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Original Article

Resolvin D1 regulates epithelial ion transport and inflammation in cystic fibrosis airways☆

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Abstract

Background: Cystic Fibrosis (CF) lung disease is characterised by dysregulated ion transport that promotes chronic bacterial infection and inflammation. The impact of the specialised pro-resolution mediator resolvin D1 (RvD1) on airway surface liquid (ASL) dynamics and innate defence had not yet been investigated in CF airways.
Methods: Ex vivo studies were performed on primary cultures of alveolar macrophages and bronchial epithelial cells from children with CF and in human bronchial epithelial cell lines; *in vivo* studies were performed in homozygous F508del-CFTR mice treated with vehicle control or RvD1 (1–100 nM).
Results: RvD1 increased the CF ASL height in human bronchial epithelium and restored the nasal trans-epithelial potential difference in CF mice by decreasing the amiloride-sensitive Na^+ absorption and stimulating CFTR-independent Cl^- secretion. RvD1 decreased TNFα induced IL-8 secretion and enhanced the phagocytic and bacterial killing capacity of human CF alveolar macrophages.
Conclusion: RvD1 resolves CF airway pathogenesis and has therapeutic potential in CF lung disease.

Keywords: Resolvin D1; Airway surface liquid layer; ENaC; F508del-CFTR; CF mice; CF alveolar macrophages

☆ Author Contributions
Conception and design of the work: FR, PMN, BJH and VU
Data collection: FR, GH, AH, AS, AM, CFT, MH
Data analysis and interpretation: FR, GH, AH, AS, AM, CFT, MH, ISG, BJH, PMN, and VU
VU, PMN and BJH supervised the PhD of FR who performed most of the *in vitro* studies
VU supervised the *in vivo* studies
Drafting the article: FR and VU
Critical revision of the article: FR, BJH, PMN, and VU
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1. Introduction

Efficient mucociliary clearance relies on adequate hydration of the airway surface liquid (ASL). This is achieved through a balance between sodium absorption, mediated by the Epithelial Sodium Channel (ENaC), and chloride secretion via CFTR and calcium-activated chloride channels. In cystic fibrosis (CF), this ion transport equilibrium is impaired, leading to a reduced ASL height that favours chronic bacterial infection and persistent inflammation [1].

Despite the robust inflammatory response, CF lungs fail to clear bacteria and are more susceptible to infections. *Pseudomonas aeruginosa* is a key CF pathogen and its early acquisition is predictive of an accelerated decline in lung function [2]. Impaired alveolar macrophage-mediated phagocytosis and bacterial killing have been reported in CF patients [3]. Moreover, one of the consequences of the excessive activation of the inflammation in patients is the production of terminal electron acceptors for anaerobic respiration that allow *P. aeruginosa* to persist and outcompete other pathogens [4]. Recent studies in young children with CF have identified neutrophil elastase, as a key risk factor for the onset and early progression of CF lung disease [5] that could contribute to Na^+ hyper-absorption in CF airways by stimulating ENaC activity [6].

Several reports provide evidence for a correlation between chronic inflammatory disease and abnormal production or activity of the specialised pro-resolution lipid mediators (SPMs) including resolvins and lipoxins [7]. Previous reports have suggested that Resolvin D1 (RvD1) is abnormally produced in CF [8,9] and there is a significant correlation between the levels of RvD1 in plasma and sputum of CF patients with the biomarkers of inflammation (IL8 and IL1β) and lung function [10].

SPMs have been shown to halt neutrophil infiltration, enhance macrophage phagocytosis of apoptotic neutrophils and attenuate NFκB activation in mouse models of lung inflammation [11]. Moreover, RvD1 promoted differentiation of alternatively activated (M2) macrophages, improved bacterial killing and the containment of a bacterial challenge in mouse models of lung infection by *P. aeruginosa* [12].

In this study, we demonstrate, that RvD1 produces restorative effects on key aspects of CF lung disease specifically; airway epithelial ion transport and surface liquid height, NFκB-mediated inflammation and CF macrophage phagocytosis activity.

2. Methods

2.1. Clinical samples

Bronchoalveolar lavage fluid (BAL) and bronchial brushings were collected through the *Study of Host Immunity and Early Lung Disease in* CF [9]. Studies were carried out in accordance with European community guidelines and approved by the Research Ethics Committee of Our Lady's Children's Hospital Crumlin (Dublin).

2.2. Human airway epithelial cell culture

Primary cultures of bronchial epithelial cells were grown from bronchial brushings or biopsies obtained from 5 healthy donors and 6 children with CF (4 F508del-CFTR homozygous and 2 F508del-CFTR heterozygous (F508del/2789 + 5G > A and F508del/H199Y)). The CF epithelia showed similar electrophysiological profiles in untreated conditions. Human bronchial epithelial cell lines were also used; Non-CF NuLi-1 and CF (F508del homozygous) CuFi-1 [13]. Epithelial cells were cultured on permeable supports under an air-liquid interface until reaching a high trans-eithelial electrical resistance, (TEER >700 Ω/cm^2) [14].

2.3. Airway surface liquid (ASL) height measurements

Texas red (2 mg/ml, Invitrogen) was applied to the ASL of bronchial epithelial cells, 24 h prior imaging and Perfluorocarbon-72 (3 M, St. Paul, USA) was added before acquisition to prevent evaporation. The ASL images were captured with a Zeiss LSM 510 Meta microscope (40×) and analysed using Zeiss LSM Image Browser. Each biological repeat represents the mean of 27 ASL height measurements per culture insert.

2.4. Nasal potential difference measurements

Nasal potential difference measurements were performed on homozygous F508del-CFTR mice (FVB/N) and their wild-type normal homozygous littermates (WT) as previously described [15] and approved by the ethics committee of Necker Hospital (Paris, France) and conformed to European Community regulations for the use of animals in research (authorization no. P2.AE.092.09). Changes in nasal V_{TE} obtained after amiloride 100 μM and low Cl^- solution perfusion reflect the ionic current contribution of Na^+ absorption via ENaC and Cl^- secretion, respectively (see on line supplement).

2.5. Short-circuit current (I_{SC}) recordings

Differentiated human bronchial epithelia were mounted in Ussing chambers and short-circuit-current SCC was measured under voltage clamp conditions and a Cl^- gradient across the epithelium (see online Supplement). The SCC decreased after amiloride (100 μM) and increased after forskolin (10 μM)/IBMX (100 μM) treatment. The use of these drugs served as an indicator of SCC changes reflecting ENaC and CFTR activity, respectively.

2.6. Enriching primary alveolar macrophages

Alveolar macrophages (AM) were isolated from the BAL of 3 CF female children (<6y, F508del homozygous), re-suspended in primary AM medium (*online data*), plated in 96 well plates and incubated (humidified, 37.2 °C, 21% oxygen, 5% CO_2) overnight. The following morning, non-adherent cells were aspirated and

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