associated with declines in pulmonary function. We hypothesized that surfactant dysfunction in CF is due to an excess of cholesterol with an interaction with oxidation.
Methods: Surfactant was extracted from bronchial lavage fluid from children with CF and surface tension, and lipid content, inflammatory cells and microbial flora were determined. Dysfunctional surfactant samples were re-tested with a lipid-sequestering agent, methyl-β-cyclodextrin (MβCD).
Results: CF surfactant samples were unable to sustain a normal low surface tension. MβCD restored surfactant function in a majority of samples.Mechanistic studies showed that the dysfunction was due to a combination of elevated cholesterol and an interaction with oxidized phospholipids and their pro-inflammatory hydrolysis products.
Conclusion: We confirm that CF patients have impaired airway surfactant function which could be restored with MβCD. These findings have implications for improving lung function and mitigating inflammation in patients with CF.

Keywords: Cystic fibrosis; Lung surfactant; Cholesterol, oxidative stress; Surface tension; Phospholipids; Free fatty acids; Inflammation; Infection; Genotypes

☆ Author contributions: Drs Gunasekara, Schoel, Al-Saiedy, Pratt, and Yang performed the experiments, data acquisition and analysis, and drafting of the manuscript. DrsAmrein and Green formulated the scientific question, designed the study, developed the methodology, drafted, edited the manuscript and provided final approval. Drs Bjornson, Brindle, Mitchell, and Montgomery recruited subjects, obtained consents, provided clinical input, conducted lung lavages, collected the research samples and contributed to editing and drafting the manuscript. Drs.Bjornson and Montgomery also provided genotype information on the CF patients. Ms. Keys performed the live cell and electron microscopy studies on patient samples, wrote the relevant methodology and contributed to the draft manuscript. Ms. Shrestha provided statistical support, data analysis, and revisions of the manuscript. Dr. Dennis provided data and expertise on methods for delivering cyclodextrins to patients.

☆☆ All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

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1. Introduction

Pulmonary surfactant is a protein-lipid mixture secreted by type-II alveolar epithelial cells which spread as a continuous film at the air–liquid interface and extends from the alveoli to the pharynx [1]. This continuous film maintains a surface tension gradient reaching near zero in the alveoli [2] to the trachea wherethe surface tension is approximately 32 dynes per cm [3]. Airway surfactant is a major factor that maintains small airway patency. Inflammation in distal airways with impaired surfactant function is thought to be a primary mechanism for small airway collapse in cystic fibrosis (CF)[4]. In vitro testing of pediatric CF surfactant samples, obtained largely from small airways, showed that the ability of the surfactant to maintain patency of a capillary tube was markedly reduced [4], a finding that may account for a significant degree of the airflow obstruction in CF, particularly in pediatric patients before development of fixed airway structural damage. Impairment of lung function in CF is also related to other factors including plasma phosphatidylcholine, liver phospholipid homeostasis and nutrition [5].

The current study explores the mechanisms that contribute to surfactant dysfunction in pediatric patients with CF and an approach to treat the abnormality. We compared CF patients toa group of lung-healthy children. We show that the basic abnormality in CF lung surfactant involves elevated surfactant cholesterol (likely genetically determined), with a further deleterious interaction between cholesterol and oxidized unsaturated phospholipids (likely due to infection). All of these abnormalities were reversed by methyl-β-cyclodextrin (MβCD).

2. Methods and materials

2.1. Patient population

The patient population was obtained from the Cystic Fibrosis Clinic at the Alberta Children's Hospital (Calgary, AB, Canada) where bronchial lavage fluid (BLF) is part of patient care. A total of 50 CF patients were enrolled in the study. Twenty-six of the BLF samples contained sufficient surfactant volume for functional analysis. BLFs were also obtained from 9 patients without CF, with a variety of conditions requiring investigative bronchoscopy including foreign body removal, laryngomalacia, hemoptysis and suspected immotile cilia syndrome. These subjects, called lung-healthy controls, had no evidence of current respiratory infection. Ethical approval required that only surplus BLF was used; consequently, most of the samples were small, many with insufficient surface active material for functional testing. A flow chart for the patient groups with sufficient sample for at least one test is shown in Fig. 1. Details of bronchoscopy and BLF procedure are included in online supplementary materials, Fig. S1.

Approval of the research protocol was obtained from theUniversity of Calgary Child Health Scientific Review Committee/Conjoint Health Research Ethics Board (Calgary, AB, Canada).

2.2. Cellular and microbiological analysis of BLF

Routine cell differentials and microbial culture were determined by Calgary Laboratory Services (CLS). Surplus BLF was centrifuged and live cells imaged with a Richardson RTM-3 highresolution (120 nm optical resolution) light microscope, with fluorescence capability [6].A portion of the cellular pellet was fixed in 2.5% glutaraldehyde and processed routinely for transmission electron microscopy and examined in a Hitachi H7650 electron microscope.

2.3. Biochemical characterization of BLF

An aliquot of the supernatant from the BLF was used to assay for lipid and protein content.Details of the methodology are included in the online supplementary materials.

2.4. Surface activity assessment

From each clinical sample, given the young age of this pediatric population, approximately 1 ml of BLF supernatant was available for surfactant isolation. Duplicate samples from the same patient were pooled when available. Samples were centrifuged at 400*g* for 10 min at 4 °C to separate the supernatant from the cellular components and cell debris. The supernatants were then centrifuged at 40,000*g* for 30 min at 4 °C to isolate the highly surface active large aggregate (LA) fraction, as previously described [7]. Care was taken to remove as much of the supernatant as possible and the remaining highly concentrated portion of lung surfactant was used for surface tension measurements.

Surface activity of surfactant was determined with a computer-controlled captive bubble surfactometer (CBS) [2,8]. To evaluate the effect of methyl-β-cyclodextrin (MβCD), powdered MβCD (Sigma Aldrich, Catalogue-Nr. C4555) was dissolved in HEPES buffer to a final concentration of 40 mg/ml and added to the CBS chamber prior to the addition of surfactant.

2.5. Model surfactant studies

2.5.1. Materials

Bovine Lipid Extract Surfactant (BLES) was donated by BLESBiochemicals Inc. (London, ON, Canada). 1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (PLPC) and bovine heart cardiolipin (CL, predominantly 1′,3′-Bis[1,2-dilinoleoyl-*sn*-glycero-3-phospho]-*sn*-glycerol) were purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). All lipids were stored at −20 °C under nitrogen. Surfactant mixtures were stored at 4 °C under nitrogen and used within three days. Buffers containing HEPES were stored in the dark at 4 °C to avoid generating H_2O_2.

2.5.2. In vitro oxidation

BLES was exposed to hydroxyl radicals generated from Fenton-like chemistry for 24 h to produce oxidized BLES (oxBLES) as described by Manzanares et al. [9]. PLPC and CL