

Original article

Eosinophilic bronchitis in asthma: A model for establishing dose-response and relative potency of inhaled corticosteroids

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Background: Newer generations and formulations of inhaled corticosteroids have necessitated the development of a clinically relevant model to compare their clinical potency.

Objective: We evaluated whether sputum eosinophil counts could demonstrate a dose-response to inhaled corticosteroids, and compared the response with other inflammatory markers. **Methods:** Fourteen steroid-naïve patients with asthma with an initial sputum eosinophilia of $\geq 2.5\%$ entered a 6-week sequential, placebo-controlled, patient-blinded, cumulative dose-response study. After 7 days of placebo, they received incremental doses of fluticasone propionate (FP), 50, 100, 200, and 400 $\mu\text{g}/\text{d}$, each for 7 days. Measurements were made of sputum and blood eosinophils, exhaled nitric oxide, spirometry, airway responsiveness to methacholine (methacholine PC_{20}), and symptom scores before and after each dose.

Results: Sputum eosinophils and exhaled nitric oxide were extremely sensitive to the effects of FP, and exhibited significant dose-dependent reductions of 99.4% and 99.8 parts per billion, respectively, where each variable was expressed per 100 $\mu\text{g}/\text{d}$ FP. This compared with a 0.5 doubling dose increase of airway responsiveness to methacholine and a 0.3 decrease in symptom scores. Airway responsiveness to methacholine was the only variable that increased throughout the study.

Conclusion: These results suggest that the model of eosinophilic bronchitis could be used to compare the effect of cumulative doses of an inhaled corticosteroid delivered by different types

of delivery systems or preparations using a relatively small number of patients.

Clinical implications: Future clinical studies based on this model will allow clinicians to make informed decisions regarding the relative potencies of different inhaled corticosteroids. (*J Allergy Clin Immunol* ■■■■;■■■:■■■-■■■.)

Key words: Asthma, inhaled corticosteroids, fluticasone propionate, relative potency, sputum eosinophils

Inhaled corticosteroids (ICSs) are regarded the most effective anti-inflammatory therapy for asthma,¹ and significantly improve symptoms,²⁻⁴ exacerbations,^{5,6} mortality,^{3,4} airway inflammation,^{2,3} and airway function.⁷ There are now several different ICSs available as well as different formulations and delivery devices,⁸ and there is a need, therefore, to determine their relative clinical potency and to establish methods to do this with small numbers of patients.^{9,10}

Measurement of relative potency requires the construction of dose-response curves. This has not been readily achieved with physiologic measurements that are improved indirectly by corticosteroid treatment. An alternative variable, which is directly affected by corticosteroid treatment, is the sputum eosinophil count, which, when increased, is a sensitive and responsive marker of corticosteroid effect.¹¹⁻¹⁵ Treatment strategies in which corticosteroid dose is adjusted on the basis of sputum eosinophil counts have been shown to be superior, in terms of relevant clinical outcomes, to current guideline-based treatment strategies.^{16,17}

Demonstration of a dose-response relationship is necessary to select the appropriate doses for comparison of clinical efficacies of different ICS preparations or the same ICS delivered by different devices.^{18,19} The sample size required can be calculated by using an estimate of the slope and variability of the dose-response relationship. It is hypothesized that the beneficial clinical effects of ICS are related, at least in part, to the attenuation of airway inflammation,²⁰ and this can be assessed conveniently and relatively noninvasively by measurements of sputum cell counts.²¹ Several studies support the concept that sputum eosinophils are highly responsive to ICS,¹¹⁻¹⁵ suggesting that they may be suitable candidates for monitoring the clinical response to ICS.

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TABLE I. Outcome measurements

	Screening	Postplacebo	Postfluticasone treatment			
		Baseline	50 µg	100 µg	200 µg	400 µg
Sputum eosinophils %	9.0 (6.4, 17.7)	9.4 (5.0, 17.5)	1.7 (0.5, 6.6)	1.9 (1.2, 3.1)	0.2 (0.0, 1.3)	0.5 (0.1, 2.0)
Sputum eosinophils 10 ⁴ /g	24.6 (13.1, 46.4)	30.7 (15.3, 61.3)	4.2 (0.7, 27.3)	5.0 (2.4, 10.3)	0.3 (0.0, 4.6)	0.9 (0.1, 6.4)
eNO ppb	25.1 (15.5, 41.7)	26.9 (17.4, 42.7)	16.2 (9.8, 25.7)	13.2 (8.3, 17.4)	12.3 (7.8, 19.1)	10.5 (6.9, 16.2)
PC ₂₀ mg/mL	0.31 (0.13-0.72)	0.28 (0.13, 0.57)	0.47 (0.24, 0.95)	0.59 (0.25, 1.39)	1.1 (0.47, 2.62)	1.54 (0.59, 4.0)
FEV ₁ % predicted	80.6 (23.8)	82.0 (25.8)	89.8 (18.1)	91.7 (7.7)	94 (19.0)	95.2 (17.6)
Blood eosinophils %	6.1 (4.5, 8.3)	5.4 (3.5, 8.1)	5.0 (3.9, 6.4)	4.6 (3.4, 6.3)	4.2 (3.2, 5.7)	3.6 (2.7, 4.7)
Sputum ECP µg/L	0.31 (0.13, 0.72)	0.28 (0.13, 0.57)	0.47 (0.24, 0.95)	0.59 (0.25, 1.39)	1.1 (0.47, 2.62)	1.54 (0.59, 4.0)
Sputum tryptase µg/L	7.7 (2.0, 29.5)	10.1 (4.3, 23.7)	6.7 (3.0, 14.6)	6.4 (2.5, 16.3)	7.4 (3.3, 16.7)	10.1 (3.7, 28.2)
Sputum fibrinogen µg/L	931 (450, 1928)	638 (272, 1499)	677 (347, 1321)	692 (312, 1531)	560 (274, 1146)	499 (304, 820)
Symptom score	6.2 (0.9)	6.6 (1.2)	7.5 (1.0)	8.1 (0.7)	7.8 (1.0)	8.3 (0.7)

Expressed as geometric mean (95% confidence limits), except symptom score, which is expressed as arithmetic mean (SD).

Abbreviations used

ECP: Eosinophil cationic protein

eNO: Exhaled nitric oxide

FP: Fluticasone propionate

ICS: Inhaled corticosteroid

We therefore investigated the possibility that eosinophilic bronchitis, as measured by induced sputum eosinophils, could be used to examine the inhaled fluticasone propionate (FP) dose-response effect. Secondary outcomes were the effects on other inflammatory markers, specifically blood eosinophils, sputum eosinophil cationic protein (ECP), tryptase, fibrinogen, and exhaled nitric oxide (eNO). In addition, we examined airway responsiveness to methacholine, FEV₁, and symptom scores.

METHODS

Patients

Fourteen nonsmoking adults with asthma were recruited by advertising in local media and pharmacies, as well as from outpatient clinics of the hospital. Asthma was diagnosed according to established guidelines.²² Additional clinical details are provided in the online repository at www.jacionline.org. For the purpose of the study, sputum eosinophilia was defined as $\geq 2.5\%$ of the inflammatory cells present on induced sputum analysis, and only those patients conforming to this at both the screening and subsequent baseline visit were included in the study. The study was approved by the hospital Research Ethics Board, and all of the patients gave written informed consent.

Study design and protocol

The study consisted of a 6-week, sequential, placebo-controlled, single-blind (patient level), cumulative dose-response design. Patients identified as suitable at the screening visit by the presence of a sputum eosinophilia were given placebo for a week, after which sputum induction was repeated. If sputum eosinophilia was still present, they were given increasing doses, in sequential order, by week, of 50, 100, 200, and 400 µg/d of FP, delivered via an Aerochamber. The inhalers used in this study were specifically formulated for the study to contain placebo or 25 µg or 50 µg of FP and were identical

in appearance (GlaxoSmithKline Canada, Mississauga, Ontario, Canada), allowing patients to remain blind to the treatment allocation. Additional details are provided in the [Methods](#) section of the Online Repository at www.jacionline.org.

Laboratory procedures

Details on assessment of symptom scores, allergen skin testing, blood counts, spirometry, sputum induction and examination, and eNO are provided in the [Methods](#) section of the Online Repository at www.jacionline.org.

Statistical analysis

The sample size of 14 patients ensured the study was powered at $>80\%$ to detect a 2-fold difference of sputum eosinophil counts among 3 doses, with a 2-tailed α of 0.05. Data were analyzed by using SPSS for Windows, Release 10.05 (SPSS Inc, Chicago, Ill). Descriptive statistics were used to summarize the baseline characteristics of the study patients. Measures of central tendency and dispersion were expressed as the geometric mean and 95% confidence limits after logarithmic transformation for all variables apart from age, symptom scores, and spirometric values, which were expressed as arithmetic mean and SD. Logarithmic transformation was to the base 10, except for methacholine PC₂₀ measurements, which were to the base 2; this allowed differences between PC₂₀ values to be expressed as doubling concentrations.²³

After each treatment period, variables were compared with placebo treatment (baseline). Repeated-measures ANOVA was used to test for within-subject differences at each visit, with Bonferroni correction (set at 95% CI) for multiple comparisons. Spearman rank correlations were used to measure relationships between variables. Regression analysis (with dose, period, and dose-period interaction as within-patient factors in the model) was used to analyze the effect of treatment dose to establish whether individual patient response slopes for each outcome variable were significantly different from 0, and whether these slopes were significantly different from each other. Two-tailed P values $< .05$ were considered to be significant.

RESULTS

Effects on markers of airway inflammation

When related to baseline, the percentage of sputum eosinophils were significantly lower after treatment with 100, 200, and 400 µg FP/d for 1 week, absolute eosinophil counts only after 200 and 400 µg FP, eNO concentration

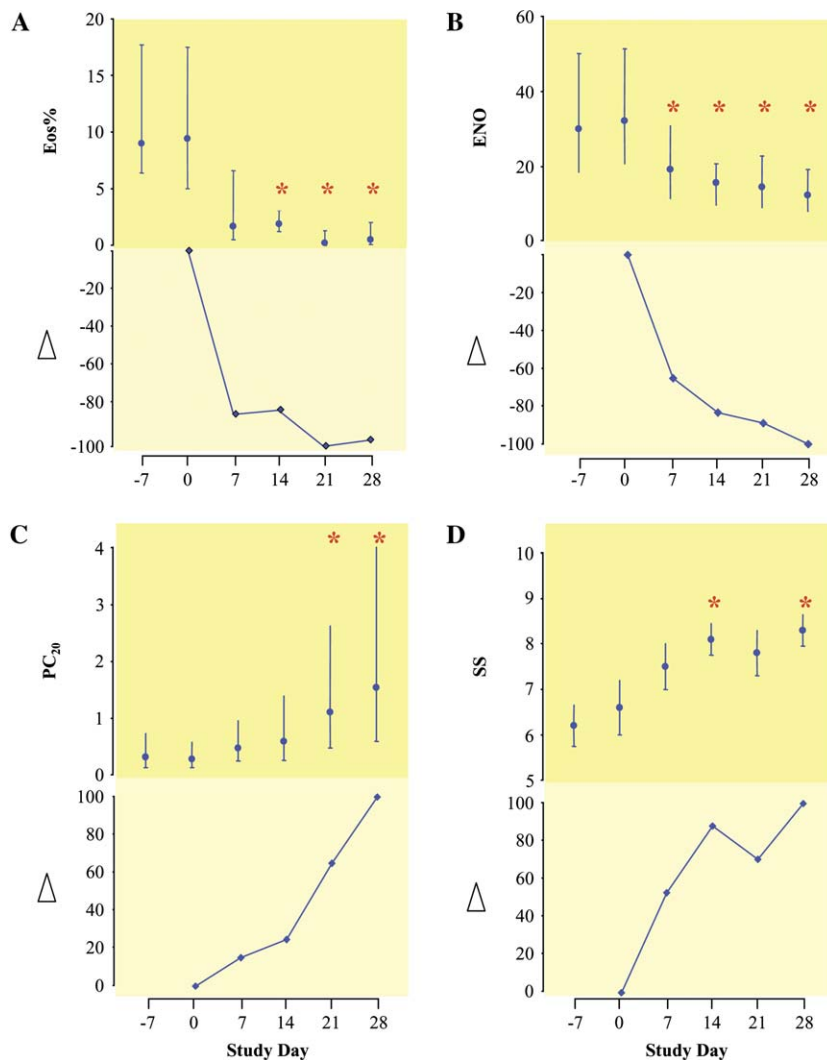


FIG 1. A, Percent sputum eosinophils (Eos%). B, eNO (ppb). C, Methacholine PC₂₀ (mg/mL). D, Symptom score (SS). Solid circles, geometric mean; vertical lines, CIs; solid diamonds, change from baseline expressed as % of total change (Δ). * $P < .05$ compared with baseline.

after treatment with FP at all time points, and symptom scores after 100 and 400 μg FP ($P < .05$). Methacholine PC₂₀ increased after treatment with 50 μg , 100 μg , 200 μg , and 400 μg FP by 0.19, 0.31, 0.82, and 1.26 doubling doses, respectively, and was significantly different from baseline after treatment with 200 μg and 400 μg FP ($P < .05$; Table I, Fig 1). Blood eosinophils, sputum ECP, tryptase, fibrinogen, and FEV₁ % predicted were not significantly different from baseline at any visit.

Dose-response relationships

A dose-response relationship was explored by using linear regression only for those outcome variables significantly different from baseline on repeated-measures ANOVA (sputum eosinophils [% and absolute counts], eNO, methacholine PC₂₀, and symptom scores; Fig 2). The outcome variables for each patient were modeled with a slope and intercept and compared with a model

with a common slope and individual intercepts. Regression analyses confirmed that individual patient response slopes for each outcome variable were significantly different from 0 ($P < .01$), but were not significantly different from each other ($P > .05$), allowing estimation of a single dose-response line. Sputum eosinophils (%) showed a significant dose-response relationship, in which sputum eosinophils decreased by 99.4 % (95% CL, 99.1, 99.8) per 100 $\mu\text{g}/\text{d}$ of FP treatment. eNO decreased by 99.8 ppb (95% CL, 99.7, 99.9). Methacholine PC₂₀ showed a 0.5 doubling dose increase (95% CL, 0.4, 0.7), and symptom scores decreased by 0.3 (95% CL, 0.1, 0.5) per 100 $\mu\text{g}/\text{d}$ of FP treatment.

Correlations between inflammatory markers

The change in sputum eosinophils (expressed either as a percentage or absolute value) was significantly correlated with the changes in sputum ECP ($r_s = 0.33$, $P = .009$;

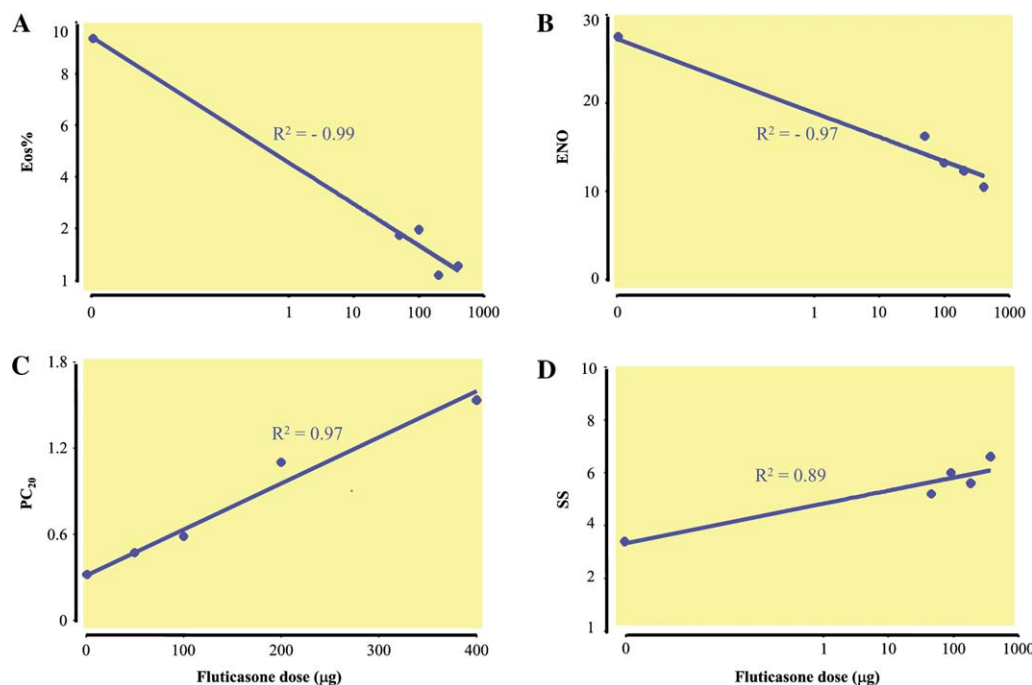


FIG 2. Outcome variables versus dose. (A) Sputum eosinophils (%) (E%), (B) eNO (ppb), (C) methacholine PC₂₀ (mg/mL; expressed as geometric mean), and (D) symptom score (SS).

$r_s = 0.56, P < .001$), eNO ($r_s = 0.42, P < .001$; $r_s = 0.36, P = .004$), and blood eosinophils ($r_s = 0.51, P < .001$; $r_s = 0.46, P < .001$). The change in absolute sputum eosinophils was significantly correlated with changes in sputum tryptase ($r_s = 0.41, P = .001$) and showed a weak negative correlation with methacholine PC₂₀ ($r_s = -0.28, P = .03$).

DISCUSSION

In this study, we have observed that 4 incremental doses of FP resulted in a significant reduction of sputum eosinophils and eNO and a significant improvement in methacholine PC₂₀ and symptom scores. In addition, the steep dose-response curves demonstrated in this study were achieved with a relatively small number of patients ($n = 14$).

Sputum eosinophils and eNO were extremely sensitive to low doses of inhaled FP, with 86.5% and 65%, respectively, of the total change from baseline occurring after treatment with the lowest dose of FP (50 μg/d; Fig 1, A and B). It is possible that had we used lower doses of inhaled FP in the study (ie, 25 μg FP/d), we might have seen a more gradual dose-response to the inhaled steroid. However, such low doses of inhaled FP are not used in current practice, and the clinical relevance and generalizability of such data would be open to question. Other investigators have previously demonstrated a dose-response relationship between eNO and ICS.^{24,25} Although we have previously observed a poor correlation between eNO measurements and sputum eosinophils while patients were being treated with ICS,²⁶ eNO measurements have

also been demonstrated to be predictive of deterioration in asthma control after ICS withdrawal.²⁴ These data support the observations that increasing doses of ICS do not have large additional benefits in terms of further reducing inflammatory markers or improving symptom scores^{5,6,27} in patients who are not on inhaled corticosteroids and need only low-dose inhaled corticosteroid therapy. This may not apply to patients who are relatively less corticosteroid responsive and need higher doses to control eosinophilic bronchitis. In contrast, methacholine PC₂₀ measurements increased steadily throughout the study (Fig 1, C), unlike previous studies in which a significant increase in methacholine PC₂₀ was demonstrated only after high doses or several weeks of treatment—for example, 8 weeks of treatment with 2000 μg/d FP,²⁸ 6 weeks of treatment with 500 μg/d FP,²⁹ or 3 months with 2000 μg/d FP.³⁰ The reasons for the response to methacholine PC₂₀ are not clear but may relate to the fact that the patients in the current study were steroid-naïve and had active eosinophilic bronchitis at baseline.

It is not surprising that FEV₁ % predicted did not show a significant difference from baseline, because a dose-response effect of ICS on FEV₁ requires large numbers of patients,^{6,7,31,32} and pulmonary function tests appear to be less sensitive to the effects of ICS.²⁰ Similar to findings in other studies,³³⁻³⁵ sputum ECP concentration was also less sensitive to change than sputum eosinophils.

The exquisite sensitivity of sputum eosinophils and eNO to ICS is consistent with meta-analyses showing that 80% of the benefit at 1000 μg/d FP is achieved at doses of 70 to 170 μg/d,³⁶ and in the majority of patients with asthma, there is little benefit in increasing the dose of FP

beyond 200 $\mu\text{g}/\text{d}$.³⁷ Another study has shown that FP at a dose of 50 μg twice daily was effective in maintaining control in a group of patients with moderate asthma,³⁸ and Szefer et al³⁹ reported that near maximum response in sputum eosinophils and eNO occurred with the lowest dose (88 $\mu\text{g}/\text{d}$) of FP used in their study. Although FP has a potency of approximately twice that of budesonide and beclomethasone dipropionate (see reviews^{9,20}), a meta-analysis of clinical trials with budesonide indicated that most of the therapeutic benefit of budesonide is achieved with a total daily dose of 400 μg ,⁴⁰ strongly consistent with the fluticasone analyses. However, these findings cannot be generalized to all subjects, because some require higher doses of ICS or even prednisone.

Clinical models using outcomes such as the dose-dependent effects of ICS in improving lung function, symptom scores, nocturnal awakening, frequency of rescue β_2 -agonist therapy, airway hyperresponsiveness, and response to allergen challenge^{6,7,41,42} are downstream of the pharmacologic effect and are only surrogate markers of anti-inflammatory activity. The model described in this study, based on sputum eosinophils and therefore directly measuring airway inflammation, has the advantage over other clinical models in which it is unclear that the mechanisms resulting in clinical efficacy necessarily translate into effectiveness in practice, and it has been shown that treatment tailored toward reducing sputum eosinophils has resulted in improved management of asthma.¹⁶ One of the exceptions to this is airway hyperresponsiveness, where treatment tailored toward reducing it has been shown to result in improved clinical outcomes.⁴³ Interestingly, our study demonstrated a dose-response of methacholine PC₂₀ to FP, with the change in methacholine PC₂₀ at each visit more uniform than that of sputum eosinophils and eNO. In addition, methacholine PC₂₀ appeared to improve even after the airway eosinophilia had been markedly attenuated. Thus, methacholine PC₂₀ may be a more useful marker of inhaled corticosteroid relative potencies, in that there is a measurable response with a wide range of FP doses, at least in this clinical study model. Although methacholine PC₂₀ may demonstrate a more sustained dose-response to inhaled corticosteroids, this response is delayed compared with that of sputum eosinophils and eNO, likely because methacholine PC₂₀ is a downstream marker of the direct anti-inflammatory pharmacologic effects of inhaled corticosteroids. As such, if methacholine PC₂₀ were to be the primary outcome in this type of study, the study duration would need to be increased, making it logistically more difficult to complete.

A criticism of this model is that the increasing dose design is likely to result in carryover effects that are not discernible with order. These carryover effects may be similar between different preparations, but because this is unknown, it may be a confounding factor in the model. In another model in which ICS was sequentially increased every 3 weeks without washout periods, Wilson and Lipworth⁴⁴ reported a dose-response of budesonide on bronchial hyperactivity and serum ECP, but eNO response showed a plateau early on in treatment. The

dose-response to bronchial hyperresponsiveness is consistent with our findings in which methacholine PC₂₀ continued to increase throughout the study. Serum ECP may not be a sensitive enough marker for relatively short periods of ICS treatment as in our study. The use of a parallel study design would avoid carryover effects, but would also necessitate using a larger patient sample size.

The need to obtain patients who were steroid-naïve and demonstrated a sputum eosinophilia at both the screening and baseline visits resulted in relatively slow recruitment; however, most steroid-naïve subjects demonstrated a sputum eosinophilia at the first visit, and only 1 did not have persistent sputum eosinophilia at the second visit. This preselection of patients is the likely reason that a relatively small sample size can be used in this model. A recent study using a model of steroid withdrawal had a sample size of 65 subjects.²⁴

In summary, we have demonstrated a dose-response effect of FP on sputum eosinophils, eNO, methacholine PC₂₀, and symptom scores. This suggests that eosinophilic bronchitis in patients with uncontrolled asthma may be a useful model for assessing and performing dose-response and relative potency studies on different ICS compounds and delivery devices.

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