Protective effects of fluticasone on allergen-induced airway responses and sputum inflammatory markers

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BACKGROUND: A direct comparison of the protective effects of single and regular doses of inhaled glucocorticoid on allergen-induced asthmatic responses and inflammation has not been made.

OBJECTIVE: To compare the effects of pretreatment with fluticasone 250 µg 30 min before allergen inhalation and two weeks of 250 µg twice daily (last dose 24 h before challenge) with single and regular (twice daily) placebo doses on early and late asthmatic responses, induced sputum cell counts and measures of eosinophil activation at 7 h and 24 h, and methacholine airway responsiveness at 24 h.

PATIENTS AND METHODS: Ten mild asthmatic patients were studied in a randomized, double-blind, placebo controlled crossover study.

RESULTS: Regular fluticasone increased the baseline mean provocative concentration of methacholine to cause a 20% fall (PC_{20}) in forced expiratory volume in 1 s (FEV_1) from 2.6 to 6.4 mg/mL ($P<0.05$) and lowered the eosinophil count from 3.1% to 0.4% ($P<0.05$) compared with regular placebo. Neither single nor regular fluticasone had any effect on the early asthmatic response. Single fluticasone attenuated the late asthmatic response, the mean ± SEM maximum percentage fall in FEV_1 (10.8±3.6 compared with single placebo 18.8±3.5, $P=0.03$), the allergen-induced increase of airway responsiveness ($P<0.05$), and the eosinophilia ($P<0.005$) and activated eosinophils at 7 h ($P<0.01$) but not at 24 h. Regular fluticasone also attenuated the late asthmatic response (11.1±2.5) compared with regular placebo (19.6±4.5), but this was not statistically significant and did not protect against the induced increase in airway responsiveness or the sputum eosinophilia.

CONCLUSION: Two weeks of regular inhaled fluticasone discontinued 24 h before allergen challenge does not offer any additional protection against the early or late asthmatic responses, increased airway responsiveness or sputum eosinophilia compared with a single dose of 250 µg immediately before allergen challenge, despite increasing baseline PC_{20} and decreasing sputum eosinophilia prechallenge. The significance of the protective effect of a single dose of inhaled steroid before an allergen inhalation and the duration of the protective effect need further investigation.

Key Words: *Airway inflammation; Airway responsiveness; Allergen inhalation; Fluticasone; Induced sputum*

Pour le résumé, voir page suivante

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Effets protecteurs de la fluticasone sur les réactions des voies aériennes à un allergène et les marqueurs inflammatoires dans les sécrétions

CONTEXTE : L'effet protecteur des glucocorticoïdes inhalés en dose unique ou régulière sur les réactions asthmatiques à un allergène et l'inflammation n'a jamais fait l'objet de comparaison directe.
OBJECTIF : Comparer l'effet de la fluticasone, 250 µg, 30 min avant l'inhalation d'un allergène et 250 µg, 2 fois par jour (dernière dose 24 h avant le test de provocation) durant deux semaines à celui d'un placebo administré en dose unique et en dose régulière sur les réactions asthmatiques précoces et tardives, la numération globulaire dans les expectorations provoquées et l'activation des éosinophiles au bout de 7 h et de 24 h, ainsi que sur la réactivité des voies aériennes (VA) à la méthacholine au bout de 24 h.
PATIENTS ET MÉTHODE : Essai croisé, à double insu, mené sur échantillon aléatoire et contrôlé contre placebo auprès de 10 patients souffrant d'asthme léger.
RÉSULTATS : La prise régulière de fluticasone s'est traduite par une augmentation de la concentration provocatrice initiale moyenne de métacholine susceptible d'entraîner une chute de 20 % (PC_{20}) du volume expiratoire maximum-seconde (VEMS) de 2,6 à 6,4 mg/ml (P<0,05) et une diminution du nombre d'éosinophiles de 3,1 % à 0,4% (P<0,05) comparativement au placebo pris en dose régulière. La fluticasone prise en dose unique ou en dose régulière n'a eu aucun effet sur les premières réactions asthmatiques. Par contre, la dose unique de fluticasone a permis de diminuer les réactions asthmatiques tardives, le pourcentage maximal moyen ± erreur type de la chute du VEMS (10,8±3,6 comparativement à la dose unique du placebo 18,8±3,5; P=0,03), l'augmentation de la réactivité des VA (P<0,05), l'éosinophilie (P<0,005) et l'activation des éosinophiles au bout de 7 h (P<0,01) mais pas au bout de 24 h. La prise régulière de fluticasone a aussi permis d'atténuer les réactions asthmatiques tardives (11,1±2,5) comparativement à la prise régulière du placebo (19,6±4,5), mais la différence n'était pas significative et le traitement n'a pas eu d'effet protecteur contre l'augmentation de la réactivité des VA ou l'éosinophilie dans les expectorations.
CONCLUSION : La prise régulière de fluticasone durant deux semaines mais interrompue 24 h avant le test de provocation à l'allergène n'offre pas de protection supplémentaire contre les réactions asthmatiques précoces ou tardives, la réactivité des VA ou l'éosinophilie dans les expectorations comparativement à la dose unique de 250 µg de fluticasone prise immédiatement avant l'exposition à l'allergène, et ce, malgré une augmentation du PC_{20} de base et une diminution de l'éosinophilie dans les expectorations avant le test de provocation. La portée de l'effet protecteur de la dose unique de stéroïde en inhalation avant l'exposition à un allergène et la durée de l'effet protecteur doivent faire l'objet d'études plus poussées.

The effects of inhaled allergens have been examined in the laboratory by allergen inhalation tests (1). These tests cause early and late asthmatic responses, increases in airway responsiveness to methacholine and an eosinophilic bronchitis, which can be measured by the examination of induced sputum (2). Allergen inhalation tests have also been used to investigate the effects of treatment with inhaled corticosteroids. Pretreatment with a single dose of inhaled beclomethasone inhibits the late asthmatic response and the increase in airway responsiveness, but has no effect on the early asthmatic response or sputum eosinophilia (3,4). Regular treatment with budesonide for seven days or with fluticasone for two weeks also inhibits the early asthmatic response (5,6), sputum eosinophilia and activated eosinophils when such treatment is stopped 30 min to 12 h before allergen inhalation (5). However, the effects of a single dose of corticosteroid have not been compared with the effects of regular treatment in the same patients.

Therefore, fluticasone was used to compare the effects of pretreatment with a single dose of 250 µg 30 min before allergen challenge with regular treatment with 250 µg twice daily for two weeks, with the last dose given 24 h before allergen inhalation. Fluticasone propionate is reported to have twice the topical anti-inflammatory potency of beclomethasone diproprionate (7). Its rate of dissociation from the glucocorticoid receptor is slower than beclomethasone, resulting in a longer corticosteroid-receptor complex half-life of about 10 h (8) and, therefore, a potentially longer anti-inflammatory effect after discontinuation of the drug. It was hypothesized that the regular treatment with fluticasone should produce a reduction in basal airway inflammation and airway responsiveness, and should, therefore, offer greater pro-

TABLE 1
Patient characteristics at screening visit

Patient	Age (years)	Sex	Height (cm)	Weight (kg)	FEV_1 (% pred)	PC_{20} (mg/mL)	Allergen (dilution)
1	19	F	178	73	95	4.7	Cat (1:16)
2	34	M	172	70	75	1.6	DP (1:512)
3	20	M	180	90	81	0.4	DF (1:1024)
4	48	M	180	72	92	1.5	RW (1:16)
5	21	M	183	82	102	2.2	RW (1:32)
6	21	F	160	53	94	2.9	RW (1:16)
7	20	M	183	77	106	5.8	RW (1:256)
8	21	F	185	75	90	0.3	RW (1:256)
9	23	F	167	66	82	3.0	RW (1:1024)
10	23	M	173	56	103	3.2	RW (1:2040)

Allergen dilution is that causing a fall in forced expiratory volume in 1 s (FEV_1) of at least 20%; % pred Percentage of predicted value; Cat Cat hair; DF Dermatophagoides farinae; DP Dermatophagoides pteronyssinus; PC_{20} Provocation concentration of methacholine to cause a fall in FEV_1 of 20%; RW Ragweed

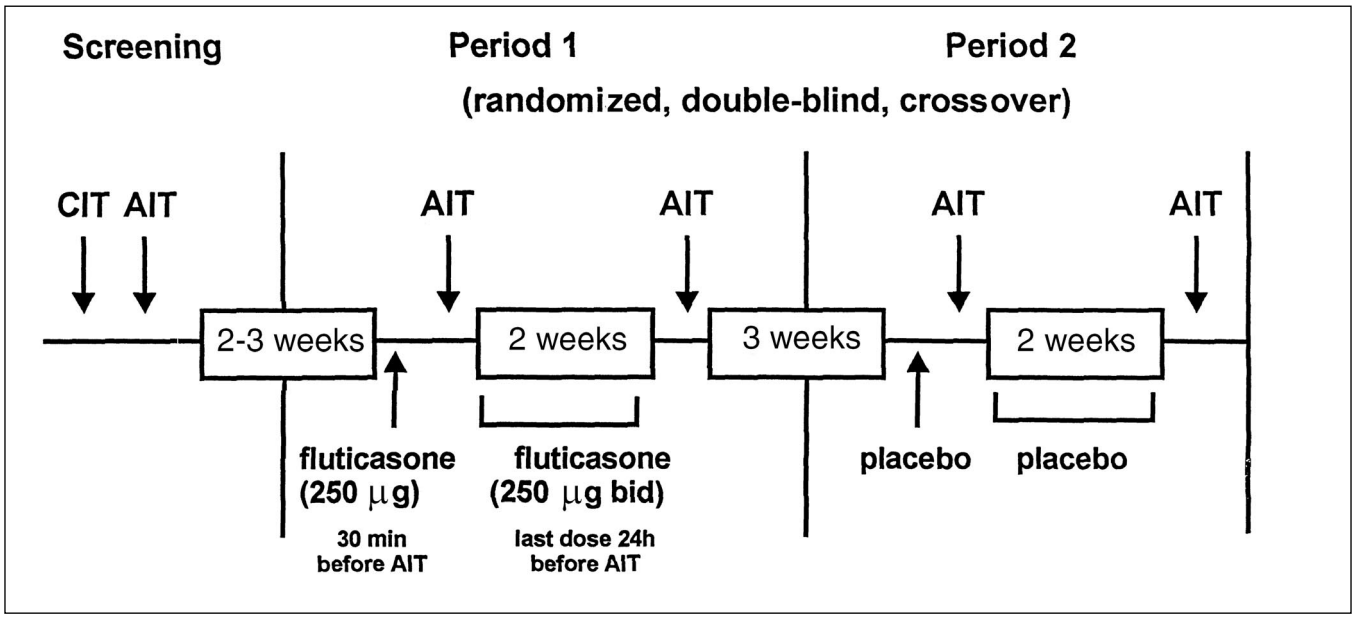

Figure 1) *Study design. AIT Allergen inhalation test; CIT Control inhalation test*

tection against the allergen-induced airway response and eosinophilic inflammation than a single dose given immediately before allergen inhalation.

PATIENTS AND METHODS

Patients: Ten atopic asthmatic patients (Table 1) were recruited from the Firestone Chest Clinic of St Joseph's Hospital, McMaster University Health Sciences Centre and from responses to an advertisement. All patients had mild stable asthma as indicated by few or no symptoms, treatment only with inhaled short acting beta$_2$-agonist when needed (not daily), a forced expiratory volume in 1 s (FEV_1) of at least 70% predicted and moderate to borderline methacholine airway hyperresponsiveness. All patients were nonsmokers and were studied out of their allergy season, and none had had a respiratory infection in the preceding month. Beta-agonist was withheld for at least 8 h before each visit, and participants were instructed to refrain from rigorous exercise and caffeine in the morning before visits to the laboratory. The study was approved by the ethics committees of both hospitals and the patients gave signed consent to participate.

Study design: This was a two-period, randomized, double-blind, placebo controlled crossover study conducted between May 1997 and March 1998 (Figure 1). An initial screening period of four visits to identify subjects with a dual response to inhaled allergen was followed two to three weeks later by two crossover control and treatment periods, each of 19 days duration, separated by a washout period of three weeks. Apart from the study medications, the only drug allowed was inhaled salbutamol when required. Randomization to the treatment order was achieved with computer-generated randomization codes, maintained off-site to ensure allocation concealment. The fluticasone and placebo inhalers looked identical; however, complete blinding was not possible for two reasons: single treatment with either placebo or fluticasone was always followed by regular treatment with the same drug, and there was a high likelihood of the investigator observing an effect of the active drug on the airway responses.

On the first day of the screening period, clinical and demographic characteristics were documented, and allergy skin prick tests were performed. A saline inhalation test was then carried out, and sputum was induced 7 h later. On the second day, a methacholine inhalation test and sputum induction were performed. On the third day, an allergen test was carried out to identify the dose of the allergen required to cause early and late asthmatic responses, and sputum was induced at 7 h. On the fourth day, methacholine inhalation and sputum induction were repeated.

On the first day of period 1, a methacholine test and sputum induction were performed between 16:00 and 17:00. On the second day, FEV_1 was measured, and two puffs of either the active drug (125 μg/puff) or placebo were inhaled. An allergen inhalation test was performed after 30 min, FEV_1 was recorded throughout the day and sputum was induced at 7 h. On the third day, a methacholine test and sputum induction were performed 24 h after the allergen test. Starting from the evening of the third day, patients took two puffs twice daily of the same medication for 14 days, the last dose being on the morning of the 17th day and 24 h before the next allergen challenge. A methacholine test and sputum induction were performed between 16:00 and 17:00. Compliance was checked by measuring plasma fluticasone levels approximately 6 h after the last dose using a Packard MultiProbe robotic sample handler (Canberra Packard Limited, United Kingdom) and analyzed by high performance liquid chromatography with tandem mass spectrometric detection using selected reaction monitoring (calibration range 20 to 1500 pg/mL). On the 18th day, 24 h after the last dose of the drug, a second allergen inhalation test was performed and sputum was induced at 7 h. On the 19th day, a methacholine

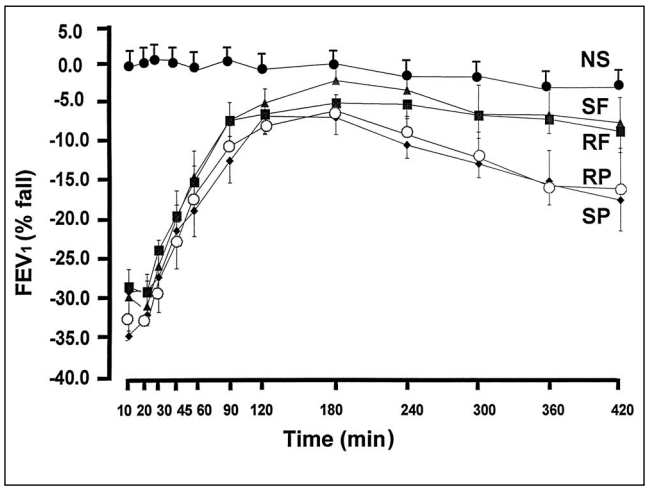

Figure 2) *Changes in forced expiratory volume in 1 s (FEV_1) (percentage fall from baseline) after normal saline (NS) and allergen inhalation preceded by single (SP) or regular placebo (RP) and single (SF) or regular fluticasone (RF). Neither SF nor RF inhibited the early asthmatic response. SF significantly attenuated the late asthmatic response; the effect of RF was not statistically significant. The effect of the control NS is also shown*

test and sputum induction were performed, 24 h after the allergen challenge (48 h after the last dose of the drug). Period 2 was the same as period 1 except that the patients received the other treatment.

Airway measurements and provocation tests: Spirometry was performed with a Koko-Trek dry rolling-seal (Trudell Medical, Canada) or Collins water-filled spirometer (Warren E Collins Inc, United States), the same type of spirometer being used for each subject on all occasions. Baseline measurements of FEV_1 and slow vital capacity were made according to the American Thoracic Society criteria (9). Methacholine inhalation tests were carried out by the method described by Juniper et al (10), and the results were expressed as the provocative concentration of methacholine required to cause a 20% fall in FEV_1 (PC_{20}) in noncumulative units.

Allergy skin tests were performed using the modified prick technique (11) with 17 common allergen extracts. Twofold dilutions of the extract selected for inhalation tests were also used to identify the lowest concentration to cause a 2 mm wheal. This concentration together with the methacholine PC_{20} was used to estimate the allergen PC_{20}, and the starting concentration for allergen inhalation was two doubling concentrations below this (12). Control and allergen inhalation tests were performed by the method described by Cockcroft et al (12) with modifications (3). FEV_1 was measured at 10, 20, 30, 45, 60, 90 and 120 min following allergen inhalation, then each hour for the next 5 h. In subsequent allergen inhalation tests, the last three doses of the allergen were administered during each treatment period.

Sputum induction and analysis: Sputum induction was performed with an aerosol of hypertonic saline as described by Pin et al (13). The collected expectorate was examined within 2 h as described by Pizzichini et al (14). Total cell count, squamous cell contamination and cell viability were recorded and cytospins prepared. One cytospin was fixed by methanol and stained by the Wright method for an overall differential cell count on at least 400 nonsquamous cells. Two other cytospins were fixed in Carnoy's fixative and stained with toludine blue and 1500 nonsquamous cells were counted for metachromatic cells. Cytospins were also prepared on aptex-coated slides and fixed in periodate-lysine-paraformaldehyde for immunocytochemical staining for eosinophil cationic protein (ECP), using a monoclonal antibody directed against cleaved ECP (EG2) (Kabi Pharmacia, Sweden). The percentage of EG2-positive cells was determined from a count of 500 cells under light microscopy. The sputum supernatant was aspirated and stored in Eppendorf tubes at –70°C for later assay of ECP. The concentration of ECP in the thawed supernatant was determined by a sensitive radioimmunoassay with a lower limit of detection of less than 2 μg/L (Kabi Pharmacia, Sweden). All sputum measurements were performed blind to the clinical details.

Statistical analysis: Sample size was estimated for an alpha of 0.05 and a beta of 0.10 (90% power). On the basis of the data of repeatability of FEV_1 measurements and sputum eosinophils, there was 90% power to detect 50% attentuation of the maximum late fall in FEV_1 and 50% change in sputum eosinophils after treatment with fluticasone. Descriptive statistics were used to summarize clinical and demographic characteristics of the sample. Grouped data were reported as the arithmetic mean ± SEM. PC_{20} was log-transformed and reported as geometric mean ± geometric SEM.

The early bronchoconstrictor response to allergen was measured as the greatest percentage change within 2 h from the mean baseline FEV_1. It was adjusted for the change in FEV_1 after saline control challenge by recording the difference between the percentage fall after allergen and after saline. The overall bronchoconstrictor response to allergen during the early phase was computed from the trapezoidal area under the FEV_1-time curve (AUC) from the start of the allergen challenge to 2 h after the last dose of the allergen. The late-phase response, representing the 3 to 7 h period after the last dose of the allergen, was calculated similarly.

Differences between the effects of single placebo and single fluticasone treatments, and regular placebo and regular fluticasone treatments on FEV_1, PC_{20} and sputum inflammatory markers were compared by repeated measures ANOVA, with treatment, sequence, period and subject as factors. Because there was no randomization between single and regular treatment within a period, their effects were compared by Wilcoxon signed-rank tests. Correlations were made by the Spearman rank method. All analyses were performed using SPSS-7.5 (SPSS Inc, United States). $P<0.05$ (two-tailed) was considered significant.

RESULTS

There was good compliance with therapy. Fluticasone levels were detected in all plasma samples when the patients were on fluticasone (mean ± SD 47.8±20.3 pg/mL) and none was detected when they were on the placebo.

TABLE 2
Effects of pretreatment and allergen inhalation on mean (±SD) provocative concentration of methacholine to cause a 20% fall in forced expiratory volume in 1 s (PC_{20}) and sputum inflammatory markers (total and differential cell counts, measures of eosinophil cell activation)

	Single placebo			Single fluticasone			Regular placebo			Regular fluticasone		
Measurement (±SD)	0	7 h	24 h	0	7 h	24 h	0	7 h	24 h	0	7 h	24 h
PC_{20} (mg/mL)	2.3±2.4	—	0.8±4.7	1.7±2.3	—	1.0±3.0	2.6±2.9	—	0.9±4.0	6.4±2.5	—	1.7±4.1
TCC x10^6/mL	3.4±2.8	6.5±3.6	9.5±7.2	4.3±2.2	8.7±6.9	10.8±9.4	3.9±3.7	12.2±9.0	8.5±5.2	4.8±5.3	9.1±6.8	11.5±9.5
Eosinophils (%)	1.0±1.0	12.7±8.9	18.4±17.0	3.1±6.4	9.6±12.9	11.2±11.4	3.1±5.3	21.3±25.0	15.1±14.3	0.4±0.8	10.7±15.1	11.3±17.0
Median value	0.8	13.5	12.7	0.5	5.4	6.0	0.8	16.4	11.9	0	5.5	2.65
ECP (µg/L)	718±961	5440±5850	5688±6049	1382±1779	3401±2840	4994±3312	1495±2488	6023±5243	7649±7032	609±827	5069±5838	5641±7798
EG-2+ (%)	1.4±1.8	10.8±7.4	14.2±11.3	1.4±1.3	3.0±2.5	7.4±4.8	3.1±4.1	17.4±19.3	15.8±10.5	0.4±0.5	4.4±4.0	6.6±4.3
MCC (%)	0.06±0.08	0.22±0.5	0.14±0.14	0.08±0.13	0.1±0.2	0.26±0.33	0.09±0.19	0.36±0.6	0.22±0.41	0.02±0.05	0.04±0.05	0.04±0.07
Neutrophils (%)	34.8±28.8	50.1±21.2	41.2±25.7	36.8±14.7	48.5±27.2	54.8±22.3	32.8±20.5	55.7±25.0	41.6±18.8	37.9±29.4	58.0±26.4	53.8±25.4
Lymphocytes (%)	1.9±0.9	1.6±1.1	2.6±2.3	2.1±1.3	2.6±2.1	2.2±1.8	2.4±1.8	2.0±1.1	3.6±1.3	0.7±0.5	1.4±1.2	1.0±0.6
Macrophages (%)	61.6±28.6	35.5±22.3	36.4±16.0	57.9±15.7	38.5±24.1	31.3±12.8	61.1±19.8	21.5±15.9	40.0±9.8	60.0±29.6	29.7±22.1	33.5±16.4

ECP Eosinophil cationic protein; EG-2+ Activated eosinophils; MCC Metachromatic cells; TCC Total cell count

Effects on FEV_1: Neither single nor regular treatment with fluticasone had any effect on the early asthmatic response compared with placebo (Figure 2). Compared with single placebo, pretreatment with a single dose of fluticasone 250 µg significantly attenuated the late asthmatic response (FEV_1 percentage fall and AUC [mean ± SEM] from 18.8%±3.5% and 3048±482 to 10.8%±3.6% and 1299±616, respectively [P<0.05]). After two weeks treatment with fluticasone, there was no significant increase in the baseline FEV_1 (4.0±0.2 L) measured 24 h after the last dose compared with either a single dose of fluticasone (3.9±0.2 L) or two weeks of regular placebo treatment (3.9±0.2 L). The regular treatment with fluticasone also attenuated the late asthmatic response (FEV_1 percentage fall from 19.6%±4.5% to 11.1%±2.5% and AUC from 2866±736 to 1563±503, compared with regular placebo), but this was not statistically significant. There was no difference between the effects of single dose or regular fluticasone treatment on late asthmatic response.

Effects on PC_{20}: Allergen inhalation caused a significant decrease in PC_{20} 24 h after both single and regular pretreatment with placebo (Table 2). A single dose of fluticasone before allergen inhalation prevented the increase in airway responsiveness. Two weeks of regular treatment significantly increased the baseline PC_{20} (P<0.05), measured about 8 h after the last dose of fluticasone, but did not prevent the allergen-induced increase in responsiveness 48 h after the last dose of the drug (Figure 3).

There was a significant correlation between the decrease in PC_{20} and the increase in sputum eosinophil count 24 h after placebo treatment followed by allergen challenge (r_s =–0.7, P=0.02). This correlation was not significant after allergen challenge preceded by fluticasone (r_s=–0.3, P=0.6).

Effects on sputum inflammatory markers: Sputum was successfully obtained in 148 of 160 (93%) inductions. The mean squamous cell contamination was less than 5%. The mean baseline sputum total and differential cell counts, eosinophil cationic protein (ECP) and activated eosinophils (EG-2+) were similar for the two study periods, with the exception of the baseline eosinophil count after two weeks of regular fluticasone, which was significantly lower than after regular placebo treatment (P<0.05) and before the single dose of fluticasone (P<0.05) (Table 2). Pretreatment with either single or regular placebo did not prevent an increase in eosinophils, ECP, EG-2+ or metachromatic cells at 7 h and 24 h after allergen challenge. However, pretreatment with a single dose of fluticasone significantly attenuated the increase in allergen-induced eosinophilia (P<0.05) and increase in EG-2+ (P<0.05) at 7 h but not at 24 h (Figure 4). Regular treatment with fluticasone for two weeks showed a similar trend, but this was not statistically significant. The sputum differential counts of neutrophils, macrophages, lymphocytes and metachromatic cells were not significantly changed by any of the inhalation tests.

DISCUSSION

The results of this study show that a single dose of fluticasone 250 µg given 30 min before allergen inhalation significantly attenuates the late asthmatic response, the increase in

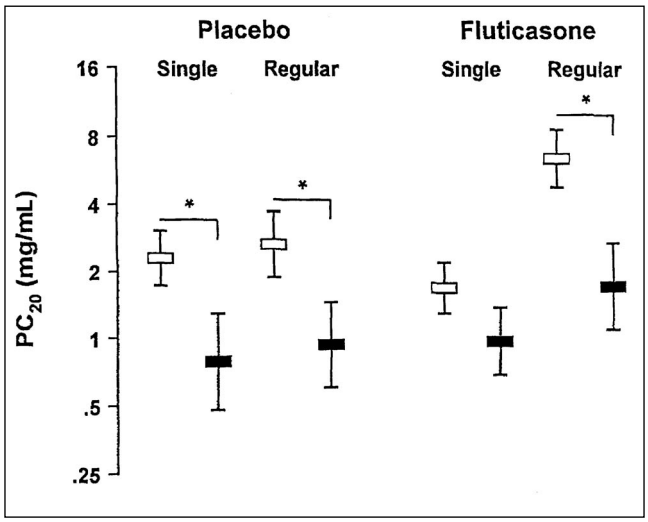

Figure 3) *Geometric mean provocative concentration of methacholine to cause a 20% fall in forced expiratory volume in 1 s (PC_{20}) pre- (open blocks) and 24 h post- (closed blocks) allergen challenge preceded by single and regular dose placebo and fluticasone treatment. The asterisks indicate P<0.05. Pretreatment with placebo did not have any protective effect on allergen-induced hyperresponsiveness. Although regular treatment with fluticasone (up to 24 h before allergen inhalation) increased the baseline PC_{20} methacholine, it failed to protect against allergen-induced hyperresponsiveness*

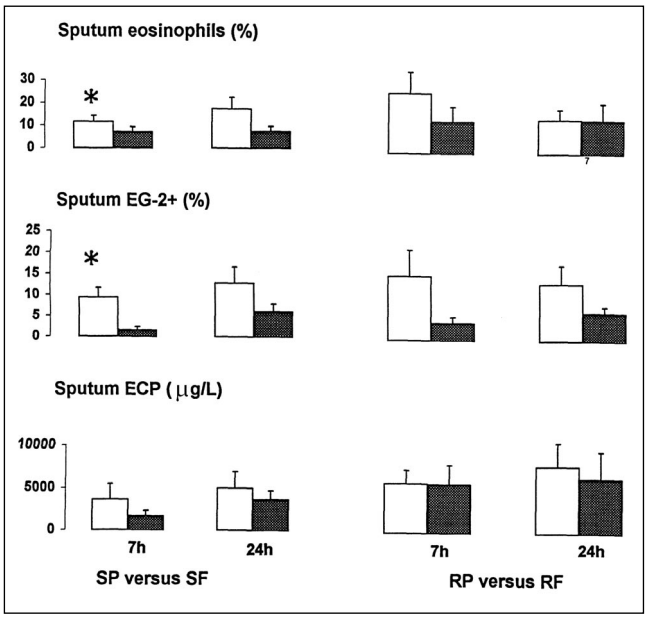

Figure 4) *Increase from baseline (mean ± SEM) in sputum eosinophils, activated eosinophils (EG-2+) and eosinophil cationic protein (ECP) 7 h and 24 h after allergen challenge preceded by placebo (open bars) and fluticasone (closed bars). The left panel shows the comparison after single doses of fluticasone (SF) and placebo (SP), and the right panel shows the comparison after regular treatment with fluticasone (RP) and placebo (RF). Pretreatment with a single dose of fluticasone immediately before an allergen inhalation significantly attenuated the allergen-induced sputum eosinophilia and eosinophil activation 7 h after the allergen inhalation. *P<0.05*

airway responsiveness and sputum eosinophilia. However, while regular treatment with fluticasone 250 μg twice daily for two weeks improved baseline methacholine hyperresponsiveness and airway eosinophilia, it did not significantly protect against the allergen-induced asthmatic or inflammatory responses. This result was unexpected and suggests that the dose of inhaled steroid must be given sooner than 24 h to allergen exposure for its protective effect to be maintained.

The protective effect of a single dose of inhaled steroid given just before allergen inhalation on the late asthmatic response has been reported previously (3,4). However, this is the first time that significant effects on allergen-induced sputum eosinophilia and activation have been observed. We used an EG2 monoclonal antibody to define eosinophil activation because of its value in distinguishing between resting and activated eosinophils (15) and because ECP, although regularly used as a marker of eosinophil activation, may not be specific to eosinophils (16). Regular treatment that is discontinued within 12 h of an allergen inhalation has also been reported to inhibit the early response (5,6) and to reduce baseline and postallergen airway hyperresponsiveness (5,17) and sputum eosinophilia (5). In the present study, as expected (17,18), regular treatment with fluticasone 250 μg twice daily for two weeks reduced baseline methacholine airway responsiveness and sputum eosinophilia. However, the regular treatment, which was discontinued 24 h before the allergen challenge, did not inhibit the early asthmatic response; although the late asthmatic response, increase in airway responsiveness and sputum eosinophilia were attenuated, these were not statistically significant. Thus, although the study was not designed to address this question, the results seem to indicate that inhaled corticosteroids, even after regular treatment and in the presence of the expected anti-inflammatory effects at baseline, need to be given closer to a proinflammatory stimulus to inhibit the early-phase bronchoconstriction and late-phase inflammatory response. Taking an earlier study (6) into consideration, the lack of significant protective effects of fluticasone on allergen-induced responses between 12 h and 24 h after discontinuation of treatment suggests that the effect on acute release of mediators of airway bronchoconstriction and inflammation after allergen challenge might be lost more quickly than the underlying anti-inflammatory effects observed on baseline readings.

Recent studies (19,20) suggest that the clinical and anti-inflammatory effects of regular treatment with fluticasone may be short lived and dose dependent (19-21). There is no information on the duration of the protective effect of inhaled steroids, which may also be dose dependent. This is an important consideration in recommending whether patients with mild asthma need regular daily therapy or short pulses of intermittent prophylactic therapy. This is the first study to report a short duration of the protective effect of an inhaled steroid on allergen inhalation. Although the study was not designed specifically to answer this question, we believe that it is an important observation of clinical relevance. Further studies are required to investigate the time and dose depend-

ency of the protective effects of inhaled corticosteroids against the effects of allergen inhalation after both single doses and regular treatment.

The inability to demonstrate a significant protective effect of regular fluticasone on allergen-induced eosinophilia, eosinophil activation and allergen-induced increase in metachromatic cells may be due to the inadequate sample size. The study was adequately powered to demonstrate a 50% attenuating effect of inhaled fluticasone on the late asthmatic response and sputum eosinophilia (22,23) compared with placebo. Posthoc analysis of the comparison between regular fluticasone and regular placebo on the attentuation of late asthmatic response and allergen-induced sputum eosinophilia at 7 h demonstrated that we had only 31% and 64% power, respectively, to demonstrate a significant change (50%). This is due to the greater variability in the responses in this study compared with the previous studies (22,23). We also recognize that the study became underpowered with multiple comparisons. Even though regular fluticasone therapy and the single-dose treatment regimens were not in random order and, therefore, could be subject to an order effect, the fact that the protective effect of two weeks' treatment was no greater than that of a single dose given before the allergen does question the duration of the protective effect of inhaled steroids.

CONCLUSIONS

A single dose of inhaled fluticasone 250 μg given 30 min before an allergen inhalation significantly protects against the allergen-induced late asthmatic response, increase in responsiveness, airway eosinophilia and eosinophil activation. However, when two weeks of regular treatment (250 μg twice daily) was discontinued 24 h before allergen challenge, no additional protective effect was seen in spite of reduced baseline airway hyperresponsiveness and eosinophilia. This suggests that the protective effect is short lasting, and emphasizes the need for regular treatment with inhaled corticosteroid to be given within 24 h of allergen exposure for it to protect against the allergen-induced responses. This time interval requires further investigation.

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