

Bronchial responsiveness to leukotriene D₄ is resistant to inhaled fluticasone propionate

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Background: Inhaled corticosteroids are highly effective in asthma, reducing inflammatory markers and bronchial hyperresponsiveness. Cysteinyl-leukotrienes are major mediators of airway obstruction and display proinflammatory effects. Although the synthesis of leukotrienes is not affected by corticosteroid treatment, the influence of corticosteroids on the leukotriene pathway remains unresolved.

Objective: We investigated whether or not bronchial responsiveness to leukotriene (LT) D₄ is reduced by fluticasone propionate in subjects with asthma.

Methods: In 13 subjects with mild asthma, inhalation challenges with methacholine and LTD₄ were performed on consecutive days before and after 2 weeks of treatment with inhaled fluticasone 500 µg, twice daily, in a double-blind, randomized, placebo-controlled study with crossover design and 3 weeks of washout between periods. Exhaled nitric oxide was measured as a marker of corticosteroid responsiveness, and baseline urinary LTE₄ concentrations as an index of cysteinyl-leukotriene biosynthesis.

Results: Fluticasone produced a significant decrease in methacholine responsiveness, corresponding to 2.6-fold shift in the PD₂₀ FEV₁, and a significant reduction in the levels of exhaled nitric oxide. By contrast, bronchial responsiveness to LTD₄ in the same subjects was unaffected by fluticasone, as were urinary LTE₄ concentrations.

Conclusion: These new data indicate that neither the biosynthesis nor the actions of leukotrienes appear to be sensitive to inhaled corticosteroids.

Clinical implications: The study provides mechanistic support for the additive therapeutic efficacy of antileukotrienes and inhaled corticosteroids in asthma. (*J Allergy Clin Immunol* 2006;118:78-83.)

Key words: Asthma, bronchoprovocation, urinary leukotriene E₄, leukotriene D₄ responsiveness, methacholine responsiveness, exhaled nitric oxide, inhaled corticosteroids

Inhaled corticosteroids (ICSs) have a broad anti-inflammatory activity, and they are the mainstay treatment for the management of asthma,¹ improving clinical symptoms, pulmonary function, quality of life, and number of exacerbations. It is also well established that ICSs reduce bronchial responsiveness to methacholine and histamine, as well as responses evoked by most indirectly acting stimuli such as adenosine 5' monophosphate, exercise, and the early (EAR) and late phase reactions (LAR) of allergen induced airway obstruction.² The molecular mechanisms whereby ICSs cause decreased bronchial responsiveness to bronchoconstrictors are, however, not fully understood.

The leukotrienes (LTs) and in particular the cysteinyl-leukotrienes (LTC₄, LTD₄, and LTE₄) mediate central components of asthma, such as bronchoconstriction, inflammatory cell infiltration, mucus production, increased vascular permeability, and smooth muscle proliferation.³ Cysteinyl-leukotrienes are biosynthesized in inflammatory cells, and their *in vivo* production can be assessed by measurement of urinary excretion of LTE₄.⁴ In subjects with asthma, the production of cysteinyl-leukotrienes is increased in response to allergen exposure and other trigger factors of asthma. Antileukotrienes, drugs that block the formation or actions of leukotrienes, protect against airway obstruction induced by the same stimuli. Antileukotrienes have during the past decade been introduced as a new treatment for asthma with beneficial properties across the spectrum of severity.^{3,5}

Despite the widespread anti-inflammatory action of ICSs, their effects on the leukotriene pathway remain uncertain. In fact, several *in vivo* studies have confirmed that treatment with inhaled or oral corticosteroids does not inhibit the biosynthesis of cysteinyl-leukotrienes in subjects with asthma.⁶⁻¹¹ Whereas the protective influence of corticosteroids on bronchial responsiveness to most bronchoconstrictors has been established, their effect on bronchial responsiveness to cysteinyl-leukotrienes in subjects with asthma has not been investigated previously. There is 1 study in subjects without asthma in which the dose-response relation for inhaled LTD₄ was unaffected by treatment with 800 µg budesonide daily for 6 days.¹²

It is intriguing that ICSs have no effect on allergen-induced formation of cysteinyl-leukotrienes^{8,9} despite these leukotrienes being the major mediators of the allergen-induced bronchoconstriction.^{13,14} We hypothesized

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Abbreviations used

- EAR: Early allergic reaction
- F_ENO: Exhaled nitric oxide
- ICS: Inhaled corticosteroid
- LAR: Late allergic reaction
- LT: Leukotriene

that treatment with an ICS would diminish bronchial responsiveness to inhaled LTD₄. Such an effect might explain the inhibitory effect of ICSs on the EAR and LAR in subjects with asthma. In fact, the overall effects of antileukotrienes and ICSs on the EAR and LAR are similar.¹⁵

Thus, in this 2-period, placebo-controlled, crossover study of 13 subjects with mild to intermittent atopic asthma, it was assessed whether 2 weeks of treatment with the potent glucocorticoid fluticasone propionate (500 µg twice daily) reduced bronchial responsiveness to LTD₄. The effect of fluticasone on LTD₄ responsiveness was compared with its effect on methacholine responsiveness as well as the levels of exhaled nitric oxide (F_ENO), serving as a marker of airway inflammation and steroid responsiveness, and urinary excretion of LTE₄, reflecting endogenous biosynthesis of cysteinyl-leukotrienes.

METHODS

Subjects

Fourteen nonsmoking subjects with stable mild atopic asthma treated only with a short-acting β₂-agonist as needed ≤ twice a week, an FEV₁ greater than 70% of predicted, and established bronchial hyperresponsiveness to methacholine (PD₂₀ FEV₁ ≤ 5579 nmol) were recruited. Exclusion criteria were significant allergen exposure, a respiratory tract infection within 6 weeks before and during the study, and use of glucocorticosteroids within 3 months. One subject failed to produce a methacholine PD₂₀ FEV₁ at the start of the crossover treatment period and was therefore excluded. Subject characteristics are displayed in Table I.

The Ethical Committee at the Karolinska Hospital approved the study (Dnr 02-207), and the subjects gave written informed consent.

Study design

The study was double-blind, randomized, placebo-controlled crossover with two 14-day treatment periods and a 3-week washout (Fig 1). Subjects received fluticasone propionate (500 µg bid) and matching placebo delivered through a dry powder inhaler (Flutide Diskus; GlaxoSmithKline, Mölndal, Sweden). After screening, eligible subjects were scheduled for 8 visits at the same time of day. Visit procedures included measurement of F_ENO, collection of urine for analysis of baseline urine-LTE₄, spirometry, and bronchoprovocation with methacholine (visits 1, 3, 5, 7) or LTD₄ (visits 2, 4, 6, 8). The methacholine and LTD₄ bronchoprovocations were performed on consecutive days before and at the end (treatment days 13 and 14) of the respective treatment, with the first dose of study medication taken in the evening after the first LTD₄ challenge at visits 2 and 6, and the last dose in the morning before the second LTD₄ challenge at visits 4 and 8.

TABLE I. Baseline characteristics of study subjects

Subject no.	Sex	Age, y	FEV ₁ , L	FEV ₁ % predicted	Skin prick test, no. positive out of 10
1	F	26	3.4	92	7
2	M	33	3.5	88	5
3	F	27	4.3	116	4
4	M	27	5.7	106	4
5	F	27	3.1	89	2
6	F	19	3.8	94	4
7	F	33	3.2	93	5
8	F	29	4.0	110	4
9	F	34	3.4	101	5
10	M	31	4.6	106	5
11	F	45	3.2	89	2
12	F	39	3.9	112	5
13	F	30	4.3	118	5
Mean ± SD	10 F/3 M	31 ± 6.4	3.9 ± 0.7	101 ± 10.9	4.4 ± 1.3

M, Male; F, female.

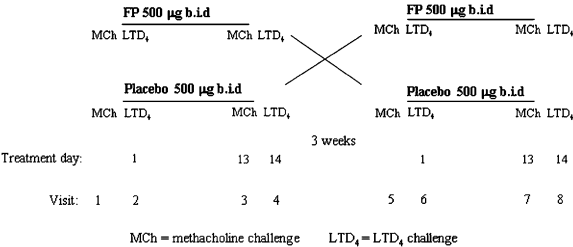


FIG 1. Design of study. FP, Fluticasone propionate.

Measurements of F_ENO and urinary-LTE₄

Exhaled nitric oxide was measured according to the recommendations published by the American Thoracic Society¹⁶ (NIOX; Aerocrine AB, Stockholm, Sweden). F_ENO was not obtained in 2 subjects because of technical problems. Urinary samples were frozen, and the concentrations of LTE₄ were determined according to a validated semiautomated enzymeimmunoassay (Cayman Chemical, Ann Arbor, Mich) methodology.¹⁷ Mean values of the 2 measurements obtained on the consecutive challenge days, before and after the respective period, were used for comparisons between treatments.

Inhalation challenge

Pulmonary function was measured as FEV₁ on a spirometer (MasterScope direct reading spirometer; software version 2.53.2; Erich Jaeger GmbH, Hoechberg, Germany), calibrated daily, and the baseline defined as the best of 3 recordings. Methacholine and LTD₄ bronchoprovocations were performed by the use of a dosimeter-controlled jet nebulizer (Spira Elektro 2; Intramedic, Bålsta, Sweden).¹⁸ Challenges always began with inhalation of the respective diluent. Provided FEV₁ did not change by more than 10%, incremental doses of the provocative agent were administered until FEV₁ had fallen by at least 20% from postdiluent baseline. For LTD₄ bronchoprovocation tests, approximately half-log increments in the cumulated dose (3, 10, 30 pmol, and so forth) were inhaled every 10 minutes (dose range, 3-335,780 pmol). This was achieved by using

TABLE II. Baseline FEV₁ (L) on the different bronchoprovocation days

Subject no.	Placebo				Fluticasone propionate			
	MCh before	LTD ₄ before	MCh after	LTD ₄ after	MCh before	LTD ₄ before	MCh after	LTD ₄ after
1	3.34	3.20	3.28	3.24	3.20	3.15	3.28	3.33
2	3.52	3.43	3.79	4.47	3.36	3.46	3.90	3.76
3	4.27	4.28	4.05	4.07	4.18	4.11	4.10	4.14
4	5.53	5.66	6.04	6.11	5.69	5.69	5.99	5.73
5	3.08	3.22	3.39	2.99	2.85	2.99	3.30	3.23
6	3.93	4.54	4.23	4.14	4.17	4.13	3.95	3.96
7	3.39	3.35	3.44	3.37	3.24	3.20	3.51	3.43
8	3.51	3.82	3.59	3.62	3.71	3.68	3.65	3.68
9	3.28	3.32	3.06	3.27	3.39	3.34	3.40	3.22
10	4.34	4.71	4.23	4.62	4.37	4.36	4.35	4.31
11	3.20	3.24	3.00	3.23	3.21	3.15	3.15	3.28
12	4.03	3.78	3.78	3.84	4.06	3.85	4.02	4.21
13	4.38	4.30	4.31	4.14	3.99	3.87	3.89	4.13
Mean	3.83	3.91	3.86	3.93	3.80	3.77	3.88	3.88
Range	3.08-5.53	3.20-5.66	3.00-6.04	2.99-6.11	2.85-5.69	2.99-5.69	3.15-5.99	3.22-5.73

MCh, Methacholine.

6 solutions of Good Manufacturing Practice-grade LTD₄ (sealed, color-coded vials each containing 1 mL solution; concentrations increasing by 10-fold from 42×10^{-9} mol/L to 4.2×10^{-3} mol/L; solvent water-ethanol 4:1; Cascade Biochemicals, Reading, United Kingdom) and a varying number of breaths (2-7) from each solution.

Spirometry was obtained at 5 and 10 minutes after each dose and the peak fall used for calculation of PD₂₀. Airway responsiveness to methacholine was assessed with a similar protocol but with dose increments every 3 minutes and single FEV₁ efforts. Three concentrations of methacholine chloride (6.24, 50, 400 mmol/L prepared at Norrlands University Hospital Pharmacy) and 2, 4, and 8 breaths were used to create increasing doses (range, 89-45,282 nmol). The PD₂₀ values were calculated from the cumulative-dose response curves by linear interpolation.

Statistical analysis

Baseline FEV₁ values were analyzed with 1-way repeated-measures ANOVA. The PD₂₀ measurements were log-transformed before calculations and are presented as geometric means. Paired *t* test was applied for the comparison between treatment effects on changes in LTD₄ PD₂₀, methacholine PD₂₀, and excretion of U-LTE₄, and Wilcoxon signed-rank test used for changes in F_ENO. Period and carryover effects of the drug treatments were analyzed by the methods of Hills and Armitage¹⁹ and correlations performed with Pearson product moment correlation. Power calculations and statistical evaluations were made by using Sigma Stat 3.00 by SPSS (Jandel Scientific, Sausalito, Calif). Differences were considered significant if *P* < .05.

RESULTS

The 2-week treatment with fluticasone propionate 500 µg bid did not change baseline lung function (Table II). Thus, prechallenge FEV₁ values did not differ among the 8 bronchoprovocation days.

The levels of F_ENO were, however, significantly depressed after treatment with fluticasone propionate, indicating that the subjects were compliant with the study medication. The reduction (ie, before minus after treatment)

in F_ENO was 22.0 (range, -1.7 to 154) ppb from a median baseline of 40.4 after fluticasone propionate and 1.8 (range, -7.3 to 24.0) ppb from a median baseline of 29.9 after placebo (*n* = 11; *P* < .01 between treatments; Fig 2).

Airway responsiveness to methacholine was also significantly attenuated. Fluticasone propionate caused a 2.6-fold shift in PD₂₀ for methacholine (Fig 3). The mean (±SD) log shift in PD₂₀ was thus 0.41 (±0.43) from a geometric mean baseline of 1148 nmol after fluticasone, and 0.02 (±0.32) from a geometric mean baseline of 1349 nmol after placebo (*P* < .05 between treatments; Fig 3). There was, however, no correlation on an individual level between a decreased responsiveness to methacholine and the decreased F_ENO value after treatment with fluticasone (*r* = -0.32; *P* = .36; *n* = 11).

By contrast, airway responsiveness to LTD₄ was unaffected, with similar results on all 4 challenge days; the mean (±SD) log shift in PD₂₀ was -0.04 (±0.30) on the fluticasone limb compared with 0.005 (±0.35) on the placebo limb (*P* = .75 between treatments; Fig 4).

Likewise, fluticasone treatment did not inhibit urinary excretion of LTE₄. The difference in U-LTE₄ concentrations before minus after treatment was 6.9 (±8.0) ng/mmol creatinine (mean ± SD) after fluticasone and 1.4 (±7.9) ng/mmol creatinine after placebo treatment (*P* = .15 between treatments; Fig 5).

Finally, there were no differences at the start of the 2 treatment periods in bronchial responsiveness to methacholine (Fig 3) and LTD₄ (Fig 4), nor in baseline F_ENO values (Fig 2). There were also no period or carryover effects in the study.

DISCUSSION

This is, to our knowledge, the first study that investigates whether bronchial responsiveness to a cysteinyl-leukotriene is decreased by treatment with an inhaled

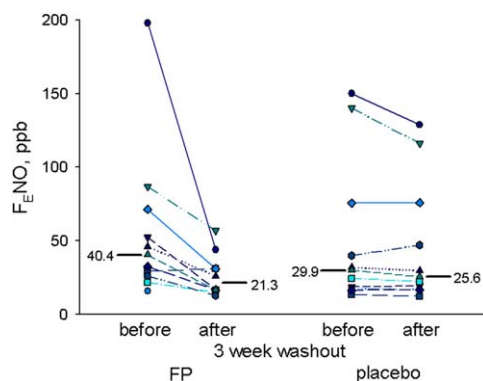


FIG 2. Fluticasone decreases $F_{E}NO$, as measured before and after 2 weeks treatment with fluticasone (500 μ g bid) or placebo. Horizontal bars indicate median values ($n = 11$; $P < .01$ between treatments). FP, Fluticasone propionate.

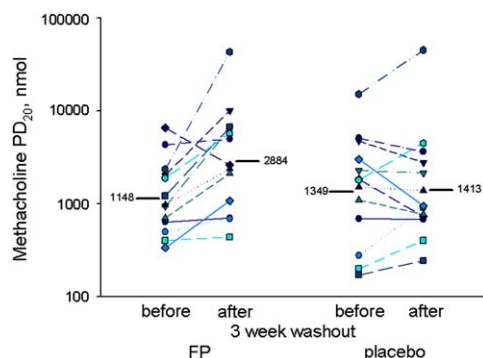


FIG 3. Fluticasone decreases responsiveness to methacholine, as indicated by PD_{20} values before and after 2 weeks of treatment with fluticasone (500 μ g bid) or placebo. Horizontal bars indicate geometric means ($n = 13$; $P < .05$ between treatments). FP, Fluticasone propionate.

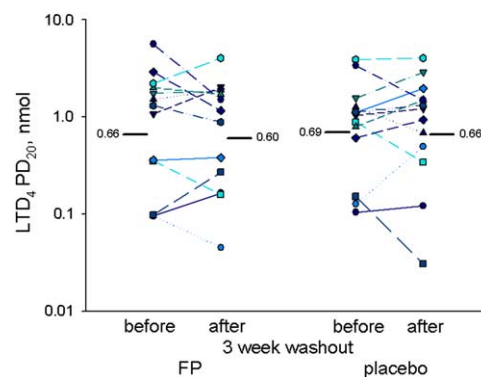


FIG 4. No change in responsiveness to LTD_4 before and after 2 weeks of treatment with fluticasone (500 μ g bid) or placebo. Horizontal bars indicate geometric means ($n = 13$; $P = .75$ between treatments). FP, Fluticasone propionate.

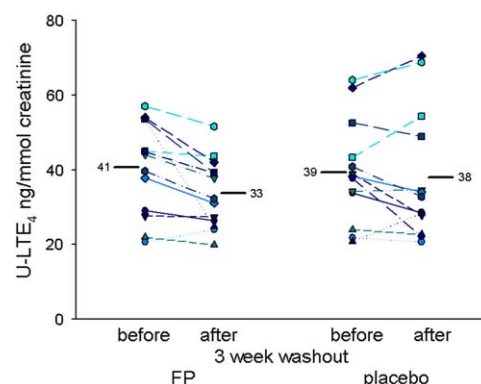


FIG 5. No change in urinary LTE_4 before and after 2 weeks of treatment with fluticasone (500 μ g bid) or placebo. Horizontal bars indicate means ($n = 13$; $P = .15$ between treatments). FP, Fluticasone propionate.

glucocorticosteroid in subjects with asthma. We found that 2 weeks of treatment with the potent glucocorticosteroid fluticasone 1000 μ g/d caused a decrease in $F_{E}NO$ and a decrease in bronchial responsiveness to methacholine. In contrast, the study could not confirm our hypothesis that ICS treatment would diminish bronchial responsiveness to inhaled LTD_4 .

The study was adequately controlled and powered ($>90\%$) to detect 0.3 log shift in LTD_4 PD_{20} , implying low risk of type II error. In fact, the repeatability of LTD_4 PD_{20} , as assessed at the start of the respective treatment periods, was excellent, with a mean difference in PD_{20} between the 2 sessions of $0.02 (\pm 0.32 [SD])$ log units. Our data are in agreement with other studies that have shown the LTD_4 challenge to be highly reproducible.^{20,21} Although the outcome was unexpected, it agrees with the result in 1 study of healthy subjects whose geometric mean LTD_4 PD_{10} was unaffected after 6 days of budesonide.¹²

Because similar 2-week courses of ICSs induce attenuation of bronchial hyperresponsiveness to most indirect

stimuli, and albeit to a lesser degree also to direct stimuli,²² the absence of an effect of fluticasone on the LTD_4 response is surprising, in particular because we at the same time in the same subjects were able to demonstrate that fluticasone caused a significant improvement of bronchial hyperresponsiveness to methacholine. Our results are in this respect completely consistent with the observations of other investigators who have evaluated a similar duration of ICS treatment on bronchial hyperresponsiveness to methacholine.^{8,23,24} The early improvement of bronchial hyperresponsiveness to histamine and methacholine appears to be correlated with the reduction in surrogate markers of airway inflammation and is therefore thought to reflect the anti-inflammatory actions of ICSs.²² In our study, $F_{E}NO$ was consistently reduced by fluticasone, supporting that the treatment had the expected anti-inflammatory effect. In addition, the reduction of $F_{E}NO$ documents that the subjects were compliant with the study medication.

Before this study, investigations of how glucocorticosteroids influence the leukotriene pathway have focused

on whether production of leukotrienes is inhibited by glucocorticosteroids. There is thus extensive evidence that glucocorticosteroids do not block the biosynthesis or release of cysteinyl-leukotrienes *in vivo*.⁶⁻¹¹ This was confirmed in the current study inasmuch that fluticasone caused no significant reduction in basal urinary LTE₄ excretion. Because we in the same group of subjects with asthma discovered that fluticasone did also not change bronchial responsiveness to LTD₄, it seems that the leukotriene-dependent parts of bronchoconstriction and airway inflammation are uniquely resistant to the anti-inflammatory effects of glucocorticosteroids. The additive therapeutic effect of antileukotrienes in glucocorticosteroid-treated subjects with asthma is established,²⁵⁻²⁷ and has sometimes been found quite remarkable in subjects with more severe asthma varieties.^{11,28} Taken together, we now conclude that the additive therapeutic effects of ICSs and antileukotrienes relate to glucocorticosteroid resistance of the leukotriene pathway at 2 levels. Thus, neither the release nor the actions of leukotrienes appear to be sensitive to ICSs, strengthening the rationale for combining glucocorticosteroids and antileukotrienes in the treatment of asthma.

The relation of our finding to the mechanisms in allergen-induced EAR and LAR is, however, less obvious. Thus, cysteinyl-leukotrienes are established as the major mediators of both the EAR and the LAR.^{13,14,29,30} Furthermore, glucocorticosteroids inhibit the allergen-induced airway obstruction to a similar, although not identical, degree as leukotriene antagonism.¹⁵ Thus, single doses of ICSs have less effect on the EAR than leukotriene antagonists, and their effect is more prominent on the LAR. Nevertheless, using the same dose (500 µg bid) and duration of fluticasone treatment, O'Shaughnessy et al⁸ showed that both the allergen-induced EAR and LAR were profoundly inhibited, and this inhibition was not associated with a reduction in allergen-induced urinary LTE₄ excretion. We therefore hypothesized that the protective effect of fluticasone in the allergen challenge might relate to inhibition of the actions of cysteinyl-leukotrienes, rather than to inhibition of their formation. In another allergen challenge study, Leigh et al¹⁵ demonstrated significant attenuation of the EAR and LAR afforded by the antileukotriene, montelukast, and the ICS, budesonide, but the 2 treatments in combination were not additive, also suggesting a common target of action for both interventions, such as the leukotriene pathway. The findings in this study, however, argue against the possibility that at least part of the effects of ICSs on EAR and LAR relate to blockade of the actions of cysteinyl-leukotrienes in the airways.

It may be speculated that the protective effect of glucocorticosteroids on the EAR and LAR is a consequence of reduced responsiveness to, in particular, histamine. We have previously shown that histamine and leukotrienes together account for about 75% of the EAR and LAR.¹³ Presumably leukotrienes, and other released mediators, synergize with histamine. Thus, reduction of responsiveness to 1 mediator (histamine) by glucocorticosteroids may be sufficient to have significant overall

effects on the allergen-induced response, although another mediator of the same reaction (LTD₄) is unaffected by the glucocorticosteroid. It might also be that redundant mediator mechanisms come into play in the EAR and LAR during the allergen challenge, and that ICSs have complex and yet poorly understood actions on synergisms and interrelationships between inflammatory and bronchoconstrictive mediators in the airways.

In conclusion, we have shown for the first time that 2 weeks of treatment with a potent ICS does not reduce bronchial responsiveness to LTD₄. However, steroid treatment was not without pharmacologic effect, causing both a reduction in bronchial responsiveness to methacholine as well as a decrease in exhaled nitric oxide. Therefore, our study provides further evidence that the cysteinyl-leukotrienes have unique actions on human airways.^{18,31-33}

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