

Evaluation of single-dose inhaled corticosteroid activity with an allergen challenge model

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Background: Inhaled corticosteroids are the most commonly used antiinflammatory agents for asthma. There is no simple way to compare objectively the relative potency of inhaled corticosteroids. The allergen-induced late asthmatic response (LAR) can be suppressed by a single dose of inhaled corticosteroid.

Objective: This study was undertaken to evaluate LAR as a model for the determination of the relative potency of single doses of inhaled corticosteroids.

Methods: We compared doses of 200 and 800 µg of a highly active inhaled corticosteroid (budesonide) with placebo and a marginally active investigational inhaled corticosteroid (D5159). Ten atopic patients with asthma completed a randomized, double-blind, double-dummy, multicenter, four-way, crossover trial. A standardized allergen challenge with the identical dose of allergen was performed 10 minutes after each of four blinded, single-dose treatments: 200 µg of budesonide, 800 µg of budesonide, 8 mg of D5159, and placebo, all administered from Turbuhaler. The LAR was recorded as the maximum percent fall in FEV₁ between 4 and 7 hours, and the allergen-induced increase in methacholine airway responsiveness at 24 hours was recorded as the Δ log PC₂₀ from the day before to the day after allergen challenge. **Results:** There were no significant differences in the early asthmatic responses during the 4 days; the mean maximum percent in FEV₁ fall ranged between 19.5% and 22%. D5159 produced a slight inhibition of the LAR with maximum percent fall in FEV₁ recorded as 28.8% ± 5.0% for D5159 versus 34.1% ± 4.8% for placebo (*p* < 0.05). There was a greater reduction recorded after administration of the two doses of budesonide. The mean LAR was 15.1% ± 3.8% for 200 µg of budesonide and 11.2% ± 2.3% for 800 µg of budesonide (*p* < 0.01 compared with placebo and D5159). The two doses of budesonide were not statistically different. Airway responsiveness to methacholine increased by 1.07 doubling doses 24 hours after allergen challenge. This increased airway respon-

siveness was slightly, but not significantly, reduced by the three active treatments (0.6 to 0.91 doubling doses).

Conclusion: The allergen-induced LAR model was able to differentiate a single dose of an active inhaled corticosteroid from placebo and a highly potent inhaled corticosteroid from a weak inhaled corticosteroid. The model did not differentiate between 2 fourfold doses of the highly active inhaled corticosteroid (at the doses used in this study), neither for the fall in FEV₁ nor for the increase in airway hyperresponsiveness. (*J Allergy Clin Immunol* 1997;100:65-70.)

Key words: Allergen-induced late response, inhaled corticosteroid, budesonide

There are currently no established methods to determine bioequivalence or relative potency of different inhaled corticosteroid preparations.¹ There are several different corticosteroids used for treatment of airway inflammation in asthma. Some of these will soon lose patent rights and will be able to be produced as generic brands. Others are likely to be marketed soon with new delivery devices, such as dry powder devices or devices containing non-freon propellants. Because of these changes, as well as the need to compare new and old inhaled corticosteroids, a simple method to study relative potency of these preparations would be desirable.

One possible method for investigation of the activity of inhaled corticosteroids is the inhibitory effect on the allergen-induced late asthmatic response (LAR), which is thought to be caused by recruitment of inflammatory cells into the airway.^{2,3} Allergen challenge in the laboratory is a well-established technique.^{3,4} It causes early asthmatic response (EAR) within the first 60 to 90 minutes, LAR between 3 and 8 hours, and increases in methacholine airway responsiveness for several days thereafter.⁵ Similar changes are seen in seasonal allergen exposure.⁶ The LAR is highly reproducible,⁷ providing a potential model for the study of medication effects in small numbers of subjects. It is attenuated by a single, relatively low dose of inhaled beclomethasone dipropionate (BDP) (200 µg).^{8,9} In this study we have investigated the single-dose inhaled corticosteroid inhibition of allergen-induced LAR as a model for the detection of differences in inhaled corticosteroids of different potency. We compared a highly clinically active inhaled corticosteroid (budesonide) with a relatively weak inves-

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Abbreviations used

BDP: Beclomethasone dipropionate
 EAR: Early asthmatic response
 LAR: Late asthmatic response

tigational inhaled corticosteroid (D5159) and with placebo. We also compared two four fold doses of budesonide (200 and 800 μ g) chosen from within the usual clinical dosing range.

METHODS**Study design**

Subjects were enrolled in the study if the screening allergen challenge revealed a dual asthmatic response with an LAR exhibiting a greater than 15% decrease in FEV₁. The study was a four-way, double-blind, double-dummy, crossover trial with treatment periods separated by at least 1 week of washout. Each treatment period consisted of three visits on consecutive days. Methacholine challenge was done on the first day. Twenty-four hours later, allergen challenge was done 10 minutes after administration of the blinded medications. Methacholine challenge was repeated 24 hours after allergen challenge. Before each allergen challenge, the methacholine PC₂₀ was required to be within one doubling concentration of baseline; if not, the next treatment period was deferred for 1 week. The goal of this investigation was to administer the same dose of allergen to each subject after each of the four treatments.

Subjects

Atopic subjects with asthma (patients and research volunteers at the three university centers) were enrolled in this study. All subjects were atopic and had a documented history of asthma. At the time of study, subjects required only intermittent treatment with an inhaled bronchodilator, were free from relevant allergen exposure for at least 4 weeks before the study and for the duration of the study, and were free from respiratory tract infections or exacerbations of asthma for at least 4 weeks before the study. No inhaled or oral antiinflammatory therapy had been used for asthma for more than 4 weeks before the study. Methacholine PC₂₀ was stable ($\pm$ one concentration) before all allergen challenges. This study was approved by the three hospital ethics committees, and all subjects gave written informed consent.

Spirometry

Spirometry was done with the same spirometer for each subject throughout the study according to American Thoracic Society standards.¹⁰ Analysis of the flow-volume loop was done to ensure technical quality. The best of three measurements of FEV₁ and forced vital capacity were recorded at each time.

Methacholine inhalation tests

Methacholine challenges were performed with the standardized 2-minute tidal breathing technique.^{11,12} Aerosols were inhaled for 2 minutes of tidal breathing from a jet nebulizer-mask system delivering an output of 0.13 ml/min. Saline solution followed by doubling concentrations of methacholine (0.03 to 8.0 mg/ml) was administered at 5-minute intervals. The FEV₁ was measured at 30 and 90 seconds after each inhalation. The percent change in FEV₁ was calculated from the lowest post-saline to the lowest postmethacholine FEV₁ at the two time

points. The test was continued until a 20% or greater decrease in FEV₁ was achieved. The methacholine PC₂₀ was then calculated from the log dose versus the response curve by an algebraic formula.¹²

Allergen inhalation tests

Diluent-controlled allergen inhalation tests were performed as previously documented.⁴ Solutions were nebulized from a Wright nebulizer (Aerosol Medical Ltd., Colchester, Essex, U.K.) providing an output of 0.13 ml/min. Subjects inhaled the solution through a mouthpiece. A Hans Rudolph valve (Hans Rudolph Inc., Kansas City, Mo.) between the nebulizer and the mouthpiece with the expiratory limb connected to two ventilator filters (Pal Biomedical Inc., Fajardo, Puerto Rico) was used to minimize extraneous allergen exposure to the subject and laboratory staff. Throughout the study, the same nebulizer was used for all challenges in the same individual. On the control day, the allergen diluent (isotonic saline solution with 0.4% phenol and 0.03% human serum albumin; Miles Hollister Stier, Etobicoke, Ontario, Canada) was administered for 2 minutes of tidal breathing on three occasions at 12 minute intervals. The FEV₁ was measured 10 minutes after each inhalation and, after the final inhalation, at 10, 20, 30, 40, 50, 60, 90, 120, 180, 240, 300, 360, and 420 minutes. On the same day, duplicate skin prick tests with the allergen to be used for inhalation were performed.¹³ The wheal was measured at 15 minutes in two perpendicular directions, and the mean wheal diameter (mean of four measurements) for each concentration was calculated. The allergen PC₂₀ was estimated with the skin prick test end point and the methacholine PC₂₀.⁴ On the second day, an allergen challenge was performed, beginning at three or four doubling concentrations below the estimated allergen PC₂₀. Doubling concentrations of allergen were administered at 12-minute intervals (as on the control day). FEV₁ was measured in triplicate after subjects rested for 30 minutes in the laboratory and then was repeated in duplicate 10 minutes after each allergen concentration. The allergen challenge was continued until there was approximately a 20% decrease in FEV₁. Thereafter, the FEV₁ was measured for 7 hours as on the control day. Allergen-induced increase in airway responsiveness to methacholine⁵ was assessed by repeating the determination of methacholine PC₂₀ 24 hours after allergen challenge and comparing it with methacholine PC₂₀ obtained 24 hours before the allergen challenge.

Medication administration

Medications were administered from Turbuhaler inhalers. Placebo, 100 μ g of budesonide per inhalation, 400 μ g of budesonide per inhalation, and 2 mg of D5159 per inhalation were supplied in Turbuhaler inhalers of identical appearance. For each treatment, two inhalations were taken from each of two blinded Turbuhaler inhalers. Four inhalations were needed to achieve the 8 mg dose of D5159. The four combinations of inhalations were as follows:

1. Budesonide, 100 μ g $\times$ 2, and placebo $\times$ 2 = budesonide, 200 μ g
2. Budesonide, 400 μ g $\times$ 2, and placebo $\times$ 2 = budesonide, 800 μ g
3. D5159, 2 mg $\times$ 2, and D5159, 2 mg $\times$ 2 = D5159 8 mg
4. Placebo $\times$ 2, and placebo $\times$ 2 = placebo

The inhalations were made from residual volume to total lung capacity at a flow rate of 60 L/min, and inspiratory flow rate was monitored with a Vitalograph portable spirometer

TABLE I. Subject characteristics

Patient No.	Age (yr)	Sex	Height (cm)	Weight (kg)	FEV ₁ (%)	Methacholine PC ₂₀ (mg/ml)	Allergen inhaled		EAR (%)	LAR (%)
							Allergen	Concentration		
1	31	F	152	63	100	1.5	HDM	1:128*	37	59*
2	24	F	169	55	95	1.2	HDM	1:8	5	20
3	27	F	160	63	103	2.1	Cat	1:4	29	20
4	24	F	160	55	94	0.7	Ragweed	1:1024	29	24
5	23	F	164	53	96	2.2	HDM	1:16	26	19
6	20	F	160	59	104	1.0	HDM	1:64	20	27
7	28	M	170	79	77	1.3	HDM	1:128	25	29
8	30	F	152	48	92	0.16	Cat	1:128	30	55
9	31	M	170	86	88	2.0	Cat	1:4	15	34
10	20	F	157	61	93	1.8	Cat	1:4†	25	28
Mean	25.8		161	62.2	94.2	1.2‡		1:32‡	24.1	31.5
SEM	1.3		2.1	3.7	2.5				2.8	4.5

EAR = percent in FEV₁ on day 2 (control screening unmedicated allergen challenge); LAR = percent in FEV₁ on day 2. HDM = House dust mite (*Dermatophagoides farinae*).

* Subject 1 completed the 4 medicated study days inhaling HDM 1:256 (dose reduced because of this large, clinically uncomfortable LAR on the screening day).

† Subject 10 completed the 4 medicated study days inhaling 1:8 (dose reduced because of large EAR on first allergen challenge after blinded treatment).

‡ Geometric mean.

TABLE II. Late asthmatic response 4 to 7 hours after the four treatments

Patient No.	LAR (maximal % fall in FEV ₁)			
	Placebo	D5159 (8 mg)	Budesonide (200 µg)	Budesonide (800 µg)
1	51.0	44.9	12.8	8.5
2	15.0	-0.3	2.6	5.1
3	18.7	14.3	2.9	0.3
4	40.3	39.3	33.0	8.6
5	12.4	11.6	-1.6	7.1
6	42.8	36.4	23.9	23.2
7	33.6	22.4	18.6	13.3
8	55.2	42.2	16.7	23.6
9	27.5	32.0	32.5	11.4
10	44.8	45.0	9.9	11.3
Mean	34.1	28.8	15.1	11.2
SEM	4.8	5.0	3.8	2.3

(Compact, Vitalograph, U.K.). Each inhalation was followed by 10 seconds of breath holding.

Analysis

The percent fall in FEV₁ was calculated from the highest prechallenge FEV₁ to the highest postchallenge FEV₁ measurement at each time interval. The allergen-induced EAR was determined as the maximum percent fall in FEV₁ during the first hour after allergen challenge, and the allergen-induced LAR was determined as the maximum percent fall in FEV₁ between 4 and 7 hours after challenge. Change in airway responsiveness to methacholine was calculated as the $\Delta \log_{10}$ PC₂₀, which was calculated by subtracting $\log_{10}$ PC₂₀ measured 24 hours after allergen inhalation from $\log_{10}$ PC₂₀ measured 24 hours before allergen inhalation. The two primary end points in this study were the LAR and $\Delta \log$ PC₂₀ methacholine. Results were analyzed by an analysis of variance model.

RESULTS

Ten subjects completed the study per protocol, receiving the same dose of allergen on all 4 blinded treatment days (Table I).

The allergen challenge results are summarized in Table II and Fig. 1. After placebo administration, the mean maximum FEV₁ decrease during EAR was 19.7% $\pm$ 3.7% (SEM), and the mean maximum FEV₁ decrease during LAR was 34.1% $\pm$ 4.8%. There was no significant difference in the EAR after the three active treatments. Values for EAR were 19.5% $\pm$ 3.8% after D5159 administration, 21.6% $\pm$ 3.7% after administration of 200 µg of budesonide, and 22.0% $\pm$ 3.6% after administration of 800 µg of budesonide ($p > 0.05$). The LAR (Table II) was significantly reduced in magnitude by the

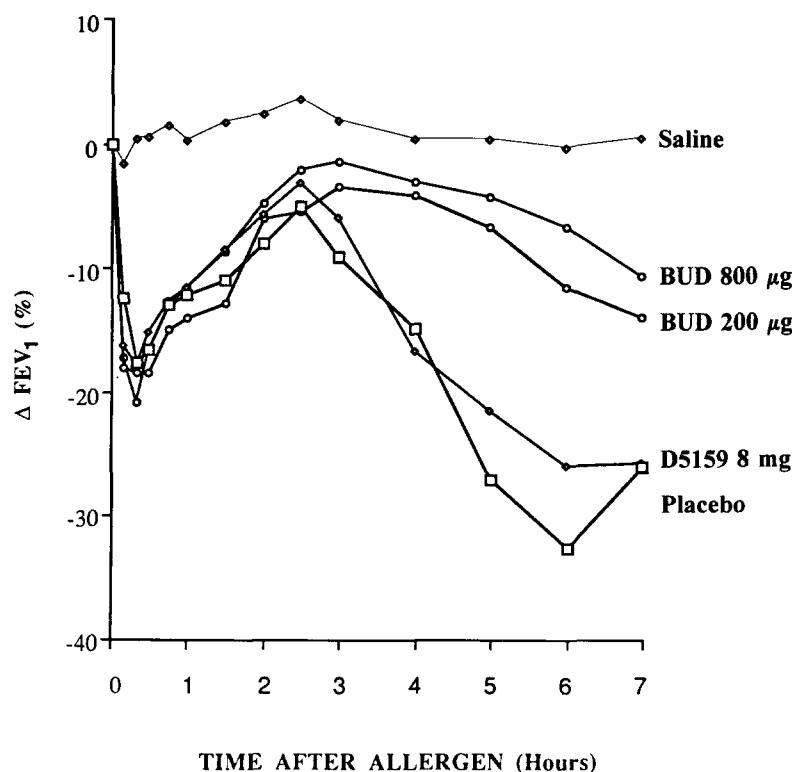


FIG. 1. Effect of treatment on allergen-induced bronchoconstriction. The y axis shows fall in FEV₁ as a percentage of baseline. The x axis shows time in hours. Symbols represent the mean values of 10 subjects evaluated. *Saline* indicates that no allergen was administered. *BUD 200* and *BUD 800* represent responses after inhaled budesonide 200 and 800 μg, respectively, given 10 minutes before challenge. *D5159* shows response after the weak corticosteroid D5159 (8 mg) administered 10 minutes before challenge. *Placebo* was administered 10 minutes before challenge.

three active treatments when compared with placebo. The LAR was $28.8\% \pm 5.0\%$ after D5159 administration ($p < 0.05$ vs placebo), $15.1\% \pm 3.8\%$ after administration of 200 μg of budesonide ($p < 0.01$ vs placebo), and $11.2\% \pm 2.3\%$ after administration of 800 μg of budesonide ($p < 0.01$ vs placebo). Both doses of budesonide were significantly more effective than D5159 ($p < 0.01$) but were not significantly different from each other ($p > 0.05$).

Allergen-induced increase in airway responsiveness to methacholine is shown in Table III. There was a significant increase in methacholine responsiveness after placebo pretreatment, $\Delta \log PC_{20} = 0.32$ (1.07 doubling concentrations, $p < 0.01$). $\Delta \log$ methacholine PC_{20} was reduced to 0.91, 0.78, and 0.60 doubling concentrations after pretreatment with D5159, administration of 200 μg of budesonide, and administration of 800 μg of budesonide, respectively. These reductions were neither statistically different from placebo nor from each other.

DISCUSSION

This is the first study to use the allergen-induced LAR to compare the activity of inhaled corticosteroids administered in a single dose. All of the inhaled corticosteroids

tested attenuated the LAR. The effect was least significant with the weak corticosteroid D5159, more significant with 200 μg of budesonide, and most significant with 800 μg of budesonide. However, the responses of the two doses of budesonide were not significantly different. This shows that the LAR differentiates between high-potency and low-potency inhaled steroids. There was a progressive reduction in allergen-induced airway responsiveness in parallel with the reduction in the LAR, but this was not statistically significant. Similar results have recently been reported for BDP.¹⁴ Single doses of BDP of 42 μg, 126 μg, and 378 μg all demonstrated potent but similar inhibition of the allergen-induced LAR in six subjects.

D5159, developed at Astra Draco, is a glucocorticoid that showed favorable topical antiinflammatory efficacy in preclinical animal models. However, it was found to have insufficient clinical efficacy in clinical trials in human subjects with asthma. Thus in this study, D5159 was included as a "negative control" antiinflammatory. This study was designed to develop a technique for finding a dose-response relationship for an effective corticosteroid, budesonide, in the usual therapeutic doses. Other than the addition of D5159 as a negative control, this study was not intended to be primarily

TABLE III. Allergen-induced increase in airway responsiveness after the four treatments

Patient No.	$\Delta \text{Log}_{10} \text{methacholine PC}_{20}$			
	Placebo	D5159 (8 mg)	Budesonide (200 μg)	Budesonide (800 μg)
1	0.62	0.42	0.30	0
2	0.23	0.06	-0.25	0.30
3	0.63	0.58	0.22	0.06
4	0.21	-0.04	0.39	0.26
5	-0.08	0.06	0.23	-0.06
6	0.35	0.53	0.23	0.65
7	0.54	0.14	0.71	0.20
8	0.03	0.08	0.30	0.20
9	0.16	0.03	0.44	0.02
10	0.53	0.88	-0.23	0.15
Mean	0.32	0.27	0.23	0.18
SEM	0.08	0.10	0.09	0.06
<i>p</i> Value	<0.01	<0.05	<0.05	<0.05

directed toward examining D5159 directly or in a comparative fashion.

The study confirms other observations on the effect of inhaled steroids on the LAR. In a previous study the LAR was reduced from 21.8% after pretreatment with placebo to 6.2% after pretreatment with 200 μg of BDP.⁸ Similar attenuation of the late response by 200 μg of BDP was reported by Wong et al.⁹ Cockcroft et al.¹⁵ showed that the late response was reduced from 26.4% after placebo administration to 7.3% when 500 μg of BDP was administered 2 hours after allergen inhalation.

The duration of therapy with inhaled corticosteroids increases the benefits to the patient.¹⁶ Regular treatment with inhaled corticosteroids (1 to 8 weeks) also reduces the EAR.^{5, 6, 16, 17} Several attempts have been made to assess a dose response of inhaled corticosteroid by using bronchoprovocation and 1 week of treatment.¹⁸⁻²⁰ De Marza et al.¹⁸ found a shallow dose response when they compared effects of administration of 200 μg of BDP twice a day with administration of 1000 μg of BDP twice a day on toluene diisocyanate-induced bronchoconstriction at 4 hours. The mean FEV₁ was 2.6 L during placebo administration, 3.3 L during administration of 200 μg of BDP, and 3.6 L during administration of 1000 μg of BDP. McCubbin et al.¹⁹ demonstrated a dose response using the EAR and three doses of each of the three inhaled corticosteroids. However, their study was nonrandomized, with the corticosteroids being administered in progressively increasing doses. By contrast, Swystun et al.²⁰ found that 1-week treatments of three different daily doses of budesonide (200 μg , 400 μg , and 800 μg) were all equally effective in inhibiting the allergen-induced EAR in a randomized study.

Inhaled budesonide caused a small but not significant attenuation of the increase in airway responsiveness. Similar results have been reported by Wong et al.⁹ after administration of 200 μg of BDP before allergen challenge. Similarly, a statistically small treatment effect ($p < 0.05$) was seen after 200 μg of BDP was adminis-

tered before allergen challenge.⁸ When 500 μg of BDP was administered 2 hours after allergen challenge, there was a borderline ($p = 0.056$) reduction in airway responsiveness.¹⁵ However, inhaled corticosteroid used regularly for 1 week before toluene diisocyanate challenge was highly effective in reducing the induced airway responsiveness.^{18, 21}

The inability to separate statistically the effect of the two doses of budesonide may be related to the high potency of this drug. The mechanism of delivery used was Turbuhaler, which provides approximately twice the lung deposition compared with metered-dose inhalers.²² This is likely to provide considerably greater deposition of inhaled corticosteroid than the 200 μg of BDP administered by the metered-dose inhalers used in previous studies. It is possible that near-maximal effects of inhaled corticosteroids are achieved when 200 μg of budesonide is administered by the Turbuhaler. In future studies this method will allow the rapid clinical assessment of inhaled corticosteroids to distinguish corticosteroids with little activity from corticosteroids with clinically significant activity. To compare two effective drugs or to show a dose-response, lower doses of inhaled corticosteroids may need to be studied.

The minor effects observed on the allergen-induced increase in airway responsiveness seen in this study are in agreement with other single-dose, pretreatment studies.^{8, 9, 15} To detect a significant difference in this parameter, much larger numbers of subjects need to be studied. However, it is unlikely that these measurements would yield information additional to that obtained from the LAR model. Indeed, using a large number of subjects would defeat part of the purpose of this study design, the establishment of a sensitive method for comparing corticosteroid activity in a limited number of subjects. For evaluation of corticosteroid bioequivalence with single-dose studies, it appears that the change in airway hyperresponsiveness is not helpful. It may be more useful in studies of longer duration (≥ 1 week).^{16, 21}

In summary, this study has shown that the LAR can differentiate between potent and weak corticosteroids. However, the effect of two doses of a high-potency inhaled corticosteroid could not be statistically differentiated. Perhaps for dose-response studies, lower doses of inhaled corticosteroid need to be used. We conclude that the LAR is a useful model for distinguishing between inhaled corticosteroids with significant and negligible activity.

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