

LETTER

The effects of an epithelial barrier protective cationic aerosol on allergen-induced airway inflammation in asthma: a randomized, placebo-controlled clinical trial

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Summary

Inhaled cationic airway lining modulator (iCALM) is a cationic aerosol therapy comprised of 1.29% calcium chloride dissolved in 0.9% isotonic saline that enhances the biophysical barrier function of the airway lining fluid and primes the host defense response. Its ability to attenuate bronchitis caused by inhaled particles was investigated using an allergen-inhalation model in a proof-of-concept study. In a randomized, double-blind, placebo-controlled cross-over trial of 6 mild atopic steroid-naïve asthmatic subjects, 3 doses of iCALM were well tolerated and they attenuated allergen-induced increase in sputum eosinophils, and levels of IL-5, MCP-1 and eotaxin. This study provides an opportunity to investigate the role of enhancing epithelial barrier to decrease airway inflammation provoked by inhaled particles in a variety of airway diseases.

Keywords allergen provocation, asthma, epithelial barrier, inhaled cationic solution, sputum eosinophils

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Introduction

The airway epithelium provides an active barrier to foreign matter and particulates via the mucociliary escalator and is known to secrete an array of immunologically active substances [1]. The airway lining fluid (ALF) acts as a natural barrier in the airway and is the first line of defence against inhaled agents including allergens, particulate and non-particulate pollutants and pathogens. Enhancing this protective barrier may be an effective strategy to attenuate the deleterious effects of a wide variety of respiratory irritants. Inhaled cationic airway lining modulator (iCALM) (PUR003) is a simple binary salt solution of 1.29% calcium chloride dissolved in 0.9% isotonic saline. The product is preservative-free and easily manufactured. Bioavailability is essentially 100% of the deposited aerosol following inhalation. Calcium alters fluid rheology by interacting with electronegative glycoproteins in the ALF, leading to a conformational change in the molecules and

transient surface gelation [2]. These changes in the biophysical properties of mucus decrease penetration of the mucus layer by particulates and microbes and limit their access to the cell surface for either attachment or internalization [3, 4].

Allergic bronchitis and infective bronchitis are the major causes of exacerbations in airway diseases such as asthma. Enhancement of the airway protective barrier by PUR003 can potentially attenuate bronchitis and thereby reduce exacerbations of asthma. As it is more difficult to study the effect on infective bronchitis, we designed this proof-of-concept study to examine whether PUR003 could prevent eosinophilic bronchitis caused by inhaled allergens. The primary objectives were to assess the safety and tolerability of inhaled PUR003 in patients with mild atopic asthma and to observe whether it would attenuate allergen-induced sputum eosinophilia. The secondary objectives were to assess allergen-induced changes in sputum cytokines.

Subjects, design and methods

Seven atopic mild steroid-naïve asthmatic subjects (three males, mean age 35 years, mean FEV1 90% predicted, geometric mean PC20 methacholine 4.4 mg/mL) who had never smoked and who gave written informed consent, completed a St Joseph's Healthcare Hamilton Research Ethics Board approved, randomized, double-blind, cross-over, three-period study comparing three doses of treatment with PUR003 (16 mg of calcium chloride in 5.5 mL of normal saline delivered by a mouth-piece over 15 min using an LC Sprint nebuliser (Pari, Midlothian, VA) or matching placebo, with at least 2 weeks of wash-out period between the treatment periods. During the first period, the subjects underwent a screening allergen inhalation [5] to identify those in whom allergen could induce at least a 50% increase in sputum eosinophil %. After 2–4 weeks, they were randomized to one of the two treatment arms using computer-derived codes that were maintained off-site by an independent third-party research assistant. For each treatment arm, subjects attended the laboratory on two consecutive days. On the first day, blood chemistry, ECG, spirometry and sputum induction were performed. Sputum was processed for quantitative cell counts [6] and the cell-free supernatant was stored at -70°C . Levels of IL-1b, IL-5, IL-6, IL-8, IL-9, IL-13, GM-CSF, MCP-1, MIP-1 α and eotaxin were assayed using multiplex bead ELISA assay (Millipore, Billerica, MA, USA). Subjects were administered the study medication at 8 am and at 8 pm and the following morning at 8 am. Spirometry was monitored at 15 min, 30 min, 1 h and 2 h after inhaling the study medication. On the second day, an allergen inhalation challenge was performed 2 h after inhaling the study medication. Spirometry was monitored for 6 h and sputum was induced at the end of the observation period. After 2–3 weeks, subjects crossed over to the second treatment arm.

The sample size predicted to be necessary to observe 50% attenuation of allergen-induced percentage of eosinophils (i.e. mean increase reduced from 19.9% to 9.95%, standard deviation 4.3%) with a power of 0.95 was six subjects with a randomized crossover study design [7]. Comparisons were made between the two treatment arms using repeated measures analysis of variance, with subjects as the between-subject factor and drug and treatment order as within-subject factors. Post hoc analysis was used to identify the source of significant variation when it was present. The criterion for significance (α) was 0.05. Analyses were performed using SPSS for Windows (ver. 10.0.5, Chicago, IL, USA).

Results

Data from one subject were excluded from the analyses of inflammatory markers before the measurements were

made as we believe that the subject violated protocol and used inhaled corticosteroid on the day of the allergen inhalation challenge in study period 2. The data from this patient were included in the safety analyses. PUR003 was well tolerated by all the subjects. It did not cause bronchoconstriction for up to 2 h following inhalation. The maximum mean decline in FEV1 observed with PUR003 was 2.7 mL compared to 2.5 mL with placebo. The mean change from baseline at 2 h post-PUR003 dosing was +1.3 mL (SD 1.5) compared to +1.0 mL (SD 1.2) post-placebo inhalation. There were no significant effects on routine blood chemistry or on a 12-lead electrocardiogram (data not shown). The mean (SD) eosinophil % in sputum at baseline was 0.6% (0.5%) and this increased to 21.6% (4.5%) 6 h after an allergen challenge. Pre-treatment with three doses of PUR003 significantly attenuated the allergen-induced increase in sputum eosinophil count compared to pre-treatment with placebo (Fig. 1). Of the 10 cytokines that we assayed, levels of IL-5, IL-9, MCP-1, eotaxin and IL-1 β increased significantly after allergen inhalation preceded by placebo pre-treatment. Pre-treatment with PUR003 significantly attenuated the allergen-induced increase in IL-5, MCP-1 and eotaxin (Table 1). Overall, there was no significant effect of PUR003 on allergen-induced maximum EAR (mean 13.5%, SD 12.5%) or LAR (mean 8.5%, SD 9.8%) compared to the maximum EAR (mean 12.1%, SD 10.2%) and the maximum LAR (mean 5.6%, SD 5.2%) following placebo inhalation. Airway hyperresponsiveness was not examined.

Discussion

This is the first clinical trial, to our knowledge, to report the efficacy of an agent to enhance the airway

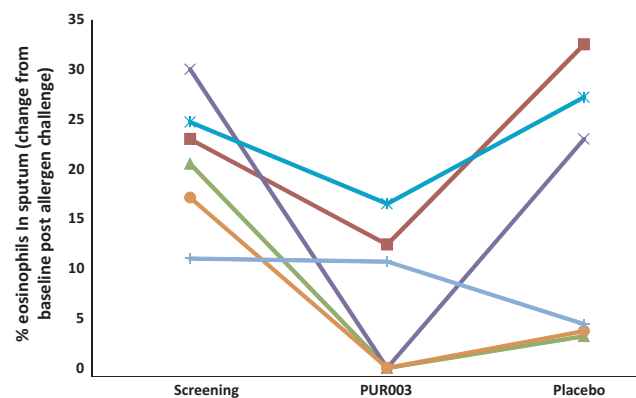


Fig. 1. Effect of PUR003 on allergen-induced sputum eosinophilia. Allergen inhalation was performed three times: during the screening period, after treatment with three doses of PUR003 and after treatment with three doses of placebo. Sputum eosinophil % was examined before and after allergen inhalation during each of these three periods. PUR003 treatment significantly attenuated the allergen-induced increase in sputum eosinophil (%).

Table 1. Table showing the levels of cytokines and % of eosinophils in sputum before and after an allergen inhalation preceded by either PUR003 or placebo

	PUR003		Placebo	
	Pre-allergen	Post-allergen	Pre-allergen	Post-allergen
Sputum supernatant				
IL-5*	10.9 (4.1)	149.9 (155.8)	15.3 (13.1)	243.7 (455.4)
MCP-1*	435.3 (473.4)	309.6 (159.2)	251.5 (126.1)	435.5 (212.5)
Eotaxin**	308 (615.8)	142.9 (234.6)	171.4 (197.8)	639.8 (1339.4)
IL-9	4.9 (8.3)	32 (18.5)	24.8 (33.4)	72.3 (22.5)
IL-13	4254.3 (5648.7)	4058.3 (4251.61)	8363.5 (8543.9)	5038.5 (6960.1)
GM-CSF	33.5 (37.1)	82.9 (41.5)	76.9 (63.4)	80.3 (52.1)
MIP-1a	34.9 (37.1)	40.3 (34.3)	62.9 (69.9)	48.8 (33.9)
IL-1b	205.6 (50.0)	469.5 (435.5)	211.1 (126.1)	411.4 (350)
IL-6	450.6 (461.3)	680 (676.4)	758.4 (897.2)	630.8 (635.1)
IL-8	4756.1 (2365.2)	6122.9 (3972.7)	10229.9 (9008)	9995.1 (10053.8)
Sputum eosinophils, %*	1.1 (3.3)	7.7 (10.5)	1.4 (0.7)	17 (14)

Pre-treatment with PUR003 attenuated allergen-induced increase in IL-5, MCP-1, eotaxin and eosinophil %. * $P < 0.05$, ** $P = 0.054$ (comparisons of the pre- and post-allergen challenge change in the listed mediators between the PUR003 and placebo treatment arms). All cytokines values are in pg/ml (mean, SD). Limits of detection for IL5 – 5 pg/mL, eotaxin – 3.2 pg/mL, MCP-1 – 3.2 pg/mL).

epithelial barrier function in patients with asthma. In this proof-of-concept study, we demonstrate that an inhaled cationic solution is safe, is well tolerated and attenuates allergen-induced airway inflammation. This provides the basis to study airway barrier function in other airway diseases such as COPD, cystic fibrosis and bronchiectasis and to design studies of inhaled interventions to prevent or attenuate infective and non-infective bronchitis.

Changes in the biophysical properties of mucus could decrease penetration of the mucus layer by bacteria or viruses and limit their access to the epithelial cell surface as demonstrated by the ability of PUR003 to inhibit the ability of multiple bacterial pathogens to move across the mucus layer compared to the saline treatment in a Costar Transwell system. We hypothesized that the enhancement of the epithelial barrier could extend to protection against inhaled allergens. Accordingly, we employed the reliable and well-characterized allergen inhalation model to examine the efficacy of PUR003 in mild asthmatics and demonstrated that inhaled pre-treatment with PUR003 significantly attenuated allergen-induced sputum eosinophilia and some of the Th2 cytokines that we measured. The effect was even comparable to what has been observed with inhaled corticosteroids [8] which are currently the most potent anti-inflammatory drugs employed to prevent allergen-induced inflammation. The effect does not appear to be allergen-specific, as the challenge agent included cat antigen in three subjects, ragweed extract in two subjects and grass pollen extract in one subject. A major limitation of our study is that we did not mimic the natural method of allergen inhalation. These

allergen proteins are carried by both small and large particles, sizes varying from 0.2 to 10 μm , depending on the stability or turbulence of indoor and outdoor air. This is a potential criticism of all currently employed drug-evaluation studies employing aqueous allergen inhalation challenges. The aqueous antigen could have seeped through the cationic lining film created by PUR003 and reached the airway epithelium and thus induced airway inflammatory and bronchoconstrictor responses. To avert a potential impact of more transportable aqueous allergen, an allergen challenge using micronized freeze-dried extracts of the antigens could have been employed. Notwithstanding this drawback, the attenuation of allergen-induced eosinophilia was significant, and we speculate that it could have been more marked with a particulate challenge, as occurs in the natural environment. We did not select patients with biphasic airway response to allergen, and therefore, we did not expect to demonstrate an attenuation of the EAR or LAR that would have required 10–12 subjects in a crossover study [9].

In summary, inhaled cationic aerosol provides a novel method to enhance airway barrier function. It was well tolerated in all subjects, without induction of bronchoconstriction. It is able to attenuate allergen-induced eosinophilic bronchitis, suggesting that this may be an effective strategy to protect against bronchitis caused by inhaled particles.

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