

Comparison of 3 different doses of budesonide and placebo on the early asthmatic response to inhaled allergen

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Background: A simple laboratory method to evaluate relative potency of inhaled corticosteroids in asthma would be valuable. Single-dose studies with the allergen-induced late asthmatic response have failed to show a useful dose-response relationship. Treatment for several days with inhaled corticosteroids will also inhibit the allergen-induced early asthmatic response.

Methods: Twelve atopic asthmatic subjects were studied during a season when no medications were required except ipratropium bromide as needed. These subjects had positive allergen and methacholine inhalation tests and FEV₁ greater than 70% of predicted value. A double-blind, randomized, cross-over study compared placebo and budesonide 100, 200, and 400 µg administered by means of Turbuhaler twice daily for 7 days with 6-day washout periods. Methacholine PC₂₀ was measured before and after 6 days of treatment, and allergen PC₁₅ was measured after 7 days of treatment.

Results: The allergen PC₁₅ ($n = 11$) was significantly larger ($P = .0001$) for all doses of budesonide compared with placebo, but there was no significant difference between the 3 doses of budesonide, and no dose response was demonstrated. The methacholine PC₂₀ was significantly larger after all budesonide treatments compared with placebo ($P = .024$), but there was no difference between the 3 doses. There was a progressive increase in the allergen PC₁₅ chronologically (sequence effect) that was not explained by improvement in FEV₁ or airway responsiveness; sequence effects were not seen for FEV₁ or for pretreatment or posttreatment methacholine PC₂₀. Statistical adjustment for sequence effect did not alter allergen PC₁₅ statistics.

Conclusion: A 7-day course of budesonide administered by means of Turbuhaler at 200, 400, or 800 µg per day provided marked and significant inhibition of the allergen-induced early asthmatic response compared with placebo. There was, however, no difference between the 3 doses. Therefore this method with these doses is not useful for providing assessment of relative potency. (*J Allergy Clin Immunol* 1998;102:363-7.)

Key words: Asthma, bronchial allergen challenge, corticosteroid, dose-response

Abbreviations used

EAR: Early asthmatic response

LAR: Late asthmatic response

PNU: Protein nitrogen unit

Evaluation of inhaled corticosteroids currently requires large, expensive clinical trials of long duration. A more efficient laboratory method for evaluating relative potency of an inhaled corticosteroid before clinical trials would be of great value for assessing new corticosteroids, new inhalation devices, and generic versions of existing corticosteroids. The relative potency of an investigational drug could be assessed by comparison with an available drug of known efficacy.

A single dose of inhaled corticosteroid has been shown to inhibit the late asthmatic response (LAR) to allergen.^{1,2} Previous studies making use of single doses of corticosteroid and the LAR to allergen failed to demonstrate a useful dose-response relationship.^{3,4} Thus a scale with which to measure the relative potencies of other therapies was not found.

It has been shown that several days of treatment with an inhaled corticosteroid can inhibit the early asthmatic response (EAR) to allergen.^{5,6} A study model making use of the EAR would have better potential to differentiate the allergen responses between different doses of corticosteroid treatment because the EAR is measured as a titrated response and thus is an open-ended scale. Other advantages of the EAR model are the greater availability of study patients, less patient discomfort, and much less laboratory testing time required. The objective of this study was to test the allergen EAR model as a method of evaluating relative potency of inhaled corticosteroids by using 1-week treatments of 3 different doses of budesonide and placebo. The principle outcome measure was the allergen PC₁₅, the provocative concentration of allergen required to produce a 15% fall in FEV₁, as measured by the allergen inhalation challenge test after the 4, 1-week treatment periods. Secondary variables evaluated were FEV₁ and methacholine PC₂₀. It was intended to demonstrate a dose-response relationship between the three doses of budesonide and the allergen PC₁₅.

METHODS

Design

A placebo-controlled, double-blind, random-order, cross-over study design was used to compare 4 1-week treatment periods of

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TABLE I. Anthropomorphic, lung function, and allergen data for 12 subjects

Subject	Age (y)	Sex	Height (cm)	Weight (kg)	FEV ₁ (L)	% Predicted	MPC ₂₀ (mg/mL)	Allergen
1	48	M	170	61	2.48	74	0.5	Grass
2	22	F	157	55	2.41	82	0.03	Cat
3	22	F	168	65	3.20	96	1.1	Birch
4	23	F	165	59	3.16	98	1.4	Weed
5	20	F	160	48	2.87	93	0.3	Horse
6	20	M	181	70	4.38	96	0.7	Weed
7	20	F	154	55	2.69	91	2.1	Grass
8	22	M	186	123	4.42	90	0.7	Grass
9	23	M	191	86	5.04	96	2.4	Cat
10	27	F	167	57	3.01	94	0.4	Grass
11	21	F	173	58	3.65	104	4.5	Horse
12	29	M	180	80	3.69	80	3.2	Cat
Mean	25		171	68	3.42	91	0.824*	
SD	7.8		12	20	0.84	8.5		

MPC₂₀, Methacholine PC₂₀.

*Geometric mean.

budesonide 200 µg per day, 400 µg per day, 800 µg per day, and placebo. The treatments were twice daily inhalations of Pulmicort Turbuhaler (Astra Draco) 100 µg, 200 µg, and 400 µg and placebo Turbuhaler taken for 7 days (ie, 14 doses) plus 1 dose taken 1 hour before the allergen challenge on Day 8 of the treatment period. Budesonide was inhaled as directed in the package insert. The treatment periods were separated by a washout of 6 or more days.

Methacholine inhalation tests were done before the study medications were started to check airway stability at the beginning of each treatment period and on Day 7 of the period 1 hour after the thirteenth dose of study medication. Allergen challenges were started 1 hour after the last dose of study inhaler on Day 8. Atrovent (Boehringer Ingelheim, Burlington, Ontario, Canada) inhalers were allowed for rescue use at a maximum of 2 times per week. All other therapy was prohibited during the study. The subjects recorded in a diary for each day of the treatment periods the occurrence of asthma symptoms, the total number of inhalations of study medication taken, and any other medications used.

Subjects

Anthropometric, lung function, and allergen data are given in Table I. Twelve subjects with mild-to-moderate atopic asthma completed the study. Thirteen subjects had been randomized to treatment, but 1 failed to respond to allergen challenge in treatment period 1 and was withdrawn.

All subjects had documented responses to specific inhalant allergens in skin prick testing and inhalation challenge testing. Values for FEV₁ were greater than 70% of that predicted, and methacholine PC₂₀ values were 8.0 mg/mL or less. Subjects were studied when they were not environmentally exposed to allergen, and thus their asthma was well-controlled, with no asthma medication requirements and no respiratory tract infection for 4 or more weeks before the start of the study. The study protocol was approved by the University of Saskatchewan Ethics Committee. All subjects gave signed, informed consent before participating in the study.

Methacholine inhalation test

The methacholine inhalation test was done by the previously described method.^{7,8} After baseline FEV₁ was recorded in triplicate, the subjects inhaled the aerosolized solution of isotonic saline from a Bennett Twin-jet nebulizer (Puritan-Bennett Corp, Carlsbad, Calif) generating an output of 0.13 mL/min by means of a loose-fitting face mask with nose occluded, for 2 minutes of tidal breathing. FEV₁ was recorded at 30 and 90 seconds after the inhalation, and

the lowest value was used as the baseline for calculations. Inhalations of methacholine (ACIC Inc, Brantford, Ontario, Canada) were done in similar fashion with the nebulizer loaded with 3 mL methacholine chloride in doubling concentrations (0.03 to 16 mg/mL) every 5 minutes until the subject had a 17% or greater fall in FEV₁. The methacholine PC₂₀ was calculated by a logarithmic dose-response curve formula.⁹

Allergen inhalation test

Before enrollment into the study, all subjects completed 2 7-hour allergen screening days in the laboratory as previously described.¹⁰ One day was the diluent inhalation day, and the second was an allergen inhalation day. Aerosols were generated with a Wright nebulizer (Aerosol Medical Ltd, Colchester, Essex, UK) calibrated to give an output of 0.13 mL/min. The nebulant was passed into a Hans-Rudolph valve (Hans Rudolph Inc, Kansas City, Mo) and to the patient through a mouthpiece. Two ventilator filters were placed in series on the expiratory side of the valve to prevent allergen aerosol from escaping into the room. On the first day, subjects inhaled diluent, 0.9% saline with 0.5% phenol, and 0.03% human serum albumin (Miles Canada Inc, Etobicoke, Ontario, Canada) for 2 minutes on 3 occasions at 12-minute intervals. The FEV₁ was initially measured in triplicate before inhalations and in duplicate after each inhalation and after the final inhalation at 10, 20, 30, 40, 50, 60, 90, 120, 180, 240, 360, and 420 minutes. The largest FEV₁ at each time was used for analysis. On this day subjects also underwent duplicate skin prick tests with doubling dilutions (1:8 to ≥1:1024) of the aqueous allergen extract (Hollister-Stier/Miles Canada Inc, Etobicoke, Ontario, Canada; and Bencard, Mississauga, Ontario, Canada) to be used for inhalation. Allergen extracts of cat hair/epithelium, horse hair, grass pollen, birch pollen, and weed pollen were diluted from stock allergenic concentrations ranging from 20,000 to 40,000 protein nitrogen units per milliliter (PNU/mL). Each wheal produced by the extract was measured in 2 perpendicular directions at 10 to 15 minutes, and the mean wheal size for each concentration was determined. The skin test endpoint was defined as the smallest concentration of allergen producing a mean wheal diameter at least 2 mm larger than that produced by the diluent. A methacholine inhalation test was also done at 7 hours on this day. The allergen PC₁₅ could then be predicted from the skin test endpoint and the methacholine PC₂₀.¹⁰

On the second day an allergen inhalation was performed in a fashion similar to the diluent inhalation, starting 3 or 4 concentrations below the predicted allergen PC₁₅. Allergen inhalations were contin-

TABLE II. FEV₁, methacholine PC₂₀, and allergen PC₁₅ results

	FEV ₁ (L) (n = 12)	Baseline MPC ₂₀ (mg/mL) (n = 12)	Day 7 MPC ₂₀ (mg/mL) (n = 12)	Day 8 Allergen PC ₁₅ * (PNU/mL) (n = 11)
Placebo	3.43 ± 0.60†	0.92‡	0.78‡	483‡
BUD 200 µg	3.51 ± 0.86	0.92‡	1.16‡	1221‡
BUD 400 µg	3.39 ± 0.87	0.84‡	1.12‡	1104‡
BUD 800 µg	3.51 ± 0.85	0.93‡	1.27‡	1441‡
ANOVA P value	.30	.77	.024	.0001

MPC₂₀, Methacholine PC₂₀; BUD, budesonide.

*Adjusted for sequence effect.

†SD.

‡Geometric mean.

ued until the FEV₁ had fallen by 15% or greater at 10 minutes, and the FEV₁ was monitored for 7 hours as on the diluent day. The allergen PC₁₅ was calculated by using the greatest percent FEV₁ decrease after allergen inhalation from the logarithmic dose versus response curve formula and reported in protein nitrogen units per milliliter.⁹

The allergen PC₁₅ was measured on Day 8 for each treatment period by using a shortened version of the allergen challenge day.⁶ The subject was allowed to leave the laboratory after the resolution of the early response, usually 30 to 60 minutes after the final allergen inhalation. Allergen inhalations began at the same concentration in all treatment periods for each subject. All subjects inhaled 800 µg budesonide before leaving the laboratory as a precaution to prevent any LARs.¹¹ On some occasions, salbutamol 100 or 200 µg was given to help reverse the fall in FEV₁ after allergen or methacholine administration.

Analysis

The primary endpoint was the allergen PC₁₅ analyzed by log transformation with a cross-over ANOVA model with patient, period, treatment, and first-order carryover effect as factors. A *P* value less than .05 was considered statistically significant. The secondary endpoints were the log methacholine PC₂₀ (Day 7) and the FEV₁ (Day 8 before allergen challenge), which were analyzed by the ANOVA model. The four pretreatment methacholine PC₂₀ values were also analyzed by ANOVA to detect time trends for change in airway function.

RESULTS

Twelve subjects completed the study per protocol. One subject (no. 12) failed to have a measurable allergen PC₁₅ for any treatment period, leaving 11 for assessment of allergen PC₁₅ and 12 for assessment of FEV₁ and methacholine PC₂₀.

Treatments on 2 occasions were extended 1 day to accommodate the participants' schedules. Subject 4 during treatment period 3 did the second methacholine test on Day 7 and the allergen challenge on Day 9; subject 9 during treatment period 1 did the second methacholine test on Day 8 and the allergen challenge on Day 9. In both cases, 17 total doses rather than 15 doses of study medication were administered. There were no serious adverse events or adverse events which lead to discontinuations.

The results are shown in Table II. All budesonide treatments caused a significant 2- to 3-fold increase in allergen PC₁₅ over placebo (*n* = 11, *P* = .0001). There was no difference in pretreatment methacholine PC₂₀ (*P* = .77). There was a significant increase in methacholine PC₂₀ on Day 7 of less than 1 doubling dose for all budesonide

onide treatments in comparison with placebo (*P* = .024). There was no significant difference for either endpoint between any of the 3 doses of budesonide. FEV₁ was unchanged by active treatment (*P* = .30).

There was a trend (*P* = .051) for a sequence effect on allergen PC₁₅ with a gradual improvement in the order of testing, approaching 1 doubling concentration, between treatment periods 1 and 4. There was no trend for a sequence effect for baseline methacholine PC₂₀ (*P* = .45), Day 7 methacholine PC₂₀ (*P* = .31), or FEV₁ (*P* = .37). When statistical adjustment was made for sequence effect on allergen PC₁₅, there remained no significant difference or trend for the effect between the 3 doses of budesonide on the allergen PC₁₅.

DISCUSSION

We have demonstrated that all 3 doses of budesonide provided marked improvement in the allergen PC₁₅, as well as a significant but lesser improvement on the methacholine PC₂₀. However, a dose-response relationship was not demonstrated in either parameter. There was no change in FEV₁ with active treatment. This could be explained by the subject population and the short duration of treatment. The mean baseline FEV₁ for this population was 91% of predicted value. Subjects were studied when they were not exposed to relevant allergens and therefore were at or near their optimum airway caliber, with little margin for improvement. There was a treatment sequence effect on the allergen PC₁₅ but not on the FEV₁ or methacholine PC₂₀. Adjustment for this effect did not alter the results of the dose-response curve.

We are aware of only 2 blinded and randomized studies looking for a dose-response effect of inhaled corticosteroids on airway response to allergen. Both studies involved single doses of inhaled corticosteroids and inhibition of the allergen-induced LAR. The first study compared efficacy among 2 doses of budesonide (200 µg and 800 µg), an investigational drug, and placebo.³ The measure of efficacy was the inhibition of the LAR by a single dose of inhaled corticosteroid given 10 minutes before the start of an allergen inhalation challenge. Both doses of budesonide significantly inhibited the LAR to allergen, but the study model failed to detect a difference between them. In a similar study 3 different doses of beclomethasone (42, 126, and 375 µg) showed excellent but indistinguishable inhibition of LAR.⁴

It is important for a study model to distinguish between various doses of a known, effective medication so that the varied responses provide a scale with which to compare the effects of other medications. The LAR as a study model is limited in its ability to demonstrate much range of response because the same dose of allergen must be administered after each treatment, and therefore an allergen dose-response cannot be measured. Only the absolute response, measured as percent fall in FEV₁ to the same dose of allergen, can be observed and allows only a small window of variability. In contrast, a study with the EAR as an outcome can measure an allergen dose-response and thus allow an open-ended, larger scale of variability in the response to therapy. The response to treatments can be measured in terms of shifts of several doubling concentrations of allergen (ie, a titrated response [PC₁₅]) instead of a simple change in percent fall in FEV₁ after the same dose of allergen measured by the LAR. This allows a magnification of the response to therapy and an increased ability to distinguish between doses.

Previous studies have demonstrated the inhibition of the EAR after 1 week of treatment with inhaled corticosteroids.^{5,6} In addition to the larger scale of measure, the EAR study model has additional advantages over the LAR model. Measurement of the EAR requires much less laboratory time, and the EAR is usually associated with less discomfort for the patient than is the LAR. There are more patients who can demonstrate an EAR compared with those who also show an LAR, and therefore there is a greater availability of potential study subjects. Furthermore, patients with an isolated EAR may have asthma that is more stable, allowing for a more reliable testing procedure and less difficulty in the interpretation of results.

The inability of our study to demonstrate a dose response may be due to the duration of treatment, the dose of drug, or the subject population. It is conceivable that a plateau of the allergen response to treatment was reached before the end of treatment and that incremental responses could be shown if the length of the treatment period was shortened or if the doses of drug were decreased. It appears that near-maximal treatment effect was reached in this population with 200 µg per day for 1 week. It should be emphasized that the subjects were studied at a time when all relevant allergen exposure (pollens, spores, and animal danders) could be avoided, and the condition of their asthma was necessarily mild to meet the study entrance criteria of no current requirement for asthma medication except occasional ipratropium bromide for symptom relief. A study model making use of a 2- to 4-day treatment period would have a better chance of distinguishing between doses because the treatment effects may not have reached plateau, and it would be even more time efficient. Decreasing the doses of drug to less than the prescribed clinical doses is certainly feasible for testing the study model. A longer treatment period may also increase the potential of separating the responses because some of the study population would show fur-

ther improvement in airway responsiveness over time of treatment.⁵ A study population that had more severe asthma would also have increased potential of showing varied responses to different doses, but an increased asthma severity would probably necessitate the therapeutic use of asthma medication in these subjects.

The allergen-induced EAR is dependent in part on airway responsiveness, which can be measured by methacholine or histamine.¹⁰ Therefore the improvement in airway responsiveness to allergen may be partly explained by improved airway responsiveness to methacholine. The improved airway response to allergen was greater than that to methacholine, and this likely reflects an additional effect, probably corticosteroid-induced suppression of airway mast cell numbers.^{12,13} The corticosteroid-induced improvement in airway responsiveness to histamine or methacholine is known to continue to increase for a period of several months or more.^{14,15} The chronology of corticosteroid-induced changes in airway mast cells is not known.

McCubbin et al¹⁶ investigated the airway response to allergen as a method for a "bioassay" for inhaled corticosteroids. Their study, however, was neither randomized nor blinded. They administered sequentially increasing doses of corticosteroids in 1-week periods with no washout, measuring the airway response to allergen at weekly intervals. Their demonstrated "dose-response" relationship could have been explained by an effect of the duration of therapy. Therefore we do not believe that their data are in conflict with ours.

The observation of a progressive increase in allergen PC₁₅ over the 4 trial periods in our study was a surprise. Previous studies making use of allergen PC₁₅ measured at approximately 2-week intervals on 4 occasions in our laboratory have not demonstrated such an effect.⁶ The sequence effect could not be attributed to a change in lung function or to an improvement in baseline airway responsiveness to methacholine and was not evident when we looked at the posttreatment methacholine PC₂₀. It is possible that there was a cumulative carry-over effect of the corticosteroid on, perhaps, mast cell number or function (some feature other than the airway responsiveness) affecting the allergen PC₁₅. Alternately, it is possible that a progressive desensitization to allergen occurred in these subjects, although this has not been demonstrated in similar repeat allergen study designs.⁶

In summary, we have investigated the allergen-induced EAR as assessed by the allergen PC₁₅ as a laboratory model to identify a dose-response relationship for inhaled corticosteroids. Although we demonstrated that inhaled corticosteroids administered for 1 week were potent inhibitors of the EAR, we failed to detect a dose-response for budesonide in doses of 200 µg, 400 µg, and 800 µg per day. Further investigation is required to determine whether adjusting the duration of treatment or the dosage of drug could make this study model a method for evaluating the relative potency of inhaled corticosteroids.

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