

Beclomethasone dipropionate blunts allergen-induced early increase in urinary LTE4

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ABSTRACT

Background The inhibitory effect of corticosteroids (CS) on the secretions of cysteinyl-leukotrienes (Cys-LTs) in asthma is controversial. The aim of this study was to evaluate the effect of CS on allergen-induced increase in urinary leukotriene E4 (uLTE4) during early (EAR) and late (LAR) asthmatic responses in mild untreated asthmatics.

Material and methods Nine subjects with mild untreated allergic asthma performed two allergen challenges, after 1-week treatment with beclomethasone dipropionate (BDP, 500 µg b.i.d) or placebo. Forced Expiratory Volume in one second 1 (FEV1) was monitored to assess EAR and LAR, and uLTE4 was measured before and during EAR and LAR.

Results After placebo, uLTE4 increased significantly during EAR, but not during and after LAR, in comparison with baseline values. Beclomethasone dipropionate induced a significant attenuation of the uLTE4 increase during EAR, in comparison with placebo, in association with a good protection of LAR ($P = 0.002$) and a mild protection of EAR ($P = 0.07$).

Conclusions Beclomethasone dipropionate blunts the early increase in uLTE4 excretion due to allergen challenge, in association with a significant effect on the severity of LAR. These data support the hypothesis that inhaled CS may inhibit the allergen-induced release of cys-LTs in asthma.

Keywords Allergen challenge, beclomethasone dipropionate, late response, urinary LTE4.

Abbreviations CS, corticosteroids; Cys-LTs, cysteinyl leukotrienes; uLTE4, urinary leukotriene E4; LTD4, leukotriene D4; EAR, early asthmatic response; LAR, late asthmatic response; BDP, beclomethasone dipropionate; FEV1, forced expiratory volume in one second; PEF, peak expiratory flux; PD20FEV1, meth, cumulative dose of methacholine producing a 20% decrease in FEV1 from baseline value; sBPT, specific bronchial challenge test; BU, biologic units; EIA, enzyme immunoassay; HPLC, high performance liquid chromatography.

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Introduction

Allergen challenge in laboratory can induce an early (EAR) and a late (LAR) airway response in atopic asthmatic subjects [1]. Both EAR and LAR are associated with an increase in markers of inflammatory cell activation, which can be measured in blood, urine and induced sputum [2]. In particular, cysteinyl-leukotrienes (Cys-LTs) are major chemical mediators of pathophysiology of asthma, and they contribute to many clinical and biological findings of the disease (like bronchoconstriction, mucous hypersecretion, eosinophil chemotaxis) [3].

Cysteinyl-leukotrienes are released during allergen challenge by several airway cells, leading to an early increase in urinary excretion of leukotriene E4 (uLTE4) [4,5], whereas the presence

of a late increase in uLTE4 excretion is controversial [4–6]. Controversial results are reported on the ability of corticosteroids (CS) to prevent the early increase of uLTE4. Some studies on humans reported minimal or no effect of oral [7] or inhaled [8] CS on the increase in uLTE4 after EAR, while other studies on animals suggested the possibility of CS to prevent the release of leukotrienes after allergen challenge [9]. More recently, some authors reported a positive effect of inhaled CS on the excretion of uLTE4 in nocturnal asthma [10], whereas other studies showed that 3–4 weeks inhaled fluticasone propionate did not reduce either uLTE4 excretion or airway reactivity to inhaled LTD4 in stable symptomatic asthmatics

[11,12]. This point is relevant, because some authors suggest that, according to these and other experimental data, corticosteroids are unable to affect the release of LTs from inflammatory cells involved in asthma [13].

We performed a double-blind placebo-controlled study to verify the effect of 1-week treatment with inhaled beclomethasone dipropionate (BDP) on allergen-induced EAR and LAR, and on the associated uLTE4 excretion. Our hypothesis was that BDP could prevent the increase in the allergen-induced excretion of uLTE4.

Subjects and methods

We studied 10 atopic asthmatic patients, recruited from our asthma clinic. One patient withdrew from the study for an acute asthma exacerbation. The main data of the nine remaining patients are reported in Table 1. Diagnosis of asthma and the level of severity (mild persistent or intermittent asthma) were performed according to the international criteria [14]. None of them had been regularly treated with CS in the last 6 months, using rescue salbutamol only (less than once a week). All subjects were sensitised to common inhalants (house dust mite in six and grass pollen in three subjects).

After a preliminary evaluation including baseline pulmonary function, methacholine challenge test, skin prick tests and a screening specific allergen challenge to select those with an EAR + LAR, subjects were randomised to receive, in a double-blind cross-over study, inhaled BDP (500 µg b.i.d) or identical placebo, for 7 days. During the night, between the sixth and the seventh day, patients were instructed to collect all the urine produced from 20:00 to 8:00 (T0). In the morning of the seventh day, subjects attended to our laboratory for the allergen challenge. Ten minutes after the last dose of drug, allergen inhalation was started, and the patient was monitored by spirometry to check for EAR and LAR, until 8 h after the last allergen dose. Patients collected urine during the first 3 h of allergen challenge (T1: 0–3 h), and separately in the interval between the 3rd and

the 8th hour after allergen challenge (T2: 3–8 h). Subjects continued to monitor peak expiratory flux at home, and they collected urine at home until 8:00 AM of the following day (T3: 8–24 h). After a wash-out period of 3–4 weeks during which patients were treated with rescue medication only, the same protocol was repeated with the alternative drug (BDP or placebo). Reporting of the study conforms to CONSORT guidelines [15]. The local Ethic Committee approved the study protocol, and each patient gave the informed consent to the study.

Specific bronchial challenge test was performed with allergens standardised in Biologic Units (BU) (NeoAbellò; Milano-Italy) using a procedure previously described [16]. Briefly, diluent and then cumulative allergen doses were administered at 10 min intervals to obtain a range of cumulative doses from 0.025 to 3.2 BU. Spirometry (Biomedin; Padova-Italy) was performed 10 min after each dose. The challenge was stopped when the FEV1 fell at least by 25% from post-diluent value. Then, FEV1 was measured at 20, 30 and 60 min, and hourly until 8 h post-challenge, to check for EAR (10 to 60 min) and LAR (3 to 8 h). The same maximal total delivered dose of allergen used in the screening test was administered in the same step-wise manner in both study days, unless the patient had a greater response to a lower dose.

Aliquots of urine were taken from each collection period. The antioxidant 4-hydroxy-TEMPO, 1 mM final concentration (Sigma, St Louis, MO, USA), was added to each urine sample before storage at –80 °C until analysis. Urinary LTE4 concentration was measured by enzyme immunoassay (EIA) after high performance liquid chromatography (HPLC) separation, according to a method previously described [5]. The recovery of the whole procedure (extraction plus separation) was 65.3 ± 15.8% (mean ± SD, *n* = 20). The lower detection limit was 11 ± 9.5 pg mL^{–1}. The intra-assay variability was 6%, and the inter-assay variability was 15%. Creatinine was measured in each urine sample by Jaffe reaction, and uLTE4 was expressed as pg mg^{–1} of creatinine.

Maximum decrease in FEV1 (ΔFEV1) during EAR and LAR is expressed as mean ± SD. The uLTE4 excretion values are expressed as mean ± SE. The uLTE4 values between BDP and placebo, and between different time-points after allergen challenge, were compared by Wilcoxon test and Friedman test respectively, whereas the comparison in the ΔFEV1 during EAR and LAR between placebo and BDP was performed using paired *t*-test. A level of significance lower than 0.05% was considered as significant.

Results

No difference was observed between placebo and BDP treatment for the baseline levels of uLTE4 (T0) (Fig. 1). After placebo, allergen challenge induced a significant increase of

Table 1 Main characteristics of the subjects

Number	9
Male/female	7/2
Age, years (M ± SD)	23.8 ± 10.1
Smoke, yes/ex/no	0/2/7
Duration of asthma, years (M ± SD)	12.5 ± 5.3
FEV1, % pred (M ± SD)	97.4 ± 9.0
PD20FEV1 meth, mg (GM and range)	0.144 (0.04–0.5)

M, mean; SD, standard deviation; GM, geometric mean; FEV1, forced expiratory volume in one second.

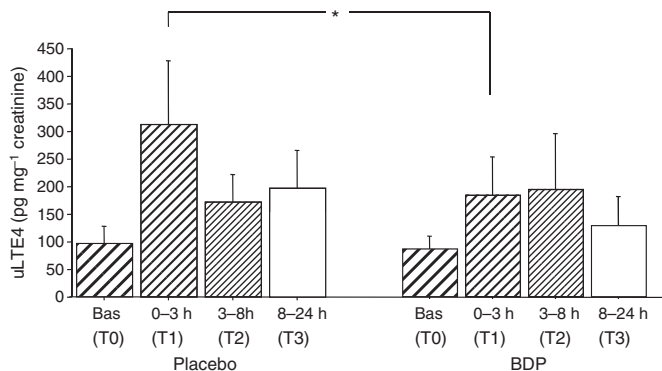


Figure 1 Mean values (standard error) of urinary LTE4 excretion before (baseline value, T0) and at different time-points after allergen challenge (T1, T2 and T3), either after placebo or beclomethasone dipropionate (BDP) treatment, * $P < 0.01$ vs. BDP.

uLTE4 during EAR (T1) compared with T0 ($P < 0.02$); uLTE4 then decreased during (T2) and after LAR (T3) ($P < 0.03$ and $P < 0.05$ vs. T1 respectively). T2 and T3 were not significantly different from T0 ($P = 0.10$ and $P = 0.07$ respectively).

After BDP, allergen challenge still induced an increase in uLTE4 in the 0–3 h (T1) sample in comparison with T0 ($P < 0.03$). However, this value was significantly lower after BDP than after placebo (Fig. 1). T2 and T3 were still not significantly different from T0 ($P = 0.07$ and $P = 0.11$ respectively) and from T1. The per cent increase from baseline in uLTE4 during EAR was also significantly lower after BDP than after placebo (81.9% vs. 208.2%, $P < 0.05$).

After placebo, all subjects showed an EAR (ΔFEV_1 : $36.2 \pm 8.3\%$), followed by a LAR (ΔFEV_1 : $36.7 \pm 12.1\%$). After BDP, there was only a mild non-significant attenuation of EAR (ΔFEV_1 : $20.7 \pm 20.8\%$, $P = 0.07$ vs. placebo), but a good protection of LAR (ΔFEV_1 : $4.7 \pm 5.2\%$, $P < 0.05$).

Discussion

The main result of our study was that inhaled BDP blunted the early increase in uLTE4 excretion after allergen challenge, in association with a mild not significant attenuation of EAR and a significant prevention of LAR. These data support the hypothesis that inhaled CS may be effective on the mechanisms leading to the increased release of Cys-LTs from the airways during allergen challenge in allergic asthmatics.

After placebo, uLTE4 excretion increased immediately after allergen challenge, but not during and after LAR, in agreement with previous observations from us and other groups [4–6]. Leukotrienes are released in the airways from resident cells (mainly mast-cells) during EAR, and their massive and rapid increase in the lung allows LTE4 to be excreted unmodified in

the urine before metabolic conjugation might occur. Cysteinyl-leukotrienes are also produced in large amount from eosinophils recruited in the airways during LAR, but increased levels of uLTE4 have not been consistently measured during LAR [4–6]. This fact could be due to the kinetic of LTs metabolism, which allows to detect increased levels of uLTE4 only when LTs are released in large amount and in a short period of time, as during EAR.

One-week treatment with inhaled BDP reduced, although not significantly, the severity of EAR, whereas it significantly decreased the early allergen-induced uLTE4 excretion. These two effects were not significantly correlated, suggesting that the effects of BDP on uLTE4 excretion is largely independent from a positive effect on EAR, which is mainly due to the release of other bronchoconstrictive mediators (histamine, prostaglandins) less sensitive to the short-term corticosteroid treatment. In effect, one-week treatment with inhaled corticosteroids partially prevents EAR, whereas different combinations of inhaled CS, anti-histamine and montelukast may result in a better attenuation of EAR [17,18]. Prostaglandin D2 release seems poorly responsive to inhaled CS, and this mediator may be responsible for the persistence of EAR despite the reduction in uLTE4. Furthermore, attenuation in uLTE4 during EAR is associated with a consistent reduction in the severity of LAR, suggesting some link between Cys-LT release during EAR and the following LAR.

Corticosteroids have been reported to be ineffective on uLTE4 excretion induced by allergen challenge. Two studies using endobronchial allergen challenge [7] and allergen inhalation [8] in allergic asthmatics have demonstrated the persistence of the allergen-induced release of LTE4 both in bronchoalveolar lavage and in urine, despite treatment with oral or inhaled CS. The difference between these studies and our results might be related to the different method of challenging (endobronchial vs. inhalation) [7] or to some difference in uLTE4 sampling time [8]. In effect, endobronchial challenge may expose lung mast-cells to a such high allergen dose, which may overcome any kind of prevention [7], and also mild difference in the sampling time (4 h vs. 3 h after allergen challenge) may blunt the possibility to see for any changes in uLTE4 [8]. Also in symptomatic asthmatics, inhaled CS induced different effects on uLTE4 excretion [10–12]. In one study, circadian rhythm of uLTE4 was modified by ICS treatment [10], and in the other two studies overnight, uLTE4 was not influenced by different doses of inhaled fluticasone in mild-to-moderate asthmatics [11,12]. The discrepancy between these results might be ascribed to a different time sampling. *In vitro* or *ex-vivo* studies on Cys-LTs release from inflammatory cells incubated with corticosteroids led also to discordant results [9,13]. Negri *et al.* demonstrated that CS do not block the acute release of Cys-LTs on the activation of blood cells

taken from normal donors [13]. However, we have previously demonstrated that CS inhibited eicosanoid production from alveolar macrophages taken from subjects with interstitial lung disease, and not from peripheral blood cells, suggesting that cell activation is required for the corticosteroid responsiveness [19].

A major clinical implication of our work is the confirmation that inhaled CS are effective on many different pathways of the allergic inflammation, including Cys-LT release. This had been suggested by many studies on asthmatic subjects showing a better efficacy of low dose inhaled CS in comparison with leukotriene receptor antagonists [20].

The number of the examined patients is low, and then the study might be underpowered for assessing a more relevant effect of BDP not only on EAR-induced uLTE4 excretion but also on LAR-induced uLTE4 excretion. Very few data were available for a correct calculation of the power of our study, and the dimension of other similar studies was limited to 10–14 subjects [7,8].

In conclusion, our study shows that inhaled BDP reduces the early increase of uLTE4 after allergen challenge in association with a partial prevention of EAR and a complete prevention of LAR, suggesting that inhaled CS may modulate the allergen-induced cascade of Cys-LTs in the airways. Considering the relevant role that these mediators have in asthma, our observation supports the efficacy of inhaled CS in the treatment of asthma.

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