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Original Article

Use of ibuprofen to assess inflammatory biomarkers in induced sputum: Implications for clinical trials in cystic fibrosis

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Abstract

Background: High-dose ibuprofen (HDI) is a clinically beneficial anti-inflammatory regimen that may be a useful reagent to study induced sputum inflammatory marker changes over short study periods appropriate for early-phase CF clinical trials.
Methods: We conducted a 28-day, open-label, randomized, controlled trial among 72 clinically stable CF subjects ($FEV_1 \geq 40\%$ predicted) randomized to HDI or routine care that assessed IL-6, IL-8, TNF-α, IL-1-β, free neutrophil elastase, and white cell counts with differentials change from baseline in induced sputum.
Results: IL-6 was the only biomarker with significant within-group change: 0.13 $\log_{10}$ pg/mL mean reduction among ibuprofen-treated subjects ($p = 0.04$); and no change in the control group. IL-6 change between groups was statistically significant ($p = 0.024$). No other inflammatory biomarker differences were observed between groups after 28 days.
Conclusion: Although we studied only one agent, HDI, these results suggest that one month may be inadequate to assess anti-inflammatory candidates using markers from induced sputum.

Keywords: Biomarkers; Cystic fibrosis; Induced sputum; Inflammation; Ibuprofen; Anti-inflammatory drugs

1. Introduction

Cystic fibrosis (CF) lung disease is characterized by a perpetuating cycle of airway obstruction, chronic bacterial infection, and excessive neutrophilic inflammation that results in bronchiectasis and progressive obstructive lung disease accounting for about 80% of the mortality [1–4]. These events begin early in life and persist even during times of clinical

Abbreviations: CF, cystic fibrosis; PMN, polymorphonuclear neutrophil; IL-8, interleukin-8; LTB_4, leukotriene B_4; HDI, high-dose ibuprofen; IL-6, interleukin-6; IL-1β, interleukin-1beta; TNF-α, tumor necrosis factor-alpha; FEV_1, forced expiratory volume in 1 s; NSAIDS, non-steroidal anti-inflammatory drugs; ANCOVA, analysis of covariance; SAE, serious adverse event; AE, adverse event; MOA, mechanism of action.

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stability [5,6]. A large burden of polymorphonuclear neutrophils (PMNs) in the CF airway is more injurious than protective, releasing proteases and oxidants that damage the airway and chemotactic factors (*e.g.*, IL-8 and LTB_4) that recruit even more PMNs into the airway [1–3]. Chronic local inflammation leads to irreversible damage to the airway and loss of lung function. Therefore, the administration of agents capable of mitigating the inflammatory response should impact CF lung disease progression and prolong survival [3,7–9].

High-dose ibuprofen (HDI) has been used to treat the airway inflammatory response in CF for over 20 years. It is the only anti-inflammatory drug recommended for use in CF [10–12]. HDI's beneficial effect was first demonstrated in a rat model of chronic *Pseudomonas aeruginosa* lung infection [13]. Oral administration of HDI decreases PMNs in both animal and human models [13,14]. The potential of HDI to modify CF lung disease was confirmed in two controlled clinical trials. CF subjects randomized to receive twice daily HDI in both 4-year and 2-year blinded trials displayed significantly lower rates of pulmonary function decline compared to subjects randomized to receive placebo [15,16]. Moreover, these findings were supported by real-world clinical use of HDI studied using the U.S. CF Foundation Patient Registry [17]. Despite demonstration of a pulmonary function benefit, HDI is not prescribed by a majority of CF clinicians [18,19]. Therefore, an alternative anti-inflammatory approach is needed.

Given that trials of anti-inflammatory agents in stable patients have required 2-4 years of study to assess safety and efficacy [15,16,20], developing a robust method to screen potential anti-inflammatory candidates for biologic activity over a much shorter time period is desirable [8]. Results from a screening study that takes only a month or two could provide a go no-go decision on whether to enter into a trial of clinical efficacy that could require years to complete [21]. Attention has been drawn to the measurement of changes in inflammatory markers found in respiratory secretions including differential white cell counts, concentrations of chemokines and cytokines such as IL-8, IL-6, IL-1β and TNF-α, and concentrations of PMN products such as free neutrophil elastase that have been studied in previous CF trials of agents that directly or indirectly affect inflammation [22–33]. However, this approach is not without challenges. First, underlying inter- and intra-subject variability in inflammatory marker concentrations must be accounted for in the study design and power analysis [34]. Second, safe and uniform access to respiratory tract secretions is required. Only a subset of persons with CF spontaneously expectorate sufficient volumes of sputum for analysis [35], and bronchoalveolar lavage is invasive and has associated morbidity [36,37]. Studies have suggested that inducing sputum by inhalation of hypertonic saline is a safe, non-invasive alternative to bronchoalveolar lavage that allows assessment of inflammatory markers in healthy CF subjects who do not spontaneously produce sputum [36–38].

To determine if measuring changes in induced sputum inflammatory markers could serve as a screening tool to identify anti-inflammatory drugs for long-term clinical trial in CF, we conducted a multi-center study of CF subjects randomized to receive routine care with or without HDI for 4 weeks. Ibuprofen was chosen as the test article because of its demonstrated efficacy on a clinically important endpoint (loss of lung function over time) in clinical trials and real-world clinical use. We hypothesized that a decrease in inflammatory markers would be observed in sputum obtained by induction with hypertonic saline after administration of HDI over a period of 4 weeks.

2. Methods

2.1. Study subjects

Subjects were recruited from 16 sites participating in the Cystic Fibrosis Foundation Therapeutics Development Network in the United States. The study protocol was approved by the Institutional Review Board at each site. Informed consent or assent was obtained from each subject and/or his/her guardian. To be included, subjects had to have confirmed diagnosis of CF, be at least 10 years of age, able to perform spirometry, and be clinically stable. Subjects <18 years of age had to have a forced expiratory volume in one second (FEV_1) of at least 50% of their predicted value at screening based on age, height, and sex [39]. Subjects 18 years of age and older were required to have $FEV_1 \geq 40\%$ predicted at screening. Individuals were excluded if they were exposed to an investigational drug, ibuprofen, other non-steroidal anti-inflammatory drugs (NSAIDS), or systemic corticosteroids within four weeks of screening. Individuals with a history of hypersensitivity to β-agonists, aspirin, or NSAIDs were also excluded, as were those with room air oxygen saturation <92% or a history of hemoptysis. Additional exclusion criteria included elevation of liver enzymes more than 3 times the upper limit of normal, creatinine > 1.8 mg/dL, inability to swallow pills, pregnancy, breastfeeding, lack of willingness to practice birth control during the study, hepatic, cardiovascular, renal, neurologic, hematologic, or peptic ulcer disease, or presence of a condition that in the opinion of the investigator would compromise the safety of the subject or the quality of the data.

2.2. Study design

This was an eight-week, open-label, randomized, controlled, parallel-group multi-center study comparing the effects of 28 days of routine care plus HDI to routine care alone on the concentration of inflammatory markers in induced sputum. Subjects in the HDI arm received 20–30 mg/kg twice daily (maximum 3200 mg/day). Ibuprofen pharmacokinetics were obtained on all subjects [40], and examined *post hoc* to ensure that peak plasma concentrations associated with long-term lung function benefit (between 50–100 μg/mL) [15] were achieved. Subjects in the routine care arm received no additional therapies to their usual maintenance therapeutic regimen.

2.3. Sputum induction

Samples of induced sputum were obtained from each subject at screening (approximately 14 days prior to treatment initiation), randomization/treatment initiation, end of treatment (28 days after