

Protection by budesonide and fluticasone on allergen-induced airway responses after discontinuation of therapy

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Background: Treatment with inhaled steroids is an effective method of reducing bronchoconstriction and airway inflammation after allergen challenge. However, the duration of the protective effects of inhaled steroids after discontinuation of therapy has not been established.

Objective: We sought to evaluate the protective effect of 1 week of inhaled steroid therapy against inhaled allergen challenge 12 hours after discontinuation of therapy.

Methods: In this randomized, double-blind, placebo-controlled crossover trial, 26 asthmatic subjects (>18 years old) not using inhaled steroids were administered 200 µg of budesonide twice daily, 200 µg of fluticasone twice daily, or placebo twice daily for 1 week. Twelve hours after discontinuation of therapy, subjects were administered an inhaled allergen challenge. Each treatment period was separated by a 3-week washout period.

Results: When compared with placebo (26% ± 14%), there was a slight but significant protection against the allergen-induced early response after fluticasone treatment (19% ± 10%, $P = .001$) but not after budesonide treatment (23% ± 13%, $P = .08$). However, when the area under the curve for the early airway response was examined, there was no difference between the 2 drugs in the amount of protection ($P = .62$). Partial protection was demonstrated against the late-response allergen-induced sputum eosinophilia with both treatments ($P = .001$). By contrast, no protection was observed against allergen-induced airway hyperresponsiveness for either treatment.

Conclusions: The protective effects of inhaled steroids against allergen-induced early responses, airway eosinophilia, and allergen-induced airway hyperresponsiveness are partially or

completely lost as early as 12 hours after discontinuation of therapy. (*J Allergy Clin Immunol* 2005;115:745-50.)

Key words: Budesonide, fluticasone, steroids, asthma, allergen challenge, bronchial challenge test, sputum, eosinophils

Asthma is characterized by reversible airway obstruction, airway hyperresponsiveness, and airway inflammation. Inhaled corticosteroids are the most clinically effective therapy available for treatment for asthma and its associated airway inflammation.¹ Allergen inhalation by subjects with atopic asthma results in the development of immediate bronchoconstriction, labeled the early airway response (EAR), which usually subsides within 3 hours.² Some subjects subsequently have a late-phase bronchoconstrictor response, the late airway response (LAR), which usually begins within 2 to 4 hours after inhaled allergen and can last 12 to 24 hours if untreated. The LAR has been associated with increased airway inflammation, including eosinophils, mast cells, and basophils,³ and the development of allergen-induced airway hyperresponsiveness.⁴ Regular treatment with inhaled corticosteroids has been shown in several studies to attenuate the allergen-induced EAR and almost completely abolish the LAR and associated airway inflammation, as well as to prevent the development of allergen-induced airway hyperresponsiveness.^{3,5-7}

The duration of protection of inhaled steroids against allergen-induced responses has not been well studied. Fluticasone propionate taken 12 hours before an allergen challenge is effective in attenuating the EAR, and a single dose of 250 µg 30 minutes before challenge attenuates the LAR.⁸ However, attenuation of the LAR, allergen-induced airway hyperresponsiveness, and inflammation were no longer present when treatment with fluticasone propionate was stopped 24 hours before allergen challenge,⁸ indicating that loss of protection might occur rapidly on cessation of treatment. By contrast, discontinuation of budesonide 12 hours before allergen challenge³ significantly attenuated the allergen-induced EAR and LAR with similar efficacy as when treatment was continued throughout the challenge period.

The concentrations of steroids in the lung tissue are determined on the basis of glucocorticoid receptor-binding affinity, lung deposition, and pulmonary residence time of the drug.⁹ Comparisons of budesonide and fluticasone propionate have shown that fluticasone has

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Abbreviations used

- AUC: Area under the curve
 EAR: Early airway response
 LAR: Late airway response
 MCh PC₂₀: Provocative challenge dose of methacholine producing a 20% decrease in FEV₁

a greater affinity for glucocorticoid receptor¹⁰ and a slower rate of systemic absorption,⁹ thereby prolonging its local availability in the lungs. Budesonide is reversibly esterified by fatty acids, resulting in intracellular storage and release, potentially making the drug available in the airway tissue for longer periods of time.¹¹ This tissue storage of budesonide might be clinically relevant if the storage results in even longer pulmonary residence.

We hypothesized that after cessation of treatment, budesonide, because of its intracellular esterification, would provide prolonged protection against allergen-induced airway responses compared with fluticasone propionate. To evaluate this, we compared the protective effects of 7 days of budesonide and fluticasone propionate stopped 12 hours before allergen challenge on allergen-induced EAR, airway hyperresponsiveness, and airway eosinophilia.

METHODS

Subjects

Twenty-six nonsmoking subjects with mild asthma were recruited. All were documented to have an allergen-induced EAR with or without a LAR in a screening challenge (Table I). Subjects had stable asthma, with FEV₁ values of greater than 70% of predicted value on all study days before allergen challenge and a baseline methacholine provocative concentration causing a 20% decrease in FEV₁ (MCh PC₂₀) of 32 mg/mL or less. Subjects used no regular asthma medication during the study other than infrequent (less than twice weekly) inhaled β_2 -agonist, which was withheld for 8 hours before each visit. They had no exposure to sensitizing allergens apart from house dust mite, had no asthma exacerbations or respiratory tract infections for 6 weeks before entering the study, and refrained from rigorous exercise, tea, or coffee on the mornings of the visits to the laboratory.

Study design

Three weeks after a screening allergen challenge, subjects returned to the laboratory for baseline methacholine challenge. If the MCh PC₂₀ concentration was within one doubling dose of the screening MCh PC₂₀ value, they were randomized to a 7-day treatment of inhaled budesonide (200 μ g twice daily administered through a Turbuhaler), inhaled fluticasone propionate (200 μ g twice daily administered through a Diskus), or inhaled placebo by using a double-dummy method of blinding. On the last day of treatment, subjects returned for a repeat methacholine challenge and sputum induction. The next day, 12 hours after the last treatment dose, subjects underwent an allergen challenge. The following day, 24 hours after allergen inhalation, the subject returned for a repeat methacholine challenge and sputum induction. The washout before

the next treatment period was at least 2 weeks but no longer than 8 weeks. The study was approved by the Research Ethics Board of the Hamilton Health Sciences Corporation and McMaster University under the ICH guidelines, and all patients provided written informed consent before starting the study.

Methacholine challenge

Methacholine inhalation challenge was performed as described by Cockcroft et al.⁴ Subjects inhaled normal saline during 2 minutes of tidal breathing from a Wright nebulizer and then doubling concentrations of methacholine phosphate. FEV₁ was measured at 30, 90, 180, and 300 seconds after each inhalation. The test was terminated when a decrease in FEV₁ of 20% of the baseline value occurred, and the methacholine PC₂₀ was calculated.

Allergen challenge

Allergen challenge was performed as described by O'Byrne and colleagues.^{2,12} Subjects were skin tested for allergies to common aeroallergens by using standardized commercially prepared allergen extracts (Omega, Montreal, Quebec, Canada). The allergen producing the largest skin wheal diameter was diluted in 0.9% saline and stored for subsequent allergen inhalation challenges. The concentration of allergen extract for inhalation was determined from a formula described by Cockcroft et al,¹³ and doubling concentrations of allergen were administered until a 20% or greater early decrease in FEV₁ at 10 minutes after allergen was reached. The FEV₁ was then measured at regular intervals until 7 hours after allergen inhalation. The EAR is the maximum percentage decrease in FEV₁ within 2 hours after allergen inhalation, and the LAR is the maximum percentage decrease in FEV₁ between 3 and 7 hours after allergen inhalation. The total area under the curve (AUC_{0-7h}; mL*s), the early area under the curve (AUC_{0-2h}; mL*s), and the late area under the curve (AUC_{3-7h}; mL*s) were calculated from the decrease in FEV₁. Subjects inhaled the same dose of allergen delivered during screening for the subsequent 3 treatment periods. During the screening period, all 26 subjects had an EAR of 20% or greater, and 11 of these subjects had an LAR of 15% or greater.

Sputum induction

Sputum was induced and processed by using the method described by Pizzichini et al.¹⁴ Cell plugs were selected, mixed with 0.1% dithiothreitol (Sputolysin; Calbiochem Corp, San Diego, Calif) and Dulbecco PBS (Life Technologies Inc, Grand Island, NY), and filtered through a 52- μ m nylon gauze (BNSH Thompson, Scarborough, Ontario, Canada), and cytospin preparations were prepared on glass slides. The total cell count was determined by using a Neubauer hemocytometer chamber (Hausser Scientific, Blue Bell, Pa) and expressed as the number of cells per milliliter of sputum, and differential cell counts were obtained from slides stained with Diff Quik (American Scientific Products, McGaw Park, Ill).

Statistical analysis

The statistical package SPSS (SPSS for Windows, v.12.0) was used to perform all the statistics. Summary statistics are expressed as the mean and SEM, except methacholine PC₂₀, which is expressed as the geometric mean and geometric SEM. PC₂₀ was calculated by means of linear interpolation of log dose-response curves and log transformed to fit a normal distribution before analysis. A fold difference change was calculated to assess the change in PC₂₀ caused by allergen challenge.¹⁵ A multiplicative ANOVA model with the factors of subject, treatment order, response (isolated early and dual), and treatment was used to compare the effects of a 7-day treatment with budesonide, fluticasone propionate, and placebo on allergen-

TABLE I. Subject characteristics at screening allergen challenge

Age (y)	Sex	MCh PC ₂₀ (mg/mL)	Allergen response	Allergen	Concentration (dilution)	Maximal decrease (%)
26	F	13.46	EAR	Cat	1:16	-21.05
24	M	2.26	LAR	Ragweed	1:128	-33.33
23	M	9.55	LAR	Cat	1:32	-51.16
26	M	5.76	EAR	Ragweed	1:32	-43.48
24	F	7.10	EAR	HDM	1:256	-26.67
40	M	27.86	LAR	HDM	1:32	-29.76
33	M	5.47	EAR	Grass	1:1024	-31.88
23	F	1.69	LAR	Cat	1:256	-20.41
22	F	13.45	EAR	HDM	1:32	-33.33
26	M	6.59	EAR	Cat	1:64	-29.03
22	F	0.91	LAR	<i>Alternaria</i> species	1:16	-26.09
23	M	2.93	EAR	HDM	1:1024	-21.88
22	F	4.86	EAR	Cat	1:16	-32.86
22	F	1.49	LAR	Ragweed	1:1024	-30.36
25	F	2.76	LAR	Ragweed	1:16	-29.63
22	M	0.15	EAR	HDM	1:2048	-21.67
22	F	0.16	LAR	Ragweed	1:256	-22.58
20	F	8.00	EAR	Ragweed	1:32	-21.43
22	F	0.12	EAR	HDM	1:512	-22.41
25	M	4.00	EAR	Ragweed	1:1024	-20.00
32	F	0.67	EAR	Cat	1:8	-26.42
24	M	16.00	EAR	HDM	1:256	-22.78
53	M	2.13	LAR	Cat	1:64	-40.30
20	F	7.46	EAR	Ragweed	1:32	-23.33
20	F	3.77	LAR	HDM	1:128	-33.33
52	F	0.91	LAR	Cat	1:16	-26.47

Maximal decrease, Maximum decrease in FEV₁ recorded during allergen challenge as a percentage of baseline FEV₁; HDM, house dust mite.

induced early and late airway bronchoconstriction (maximum percentage decrease in FEV₁ and AUC), airway hyperresponsiveness, and sputum eosinophilia.

RESULTS

The allergen-induced maximal EAR was slightly but significantly attenuated by fluticasone ($19.0\% \pm 9.7\%$, $P < .001$) but not by budesonide ($23.1\% \pm 13.2\%$, $P = .08$) when compared with placebo ($26.4\% \pm 13.5\%$, Fig 1). Similar results were obtained when the AUC_{0-2h} was analyzed. No significant difference in attenuation existed between fluticasone and budesonide ($P = .62$).

The allergen-induced maximal LAR was partially but significantly attenuated by both fluticasone ($11.0\% \pm 7.2\%$, $P = .006$) and budesonide ($13.0\% \pm 7.3\%$, $P = .03$) when compared with placebo ($19.3\% \pm 7.6\%$) in the 11 subjects who had a greater than 15% decrease in FEV₁ between 3 and 7 hours after allergen inhalation during their screening challenge. No difference in attenuation was detected between fluticasone and budesonide ($P = .46$). Similar results were obtained when the AUC_{3-7h} was analyzed.

Airway responsiveness to methacholine did not change from before to after treatment with placebo, with the MCh PC₂₀ being 2.3 mg/mL (range, 0.12-26.9 mg/mL) before treatment and 2.5 mg/mL (range, 0.07-24.0 mg/mL) after treatment ($P = .45$, Fig 2). Seven days of treatment with

either fluticasone or budesonide improved methacholine airway responsiveness. The MCh PC₂₀ significantly increased after fluticasone, from 3.0 mg/mL (range, 0.09-13.8 mg/mL) before treatment to 4.6 mg/mL (range, 0.37-37.2 mg/mL) after treatment ($P < .001$). The MCh PC₂₀ also significantly increased with budesonide, from 2.6 mg/mL (range, 0.11-25.7 mg/mL) before treatment to 4.2 mg/mL (range, 0.14-46.8 mg/mL) after treatment ($P = .001$, Fig 2).

Allergen inhalation significantly changed methacholine airway responsiveness. After placebo, the MCh PC₂₀ significantly decreased from 2.5 mg/mL (range, 0.07-24.0 mg/mL) before treatment to 1.5 mg/mL (range, 0.06-9.5 mg/mL) at 24 hours after allergen inhalation ($P = .003$, Fig 2). After fluticasone treatment, the MCh PC₂₀ significantly decreased from 4.6 mg/mL (range, 0.37-37.2 mg/mL) before treatment to 2.0 mg/mL (range, 0.03-15.8 mg/mL) after allergen ($P < .001$), which was similar to budesonide treatment, in which the MCh PC₂₀ decreased from 4.2 mg/mL (range, 0.14-46.8 mg/mL) after 7 days of treatment to 1.8 mg/mL (range, 0.13-52.5 mg/mL) 24 hours after allergen ($P < .001$). The allergen-induced change in airway responsiveness with fluticasone (0.43 [95% CI, 0.3-0.64] fold difference units) and budesonide (0.42 [95% CI, 0.32-0.56] fold difference units, $P = .46$) was similar to that with placebo (0.59 [95% CI, 0.42-0.81] fold difference units). There was no difference in the allergen-induced change in MCh PC₂₀ between fluticasone and budesonide ($P = .75$).

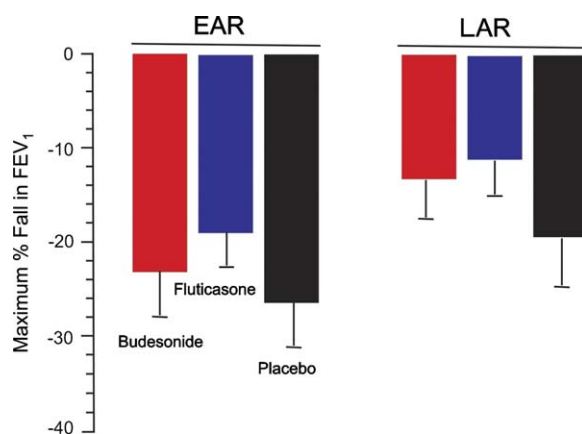


FIG 1. The maximal allergen-induced EAR (mean $\pm$ SEM) was significantly attenuated by fluticasone but not by budesonide when compared with placebo. No difference in attenuation was detectable between budesonide and placebo. The maximal allergen-induced LAR (mean $\pm$ SEM) was significantly attenuated by both fluticasone and budesonide when compared with placebo. No difference in attenuation was detectable between fluticasone and budesonide.

There was no change in sputum eosinophils after 7 days of treatment with placebo, fluticasone, or budesonide ($P = .51$, Fig 3). There was an allergen-induced increase in the percentage of sputum eosinophils 24 hours after allergen inhalation. Sputum eosinophils increased after placebo from $4.3\% \pm 1.1\%$ to $18.7\% \pm 2.5\%$, increased after fluticasone from $3.6\% \pm 0.9\%$ to $11.5\% \pm 1.7\%$ ($P < .001$), and increased after budesonide from $2.8\% \pm 0.8\%$ to $13.8\% \pm 2.1\%$ ($P < .001$). Treatment with fluticasone and budesonide significantly attenuated the allergen-induced sputum eosinophilia compared with placebo ($P = .001$, Fig 3), with no difference in attenuation between fluticasone propionate and budesonide ($P = .81$).

DISCUSSION

We have demonstrated that 1 week of treatment with either fluticasone or budesonide discontinued 12 hours before allergen challenge produced a marginal, although statistically significant, attenuation in the allergen-induced EAR (for fluticasone), LAR, and airway eosinophilia 24 hours after allergen inhalation but not allergen-induced airway hyperresponsiveness. Because no difference in attenuation of allergen-induced airway responses was observed between fluticasone and budesonide, these results suggest that both corticosteroids have a similar degree of protection within 12 hours of discontinuation of treatment, despite differences in their physical properties.

Several studies have evaluated the protective effect of inhaled corticosteroids^{16,17} on allergen-induced airway responses.^{3,5,6,8,18-24} These studies have demonstrated that when inhaled corticosteroid treatment was continued throughout the allergen challenge and 24 hours thereafter,

partial ($\leq 50\%$) attenuation of the EAR and almost complete abolition of the LAR, airway eosinophilia, and allergen-induced airway hyperresponsiveness was achieved.^{3,5,19-21,24} These studies have shown that some components of the allergen-induced airway responses are more responsive to inhaled steroids than others. The magnitude of the EAR is determined on the basis of airway mediator release and the degree of airway responsiveness (which in this study improved significantly after 1 week of treatment), and thus the minimal degree of protection against the EAR by fluticasone and budesonide was surprising. The current study suggests that the inhaled corticosteroids need to be present in the airways to afford optimal protection against the acute bronchoconstriction caused by inhaled allergen. This might be partially mediated through steroid effects on vascular permeability.²⁵

The magnitude of protection afforded by the inhaled corticosteroids on the LAR, when discontinued for 19 hours before the final measurements, are smaller in magnitude to what has been published.³ In addition, the measurements of induced sputum eosinophils and methacholine AHR made approximately 36 hours after discontinuation of treatment demonstrated a smaller than previously reported,^{3,6,8,24,26} but still significant effect on sputum eosinophils and no attenuation of allergen-induced airway hyperresponsiveness, which has consistently been shown to be abolished by treatment with inhaled corticosteroids if continued throughout the challenge.^{3,6,8,24,26} Thus the current study confirms that different components of the allergen-induced airway response are differentially sensitive to inhaled corticosteroids. It has also shown that the protective effects of inhaled corticosteroids on the EAR,²⁷ sputum eosinophilia, and allergen-induced ARH are short lived, which is consistent with a direct pharmacologic effect of corticosteroid treatment on attenuating these responses, rather than changing the state of the airway, an effect that would last longer than a direct pharmacologic effect.

The lung dose should ideally be the same to directly compare the duration of action of these steroids; however, the doses of budesonide and fluticasone required for a similar deposition in the airways is difficult to predict. Budesonide administered by means of Turbuhaler has been shown to deliver a higher dose to the lung compared with that delivered by the fluticasone propionate Diskus.⁹ However, the affinity of fluticasone for the glucocorticosteroid receptor is 2-fold that of budesonide.¹⁰ The doses of the drugs administered in this study were the minimal effective doses of fluticasone (administered through the Diskus) and budesonide (administered through the Turbuhaler) and were found to be the same ($200 \mu\text{g}$ twice daily) in adult subjects with asthma.²⁸

Formation of fatty acid esters of the unbound fraction of budesonide allows the drug to be stored intracellularly in the lungs and later released as active drug. It is believed that this intracellular fatty acid esterification of budesonide prolongs pulmonary residence time,²⁹ as well as efficacy. In rats instillation of budesonide 6 hours before LPS significantly reduced markers of inflammation compared

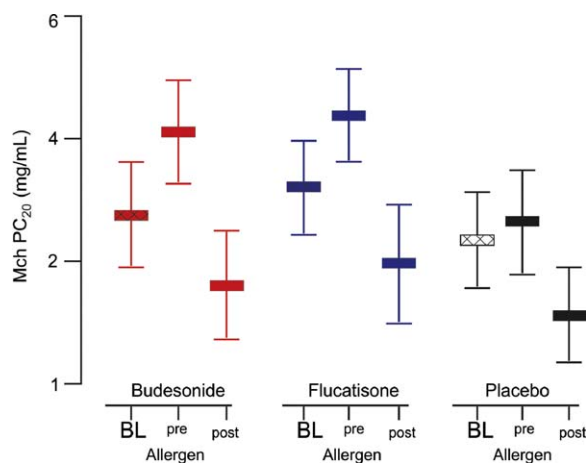


FIG 2. Airway responsiveness (MCh PC₂₀ expressed as geometric mean $\pm$ geometric SEM) for before treatment (BL), 7 days after treatment (*pre allergen*), and 24 hours after allergen inhalation (*post allergen*). Airway responsiveness improved from baseline to before allergen with inhaled steroid but not placebo treatment. However, the airway responsiveness significantly worsened in all groups, despite treatment after allergen challenge.

with instillation of fluticasone, demonstrating a more prolonged topical anti-inflammatory activity than fluticasone.³⁰ In addition, budesonide has been shown to be retained in the nasal mucosa to a greater extent than fluticasone when measured at 2 and 6 hours after delivery.³¹ This suggested that budesonide might be more efficacious for a longer duration than fluticasone after discontinuation. The current study, however, could not show any difference in efficacy in attenuating allergen-induced airway responses.

The fact that 12 hours after cessation of treatment the protective effect of fluticasone and budesonide against some components of allergen-induced airway responses were lost might have some clinical implications. This study suggests that fluticasone and budesonide treatment, when used as monotherapy, might need to be given twice daily during the allergen season to confer maximal protection. There are many studies showing the clinical benefit of once-daily budesonide dosing in improving asthma control in studies with outcome measures such as FEV₁, peak expiratory flow rate, and asthma symptoms.³²⁻³⁴ These studies have generally been in patients with milder disease whose symptoms are controlled with lower doses of inhaled corticosteroids. Other studies have suggested an improvement in asthma stability with twice-daily dosing compared with once-daily therapy in more difficult-to-control asthma.^{34,35}

In conclusion, this study, the first randomized controlled trial to evaluate the duration of the protective effects of different inhaled steroids after cessation of treatment, shows that the protection afforded by both fluticasone and budesonide against allergen-induced airway responses and airway inflammation is partially lost 12 hours after stopping treatment.

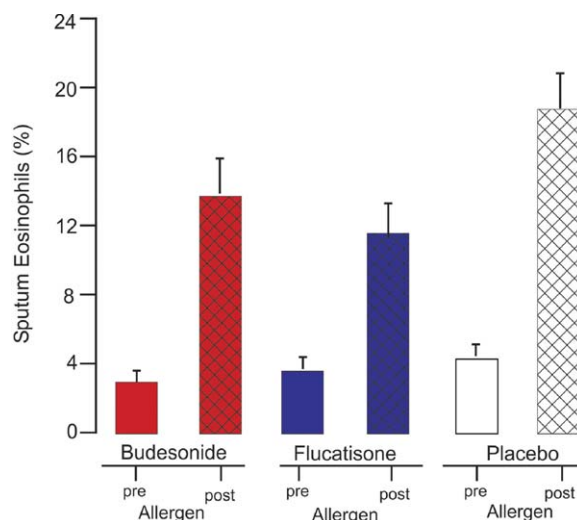


FIG 3. Changes in sputum eosinophils (mean percentage $\pm$ SEM) between 7 days after treatment (*pre allergen*) and 24 hours after allergen inhalation (*post allergen*). The allergen-induced increase in the number of sputum eosinophils was significantly decreased with fluticasone and budesonide treatment compared with placebo treatment.

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