

ORIGINAL PAPER

Intranasal steroid reduces exhaled bronchial cysteinyl leukotrienes in allergic patients

M. Failla, G. Biondi, M. Provvienza Pistorio, E. Gili, C. Mastruzzo, C. Vancheri and N. Crimi

Department of Internal and Specialistic Medicine, Section of Respiratory Medicine, University of Catania, Catania, Italy

Clinical and Experimental Allergy

Summary

Background Allergic rhinitis (AR) precedes and is often associated with bronchial asthma. Indeed, local and systemic inflammations in both conditions are very similar. Cysteinyl-leukotrienes (cys-LTs) are generated during early- and late-phase allergic reactions and induce smooth-muscle contraction, microvascular leakage, and mucous hypersecretion. Cys-LTs are detected in exhaled breath condensate (EBC) of asthmatics and regardless of bronchial symptoms, they are also found in EBC of rhinitic patients.

Objective To evaluate cys-LTs in EBC of allergic patients and to assess the activity of nasal fluticasone propionate (FP) on EBC cys-LTs levels.

Methods Cys-LTs coefficient of variation (CV) was evaluated from different EBC in 5 healthy volunteers. Cys-LTs levels from EBCs in 13 healthy controls and 56 allergic rhinitic ($n = 31$) and rhinitic/asthmatic ($n = 25$) patients were also evaluated at baseline. Subsequently patients were randomized to receive either FP 100 µg/day per nostril or placebo for 2 weeks and then re-evaluated for EBC cys-LTs.

Results The CV was 14.12%. EBC cys-LTs in allergic patients were significantly higher than in healthy subjects (70.9 vs. 20.6 pg/mL (median), $P < 0.05$), while it did not differ between asthmatic/rhinitic and purely rhinitic patients. Treatment significantly reduced cys-LTs (from 93.6 to 19.9 pg/mL, $P < 0.001$). This effect was evident both in asthmatic/rhinitic and in rhinitic patients.

Conclusion Treatment of AR with FP significantly reduces the levels of cys-LTs, major noninvasive markers of lower airway inflammation, suggesting that upper and lower airway inflammation is present and should be thus treated as a whole in subjects with AR with and without asthma.

Keywords asthma, exhaled breath condensate, intranasal steroids, leukotrienes, rhinitis

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Correspondence:

Prof. Nunzio CRIMI, MD, Dipartimento di Medicina Interna e Specialistica, Sezione di Malattie Respiratorie, Università di Catania, Ospedale 'Ascoli-Tomaselli', Via Passo Gravina, 187, 95125, Catania, Italy.
E-mail: crimi@unicit.it

Introduction

Disorders of the upper respiratory tract, particularly allergic rhinitis (AR), are commonly associated with bronchial hyperresponsiveness and asthma [1–2]. Although several studies have shown that asthma and rhinitis are characterized by a similar inflammatory process [3], pathophysiological interactions between upper and lower airways are not completely understood. It is clear that the state of the upper airways definitely influences lower airways [4]. In AR patients, signs of allergic inflammation in lower airways have been found in induced sputum [5], bronchoalveolar lavage fluid [6] and bronchial biopsy specimens from patients without any clinical evidence of bronchial asthma [7].

The same concept is emphasized by the European Community Respiratory Health Survey [8]. This study recognizes perennial rhinitis as an important risk factor for the development of subsequent bronchial asthma, with odds ratio of 11 in atopic population and 17 in non-atopic one.

Indeed, local and systemic inflammation in these two conditions are very similar and sustained by a similar inflammatory infiltrate, which includes eosinophils, mast cells, T lymphocytes and proinflammatory mediators such as histamine and cysteinyl leukotrienes [9, 10].

Braunstahl et al. [7] have demonstrated that after a nasal allergen challenge, both the number of eosinophils and the expression of adhesion molecules increase in the lower airway mucosa. Furthermore, peripheral blood eosinophils are activated and increased in number after a

segmental bronchoprovocation test in rhinitic patients [11]. All together these data support the hypothesis that one of the primary mechanism of the vertical interaction between upper and lower airways is a systemic diffusion of inflammation between the two districts [12].

These interactions are not easily investigated without invasive procedures such as bronchoscopy. Only recently new and less invasive techniques to investigate inflammation in the deep lung have been developed. Much interest has been provoked by the exploration of exhaled air gases and condensates.

The collection of exhaled breath condensate (EBC) is a simple, safe and non-invasive technique suitable for the characterization of lower airway inflammation. Furthermore, EBC sampling is easily performed without affecting ongoing inflammatory processes [13, 14]. EBC also provides access to non-volatile respiratory compounds that usually reach the lower limit of commercially available ELISA kits.

Among the compounds found in EBC, increased levels of cysteinyl leukotrienes have been detected in patients with bronchial asthma [15]. The levels of cys-LT in breath condensate are significantly elevated in patients with moderate or severe asthma. Steroid withdrawal in moderate asthma leads to worsening of asthma symptoms and further increase in the concentration of LTB₄, LTE₄, LTC₄, and LTD₄ in exhaled condensate [16]. On the other hand, in children with bronchial asthma steroid treatment succeed in reducing exhaled leukotrienes significantly [17]. Regardless of any bronchial symptom, cys-LTs are also found in EBC of rhinitic patients [18].

Treatment of allergic inflammation in the nose with topical steroids reduces asthma symptoms, airway hyper-responsiveness [19] and also hospitalization in acute asthma exacerbations [20–22]. To test the hypothesis that rhinitis therapy is able to modulate the production of inflammatory compounds in lower airways, we evaluated the levels of cys-LTs in EBC of allergic patients before and after a 2 weeks course of an intranasal steroid such

as fluticasone propionate (FP; Glaxo, Smith & Kline, Verona, Italy).

Methods

Subjects

During the pollen seasons of the years 2004 and 2005, 56 allergic adults, and 13 healthy subjects were recruited in our outpatients clinic.

Asthma and rhinitis were classified according to ARIA [23] and Global Initiative for Asthma (GINA) [24] guidelines (Table 1). Subjects with AR had a characteristic history of seasonal AR (rhinorrhoea, nasal itch, sneezing, and nasal obstruction) and positive skin-prick tests (SPTs) results (> 3 mm weal response) to common inhalant allergens. Subjects with bronchial asthma had a characteristic history of symptoms (wheezing, dyspnoea, chest tightness, and chronic cough), positive SPTs results to common inhalant allergens, and a post-dilator FEV₁ increase > 12% of the predicted. However, all of our asthmatic subjects complained of symptoms of AR. Anti-histamines were withheld for > 7 days before each visit and none had ever received immunotherapy. Throughout the study no other medication for treatment of AR or asthma, with the exception of short acting β_2 -agonists, was allowed. Healthy control subjects had no history of asthma, chronic rhinitis, atopic eczema, or other relevant disease, and their SPT results were negative. All 69 subjects were nonsmokers. None of the subjects studied had experienced a respiratory tract infection for > 6 weeks before or during the study.

This study was reviewed and approved by the Local Research Ethics Committee and all the patients gave their written consent.

Study design

After a 1-week run-in period, patients were re-evaluated and if stable they were allocated in a randomized, double-

Table 1. Patients characteristics

	Healthy volunteers	Allergic patients	Rhinitics subgroup	Asthmatics subgroup
N	13	56	31	25
Age*	31 (25–37)	27 (24–29)	26 (22–28)	28 (25–30)
Sex (M/F)	5/8	29/27	15/16	14/11
FEV ₁ % predicted*	98 (91–101)	92 (85–100)	98 (89–104)	88 (80–95)
FVC% predicted*	95 (90–99)	92 (84–100)	98 (92–105)	81 (78–91)
Post dilator FEV ₁ increase %*	1 (0–1)	4 (2–13)	2 (1–3)	14 (12–20)
ARIA (1/2/3/4) [†]	/	35/38/18/9	36/45/18/1	40/36/24/0
GINA (1/2/3/4) [‡]	/	24/21/0/0	0/0/0/0	52/48/0/0

*medians and interquartile ranges (25th–75th percentiles).

[†]percent of patients according to ARIA class (1 mild intermittent/2 mild persistent/3 moderate intermittent/4 moderate persistent).

[‡]percent of patients according to GINA class (1 mild intermittent/2 mild persistent/3 moderate persistent/4 severe persistent)

GINA, Global initiative for asthma.

blind, placebo-controlled, parallel fashion to a 2-week treatment period with 2 puffs per nostril of 50 µg FP (Glaxo Smith & Kline, Verona, Italy) once daily or placebo, delivered through a matching nebulizer device.

Exhaled breath condensates and spirometric data were collected and analysed before and after the treatment in either placebo or treatment group.

Measurements

Exhaled breath condensate was collected through a custom device. Briefly a one-way circuit was realized with polystyrene mouthpieces and non-rebreathing valves, exhaled air was then conducted in a 50 mL polypropylene sterile centrifuge tube that was inserted in a pre-cooled 15 mm thick stainless-steel tube designed to exactly receive the centrifuge tube. After rinsing their mouths, patients breathed for 10 min at tidal volume wearing a nose clip. Patients were asked to swallow their saliva periodically. Saliva when present was trapped in the lower arm of a T tube placed just after the mouthpiece. Frozen samples were immediately stored at -80°C and analysed within 2 months.

Saliva was obtained from eight patients that underwent to EBC collection. Amylase was evaluated in EBC and compared with that from saliva by means of an *in vitro* colorimetric method (Roche Diagnostics, Basel, Switzerland), in order to assess saliva contamination in the collected EBC.

Cys-LTs (LTC₄, LTD₄, and LTE₄) concentrations were measured with a specific enzyme immunoassay (Cayman Chemical, Ann Arbor, MI, USA). The lower limit of detection for this assay was 15.0 pg/mL. Spirometric parameters were obtained by means of a V-max 229 spirometer (Sensor Medics, Yorba Linda, CA, USA).

Statistical analysis

In order to assess the intra-subject reproducibility of the procedure, coefficient of variation (CV) for cys-LTs was evaluated within three different EBC obtained in different days from five healthy volunteers. Data were statistically analyzed by nonparametric tests. Patients characteristics as well as leukotrienes concentrations in EBC were expressed as medians and interquartile ranges (25th–75th percentiles). Differences among groups were analyzed by means of the Kruskal–Wallis test followed, where significant differences were found, by the Mann–Whitney U-test for comparisons among groups. Correlations between cys-LTs and ARIA and GINA severity scale were evaluated by Spearman's rank test. Results were considered significant at a value of $P < .05$. Statistical analysis was performed using GraphPad Prism 4.0 (Graphpad Software, San Diego CA, USA).

Results

Patients characteristics

Fifty-six patients, 29 males, and 27 females, aged 27 [24–29] years participated to this study (Table 1). Forty-six percent of these patients were sensitized to perennial allergens, while 80% of them were sensitized to seasonal allergens. All patients suffered from AR, while 25 of them suffered also of bronchial asthma. Purely rhinitic patients had a median FEV₁% of 98% [89–104] and a post dilator FEV₁ increase of 2% [1–3]. Asthmatic/rhinitic patients had a median FEV₁% value of 88% [80–95] and a higher post dilator FEV₁% increase: 14 [12–20]. Thirteen healthy subjects, five males and eight females, aged 31 [25–37] years with a negative history of AR or bronchial asthma, with a negative SPT to common inhalant allergens and with normal pulmonary function tests, were enrolled in this study as a control group.

Custom EBC collecting device performances

The device used through the study was examined for its performances. During the 10 min procedure, temperature in the refrigerating chamber ranged from -55 to -50°C below the room temperature. This allowed a fall in exhaled air temperature from 28 to 15°C at the exit of the one-way circuit. Our device had a production rate of $250 \pm 80 \mu\text{L}/\text{min}$ of exhaled air condensate.

The intrasubject coefficient of variation for cys-LTs levels, evaluated in five healthy subjects on three different different days, was 14.12%, which is considered acceptable for such an assay.

Saliva was trapped in a dedicated chamber in 2% of all the procedures. In the eight patients in which amylase levels were evaluated, we found a median concentrations of 9 UL [3–14 UL] in EBC, that is 0.00005 times the concentration we found in saliva of the same subjects (1.76×10^5 UL, [1.23–2.52 $\times 10^5$ UL]).

Basal cys-LTs levels

Basal cys-LTs levels were measured in 69 subjects, 56 allergic patients and 13 healthy subjects. Significantly higher values were found in allergic patients as compared with healthy subjects (70.9 [37.9–126.8] vs. 20.6 [9.3–94.5] pg/mL, $P < 0.05$). Allergic patients pooled according to their disease status, rhinitis or bronchial asthma, had similar basal cys-LT levels (65.5 [32.8–126.8] vs. 73 [55.3–127] pg/mL, $P = \text{NS}$), although a trend towards higher values was evident in asthmatic/rhinitic patients (Fig. 1). Cysteinyl leukotrienes found in EBC of allergic patients failed to correlate with ARIA and GINA guidelines severity scales, (Fig. 2).

Effect of nasal fluticasone propionate on cys-LTs levels

After a 2-week course of treatment with nasal fluticasone or placebo, cys-LTs were measured again in the EBC of allergic patients. In the treated group basal cys-LTs were significantly reduced from 93.6 [32.1–152.2] to 19.9 [11.6–34.8] pg/mL, $P < 0.001$, (Fig. 3). This effect was present and equally significant in treated rhinitic and also in treated asthmatic/rhinitic patients. Indeed, basal cys-LTs levels were reduced from 92.3 [25.6–143.8] to 20.4 [10.2–30.2] pg/mL, $P < 0.01$, after the treatment course in rhinitic patients and from 93.6 [41.9–186.5] to 19.6 [12.5–54.7] pg/mL, $P < 0.01$, in asthmatic/rhinitic patients. Post-treatment and post-placebo cys-LTs did not differ significantly between asthmatic/rhinitic patients and purely rhinitic patients. Placebo group did not show any change of EBC cys-LTs levels after the 2 weeks course of placebo (from 62.8 [39.2–106.2] to 52.6

[34.8–106.9] pg/mL, $P = \text{NS}$). According to these results in none of the placebo subgroups was evident a statistically significant difference between pre- and post-treatment cys-LTs levels.

Discussion

In the present study, we have shown that treatment of AR with the intranasal corticosteroid FP reduces cys-LT levels in lower airways of allergic patients during the pollen season. Our findings suggest that there is an important link between upper and lower airway inflammation in patients suffering AR. Furthermore, by performing subgroup analysis, we demonstrated that this effect is present in purely rhinitic as well as in asthmatic/rhinitic patients after an intranasal steroid course.

Recent data show how cys-LTs characterize different asthmatic phenotypes according to the severity status and the steroid treatment [17, 25–27]. However, only one study to date addressed the question whether cysteinyl-leukotrienes, hallmarks of allergic inflammation, are present in the EBC of rhinitic patients. Indeed, Cap et al. [18]. have shown how even rhinitic patients produce higher levels of cys-LT in the lower airways compared with healthy subjects and how cys-LTs are increased during the pollen season, regardless of bronchial symptom. Our findings confirmed that patients suffering from AR had higher baseline levels of cys-LTs compared with healthy subjects. We found similar levels of cys-LT in the EBC of both rhinitic and asthmatic/rhinitic patients. Although a trend towards increased levels of these inflammatory mediators was present in asthmatic/rhinitic patients, this was not statistically significant. It is noteworthy that the latter group of subjects was characterized by mild intermittent asthma, while rhinitic patients showed a broader range of disease severity, according to ARIA guidelines, even though no correlation was evident between disease severity and cys-LT levels found in EBC. Gratziou et al.

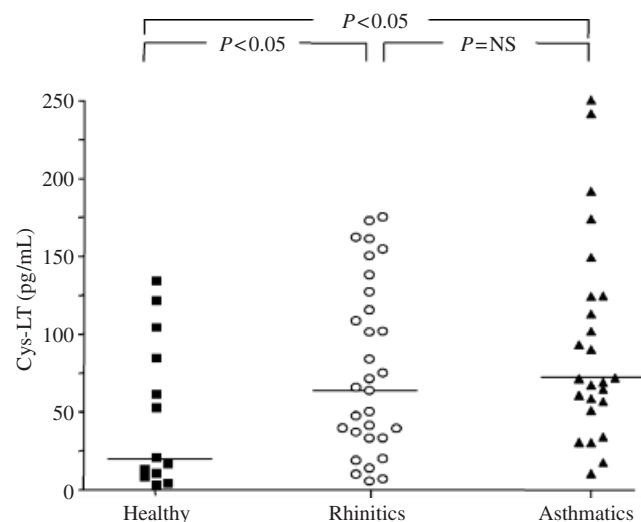


Fig. 1. Basal cysteinyl-leukotrienes (cys-LT) levels found in exhaled breath condensate of healthy and allergic subjects during the pollen season according to their disease status.

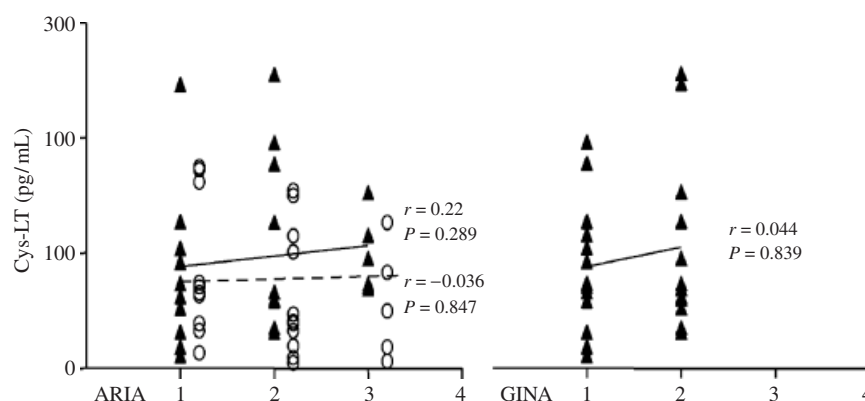


Fig. 2. Basal cysteinyl-leukotrienes (cys-LT) levels found in exhaled breath condensate of allergic subjects during the pollen season plotted against ARIA and Global initiative for asthma (GINA) scale of severity to show correlation. Blank circles represent purely rhinitic patients, filled triangles represent asthmatic/rhinitic patients.

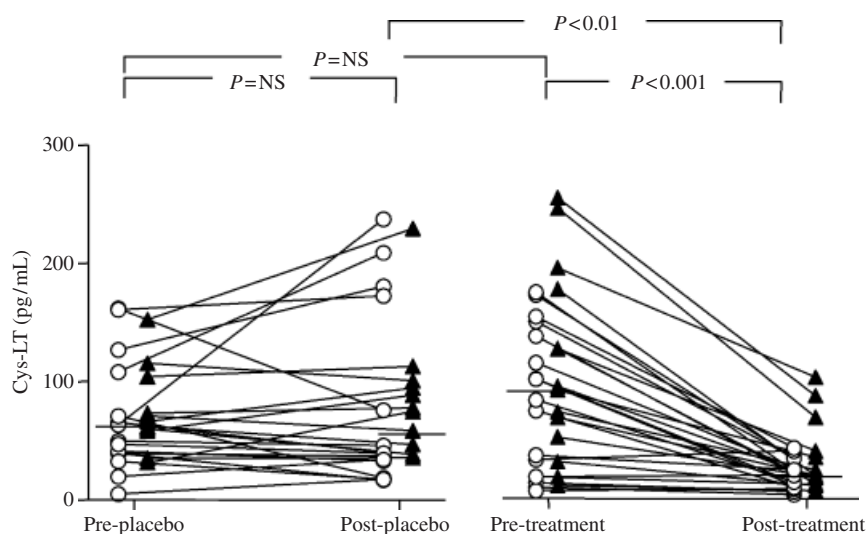


Fig. 3. Cysteinyl-leukotrienes (Cys-LT) levels found in exhaled breath condensate of the allergic group after a 2 weeks course of nasal fluticasone propionate or intranasal aerosol of placebo. Blank circles represent purely rhinitic patients, filled triangles represent asthmatic/rhinitic patients.

[28] and Lopuhaa et al.[29] have previously shown that levels of eNO did not differ between subjects with rhinitis and subjects with asthma and concomitant rhinitis. However, their results are not directly comparable with ours because of the inclusion in previous studies of patients with and without atopy and because eNO is not directly related to cys-LT production. Nevertheless, these authors certainly reinforce the concept that in rhinitis inflammation is present both in nose and bronchi, in a ratio that probably depends on several and yet undefined variables.

Different studies have already shown an influence of inhaled steroids on cys-LTs production in patients with asthma [17, 30–32] but to the best of our knowledge, no other studies have looked at the effects of an intranasal steroid treatment on cys-LTs levels in the exhaled breath condensate, in patients with AR with or without asthma.

We hypothesize that the reduction of cys-LT we found is not a consequence of nasal corticosteroid spilling into lower airways. In fact, in a previous study with intranasal radiolabelled beclomethasone dipropionate, less than 2% of the total radioactivity was detected in the chest after nasal administration [33]. Moreover, because of the low systemic bioavailability of nasal FP, it is unlikely that our findings result from a systemic action of this compound. It is conceivable that the inhibitory effect of nasal FP on EBC cys-LTs levels is related to the anti-inflammatory properties of steroids exerted on inflammatory cells present locally in upper airways, given the pivotal role played by these cells in regulating the kinetics of local but also systemic inflammation [7, 34].

We cannot exclude that some of the cys-LT measured in our EBC was of upper airway origin, despite the fact that during the procedure patients breathed with a nose-clip. Even though this is beyond the scope of our study, it is likely that resistance created by the whole circuit was

sufficient to induce the nasopharyngeal velum closure for part of the respiratory cycle, thus possibly reducing nasal air contamination. Similarly, there is little evidence that breath condensate is contaminated during the passage through the mouth. Effros et al. [35] have shown that the average amylase concentration in the condensate is less than 2×10^{-5} of that found in saliva. To avoid saliva contamination, we used a saliva trap, placed under the mouthpiece, before the banded tube. Moreover a unidirectional valve, placed at the end of this tube, physically avoided saliva spillover into condensing chamber. In our preliminary experience with a custom device, in only few procedures saliva was found in its dedicated trap, and again amylase in EBC was almost absent.

Markers of lower airway inflammation, such as exhaled NO, were significantly reduced in patients with AR, with or without asthma, after intranasal treatment with triamcinolone acetonide in a study of Sandrini et al. [36]. We confirm these results by studying a well-characterized class of inflammatory compounds: cys-LTs.

In summary, we have demonstrated that cys-LTs are increased in the exhaled breath condensate of purely rhinitic patients during the pollen season, to a level similar of that found in asthmatic/rhinitic patients, thus further supporting the link between upper and lower airways inflammatory process.

In our study, allergic patients treated with a nasal steroid had reduced levels of cys-LTs coming from the lower airways through exhaled breath. It is noteworthy that this effect was present and significant both in rhinitis and in asthma subgroups.

Our results show that upper and lower airway inflammation should be considered, and thus treated, as a whole in subjects with AR with and without asthma. Nevertheless, further studies are required to assess whether

monitoring cys-LTs found in EBC may be used to evaluate the effects of treatment or to identify patients at risk of bronchial asthma.

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