Respiratory Medicine (2012) **106**, 1139–1147

Available online at www.sciencedirect.com

SciVerse ScienceDirect

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

Ciliary beating is depressed in nasal cilia from chronic obstructive pulmonary disease subjects

Asma Yaghi [a,b], **Aisha Zaman** [a,b], **Gerard Cox** [a,b], **Myrna B. Dolovich** [a,b,*]

[a] *Department of Medicine, McMaster University, Hamilton, ON, Canada*
[b] *St. Joseph's Healthcare, Firestone Institute for Respiratory Health, Hamilton, ON, Canada*

Received 20 October 2011; accepted 4 April 2012
Available online 17 May 2012

KEYWORDS
Epithelium;
Lung disease;
Chronic obstructive;
Cilia function;
In vitro drug effects

Summary
COPD is characterized by increased cough, mucus production, and airway inflammation. Beating epithelial cell cilia contribute to mucociliary clearance with ciliary beat frequency (CBF) an important measure of cilia function. However, whether CBF varies with COPD severity is unknown. Aims: 1) to compare nasal cilia samples and their CBF from healthy non-smokers (Control), COPD and At Risk (cough and sputum production) subjects. 2) to determine the effect of pharmacologic agents that modulate mediators implicated in the pathogenesis of COPD on nasal CBF. Nasal brushings of ciliated cells were obtained from Control, At Risk and COPD subjects. Using high speed digital imaging, we measured baseline CBF *ex vivo*. Then, CBF was re-measured after 30 min perfusion with pharmacologic agents that modulate mediators implicated in COPD (salmeterol xinafoate, tiotropium bromide, licofelone, luteolin, YM976, Defensin HNP-1) and again after 30 min washout. CBF was significantly depressed in moderate and severe COPD compared to At Risk and Control subjects. There was an evident and persistent rise in CBF with all agents tested in COPD cilia except that YM976 effects persisted only in severe COPD. Only YM976 and tiotropium caused a persistent increase in CBF in At Risk cilia. The reduction of nasal CBF in moderate and severe COPD implies that impaired ciliary function may impact mucociliary clearance in COPD, potentially contributing to retention of secretions and infection. Pharmacologic agents with different mechanisms of action can increase CBF of COPD cilia. Further investigation of the signalling pathways influencing CBF of COPD cilia is needed.

* Corresponding author. McMaster University, Department of Medicine, 50 Charlton Ave East, FIRH Room T2135, Hamilton, ON, Canada L8N 4A6. Tel.: +1 905 522 1155x33597; fax: +1 905 521 6183.
E-mail address: mdolovic@mcmaster.ca (M.B. Dolovich).

0954-6111/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
doi:10.1016/j.rmed.2012.04.001

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a respiratory disorder characterized by progressive, non-reversible airflow limitation and usually is associated with a chronic inflammatory response in the airways to harmful agents e.g. tobacco smoke, pollutants and other irritants. Inflammatory cell infiltration of large airways and hyperplasia of mucus glands, contribute to two characteristic symptoms of COPD: cough and sputum production. These symptoms usually precede development of airflow obstruction by many years, sometimes identifying people at risk of developing COPD.[1,2] Recent studies demonstrated that nasal epithelial cells can be used as surrogates for lower airway epithelial cells. McDougall et al. (2008) showed that inflammatory responses of nasal epithelial cells reflect those of bronchial cells,[3] and Sridhar et al. (2008) demonstrated that smoking-induced gene expression changes in the airway are reflected in the nasal epithelium.[4] Hurst et al. reported a correlation between the degree of inflammation found in the upper and lower airways in COPD, highlighting the potential relevance of studying nasal epithelial cells in subjects with COPD.[5]

Ciliary beat frequency (CBF) and coordination are important measures of ciliary function. We found that cilia on nasal epithelial cells of 14 healthy volunteers (ages 22–59) beat with a frequency of 14.57 ± 0.88 Hz and exhibit a common metachronous beat pattern. Beat frequencies have been shown to be similar in nasal, tracheal and bronchial epithelial cells of subjects[6,7] and these frequencies were independent of age.[7,8] Differences in CBF have been documented in diverse patient populations.[9] Numerous agents have been shown to modulate CBF[10,11] with efficient mucociliary transport relying on effective regulation of ciliary beating.[12,13] *In vitro*, beta-adrenergic and cholinergic agents were demonstrated to stimulate CBF, while fluticasone, some preservatives and some bacterial toxins inhibited CBF.[10,11,14,15] However, the direct effects of pharmacologic agents on CBF in epithelial cells of COPD patients have not been well characterized. A previous study of nasal CBF in stable COPD found that CBF was reduced by 25% compared to healthy controls and that salmeterol produced a significant increase in CBF.[16]

The aims of our study were to investigate whether changes in *ex vivo* ciliary function were related to the level of severity of COPD and to investigate how currently available COPD therapies and experimental pharmacologic agents might affect CBF. Some results from these studies were previously reported as abstract(s).[17,18]

Methods

Study population

The research presented in this manuscript was done in compliance with the Helsinki Declaration. The Research Ethics Board of St. Joseph's Healthcare Hamilton (SJHH) approved the study protocols and consent forms. Male and female subjects, aged 40–75, were recruited with advertisements and from patients attending the Firestone Institute for Respiratory Health (Hamilton, ON, Canada). Subjects with differing severity of COPD as defined by the original GOLD guidelines were studied: At Risk ($n = 7$); Moderate ($n = 5$) and Severe/very severe COPD ($n = 7$).[2] Non-smoking, age-matched healthy volunteers ($n = 6$) with no history of respiratory disorders served as Controls. All subjects were not withdrawn from their medications at the time of study. All participants were non-atopic, had no rhinitis and used no nasal therapy, and were studied when clear of respiratory infections for at least four weeks (verified by nasal swabs for bacteriology/virology on the day nasal cilia were obtained). Please note that due to limited resources, our experimental design did not include comparing smoking to non-smoking subjects for each group. The comparison was for Control (Healthy non-smokers) compared to COPD subjects at different levels of COPD severity independent of whether they were smokers or ex-smokers.

Study sample collection and procedures/ assessments

After obtaining informed consent, subjects were characterized using spirometry, body plethysmography, induced sputum for cytology and bacterial analysis, allergy skin test, and nasal swab (to test for respiratory viruses).

Cilia samples were obtained before sputum induction. Subjects had two nasal brushings taken from the right or left nostril, using a 3 mm bronchial cytology brush. Each sample was suspended in 5 ml Earle's Balanced Salt Solution (EBSS) at 37 °C for further examination.

Measurement of CBF

Nasal cilia samples were allowed to settle in the perfusion chamber for 5 min, and then perfused with EBSS for 5–10 min to rinse out non-epithelial entities (blood or inflammatory cells and debris). Ciliary beat frequency (CBF) of nasal epithelial ciliated cells was determined at 37 °C using published methods[19] and as described in the online supplement (Fig. 1s). Baseline CBF was measured and results were expressed as mean frequency, in Hertz (Hz). Then cells were used for testing pharmacologic agents. CBF, epithelial cell size, coordination of beat, and ciliary length were determined using ProAnalyst (XCitex, MA, USA).

Measurements of the effect of pharmacologic agents on CBF

The protocol involved 3 sequential steps: 1) baseline perfusion with EBSS, 2) 30 min exposure perfusion to one of the test agents, 3) 30 min EBSS washout perfusion to determine the reversibility of the effects of the exposure perfusion. All measurements were taken in the absence of flow. For all epithelial samples, each CBF value was the average CBF measured from 10 to 12 individual sites of beating ciliated strips/clumps of cells. Each measurement was made from each site consisting of at least 8 to 10 intact ciliated cells. All drugs and vehicle controls (Methanol and DMSO) were prepared in Earle's Balanced Salt Solution (EBSS) and the concentrations were final chamber