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-naive patients with asthma with an initial sputum eosinophilia of ≥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 μg/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 μg/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 2006;117:989-94.)

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

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TABLE I. Outcome measurements

		Postplacebo	Postfluticasone treatment			
	Screening	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

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.

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, FEV_1, 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 PC_{20} measurements, which were to the base 2; this allowed differences between PC_{20} 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