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Methacholine Challenge as a Clinical Bioassay of Pulmonary Delivery of a Long-Acting β_2 -Adrenergic Agonist

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Abstract

Study Objective—To determine whether the methacholine challenge method used for albuterol can be applied to assess long-acting β_2 -adrenergic agonist (LABA) bioequivalence, which would require a sufficiently steep dose-response curve.

Design—Prospective, unblinded, randomized, 2-way crossover study.

Setting—University medical center clinical research laboratory.

Patients—Ten adults, aged 21–58 years, with mild asthma (forced expiratory volume in 1 sec [FEV₁] 70% predicted) and a baseline provocative concentration of methacholine required to decrease FEV₁ by 20% (PC₂₀) of 4 mg/ml or less completed the study.

Intervention—Patients were randomized to receive a single dose of either 12 or 24 μ g of formoterol delivered by a dry powder inhaler; 3–7 days later, at the same time of day, they received the other dose.

Measurements and Main Results—The FEV₁ was measured before and 1 hour after dosing, followed by performance of a methacholine challenge. Statistical analysis was performed by the 2-sample regression method for crossover studies. The dose-response curve for bronchodilation was flat; the mean $\pm$ SD increase in FEV₁ after formoterol 12 and 24 μ g was 14 $\pm$ 5% and 14 $\pm$ 8%, respectively ($p > 0.05$). In contrast, the geometric mean PC₂₀ (95% confidence interval) was 7 mg/ml (2–22 mg/ml) after the 12- μ g dose and 16 mg/ml (5–45 mg/ml) after the 24- μ g dose ($p < 0.001$).

Conclusion—Bioassay by methacholine challenge will be useful for bioequivalence studies of LABAs. A sample of at least 28 patients will be required for formoterol when methacholine challenge is performed in an optimal manner. The sample size may differ for other LABAs.

Keywords

methacholine; bioassay; formoterol; long-acting β_2 -adrenergic agonist; LABA

Inhaled long-acting β_2 -adrenergic agonists (LABAs) are recommended in the treatment of asthma as maintenance therapy when combined with an inhaled corticosteroid or when used

alone to prevent exercise-induced bronchospasm.¹ In addition, they are recommended alone or in combination with a long-acting inhaled anti-cholinergic or an inhaled corticosteroid as maintenance therapy for chronic obstructive pulmonary disease.² Formoterol, salmeterol, and arformoterol are the only LABAs currently available in the United States, and they are marketed in various formulations alone or in combination with an inhaled corticosteroid.

Although patents are still in force for several drug products containing a LABA (i.e., drug and device), the patents on the drug substance (i.e., the drug alone) have expired. Therefore, pharmaceutical firms have developed or are interested in developing alternative delivery devices for two of these drugs, and in some cases attempts are being made to develop a generic version.

Obtaining U.S. Food and Drug Administration (FDA) approval of a generic LABA includes a requirement for an in vivo dose-response study to establish bioequivalence,³ and under varying circumstances, such a study is required by the European Medicines Agency⁴ and Canada's Human Protection Branch.⁵ The purpose of such a study is to determine whether a test and reference product meet equivalence criteria based on a bioassay sensitive to differences in dose. If the study design is not sufficiently sensitive to detect a difference in two doses of the reference product, then it would not be possible to detect a difference between the test and reference products in the amount of drug delivered to the airways.⁶

In the United States, pharmacokinetic studies are not sufficient, by themselves, to establish a dose-response relationship,³ since plasma concentrations have not been proven to indicate where in the airways the drug is delivered and do not distinguish drug absorbed from the lungs and drug absorbed from the swallowed portion of the dose in the gastrointestinal tract.⁷ Thus, a pharmacodynamic end point is required to demonstrate the amount of drug delivered to the relevant receptors in the airways.⁸

Conventional bronchodilator studies, where the forced expiratory volume in 1 second (FEV₁) is measured after a dose over time, have been used, but often the dose-response curve with this pharmacodynamic end point is relatively flat.^{9, 10} That is, during typical daytime studies in patients with stable asthma, one actuation may produce the same improvement in FEV₁ as two actuations, since the response lies on the upper plateau portion of the dose-response curve.¹⁰

Dose-response studies of albuterol have demonstrated that bronchoprovocation with histamine or methacholine are the most sensitive methods of determining differences in doses when delivered by generic and brand chlorofluorocarbon (CFC) metered-dose inhalers (MDIs),¹¹ between a CFC MDI and dry powder inhaler (DPI),¹² and between CFC and hydrofluoroalkane (HFA) MDIs.¹³

Bronchoprovocation with methacholine has been used to compare the intensity and/or duration of formoterol delivered by a DPI or MDI.^{14–16} However, none of these studies has provided sufficient information to determine whether methacholine challenge can detect a 2-fold or smaller difference in dose and, thus, be useful as a bioassay to determine bioequivalence. Also, they do not provide information on the sample size required for a bioequivalence study. Since the Aerolizer (Foradil Aerolizer; Schering Corp., Kenilworth, NJ) is the only DPI formulation of formoterol available in the United States, it will serve as the reference product for subsequent studies of new delivery devices or generic formulations of this drug.

Thus, the purpose of this study was to determine whether a methacholine-based clinical bioassay can be used to evaluate bioequivalence of formoterol (Aerolizer) and other formoterol-containing formulations. This would require that a highly significant dose-

response relationship be present, which, in turn, requires a sufficiently steep dose-response slope relative to the response variability observed in the study. In addition, we propose possible improvements in study design that may further increase the statistical power of these bioequivalence methods.

Methods

Patients

The study protocol was approved by the University of Florida Institutional Review Board (IRB), and all patients read and signed an informed consent form before any procedures were begun. Since the formoterol higher dose of 24 µg was above the FDA-approved dose of 12 µg, and methacholine concentrations were above labeled concentrations, the protocol was submitted to the FDA and approved as an investigator-sponsored investigational new drug application (IND no. 101,246).

Patients aged 18–60 years with mild stable asthma were eligible for study inclusion. The protocol called for patients to have a baseline FEV₁ of 70% or greater and a provocative concentration of methacholine required to decrease FEV₁ by 20% (PC₂₀) of 4 mg/ml or less. Patients were required to be nonsmokers for at least 1 year and to have had a smoking history of not more than 10 pack-years. Women of child-bearing age were required to have had a negative pregnancy test, were not breastfeeding, and were using an acceptable method of contraception. All patients were required to be able to perform acceptable and reproducible spirometry according to the American Thoracic Society (ATS)–European Respiratory Society (ERS) guidelines.¹⁷ The inhalation technique of the DPI (Aerolizer) was reviewed on each study day and performed in accordance with the product's package insert. Patients were allowed to use an oral inhaled or intranasal corticosteroid as long as the dosage was stable for at least 4 weeks and entrance criteria were met.

Patients were excluded if they had a severe asthma exacerbation requiring hospitalization in the previous 12 months, if they required a short course of systemic corticosteroids for asthma in the previous 4 weeks, if they had a respiratory infection in the previous 4 weeks or during the study, or if they were unable to withhold drugs that might interfere with the methacholine challenge for the recommended times.¹⁸ Patients were also excluded if they had a history of any pulmonary disease other than asthma, or history of clinically significant cardiovascular, renal, neurologic, liver, or endocrine dysfunction. Patients with well-controlled hypertension, hypercholesterolemia, or diabetes mellitus were not excluded as long as their maintenance drug therapy would not interfere with the methacholine challenge (e.g., β-blockers).

Study Design

This prospective, unblinded, randomized, 2-way crossover study consisted of three visits. All study visits were either in the morning or afternoon, at the same time of day for each patient. The first visit was a screening visit to determine study eligibility and baseline PC₂₀, followed by two subsequent visits for study drug randomization and administration. The second and third visits were each scheduled 3–7 days after the previous visit. At the second visit, patients were randomized to receive a single dose of either 12 or 24 µg of formoterol delivered by a dry powder inhaler; they received the other dose 3–7 days later at the third visit. During the second and third visits, vital signs and spirometry were measured before administration of study drug. After the initial screening visit, FEV₁ had to be 60% or greater and within 10% of the previous visit on each study day and within 20% of the screening visit. Albuterol was not used for at least 6 hours, and LABAs and leukotriene modifiers were not used for at least 36 hours before each challenge. If a patient was taking an inhaled

corticosteroid, the morning dose was withheld. Patients then inhaled the single dose of formoterol 12 or 24 μg . The FEV_1 and methacholine challenge were performed 1 hour after dosing. At the conclusion of the challenge, inhaled albuterol was administered until FEV_1 returned to 90% or greater of that study day's baseline value.

Study Drug Administration

Formoterol fumarate was delivered by a DPI, 12 μg /capsule (Foradil Aerolizer). Patients inhaled rapidly and deeply through the inhaler mouthpiece and held their breath for 10 seconds. The capsule was inspected; if there was any powder remaining, the inhalation process was repeated immediately. Patients inhaled one capsule for the 12- μg nominal dose and two capsules for the 24- μg nominal dose.

Spirometry

Spirometry was performed with a pneumotach spirometer (KoKo; Quantum Research, Louisville, CO). Spirometry was performed, at least in triplicate, according to standards set by the ATS-ERS Task Force.¹⁷

Methacholine Challenge

Methacholine challenges were performed in accordance with the ATS guideline for the five-breath dosimeter method (KoKo Digidoser; Quantum Research).¹⁸ The same nebulizer was used on all three visits for a given patient. A total of three nebulizers were used in the study. Methacholine dilutions of 0.0625, 0.25, 1, 4, 16, and 64 mg/ml were prepared by the Investigational Drug Service of Shands Hospital Pharmacy at the University of Florida by using an aseptic technique, from 100-mg vials of FDA-approved methacholine chloride powder (Provocholine; Methapharm, Brantford, Canada). Each concentration contained a final volume of 2 ml dispensed in unit-dose syringes and was stored frozen for up to 5 months.¹⁹ The solutions were allowed to warm to room temperature before use, and any unused syringes were discarded after the challenge.

Data Analysis

Bronchodilator response to each dose of formoterol was calculated as the percent change in FEV_1 from the study day baseline value to 1 hour after administration, before the methacholine challenge began. The 1-hour postdose PC_{20} was interpolated by the KoKo software program from the log-linear slope of the last two concentrations and percentage decrease in FEV_1 .

Statistical Analysis

No formal sample size calculation was performed. However, based on previous experience,²⁰ it was judged that completion by 10 patients would be sufficient to meet the study objectives.

The regression method of Shuster was used to compare the natural logarithm of postdose PC_{20} values.²¹ This method takes the period 2 less period 1 difference (irrespective of treatment assignment) and compares the two treatment orderings. This effect size estimate is superior to the 1-sample t test in that it is unbiased when the actual sizes assigned to the orderings differ, is more efficient, and adjusts for carryover effects. The dependent primary variable was the difference in the change in natural log PC_{20} (period 2 less period 1). The FEV_1 was compared in the same way, except logs were not used. A p value less than 0.05 (2-sided) was considered to indicate a statistically significant difference.

Results

Between April and November 2008, 37 patients were enrolled in the study (i.e., they signed the informed consent form), but 25 were excluded during the screening visit. In two patients (patient nos. 13 and 31), the FEV₁ decreased only 17% during the screening visit. Therefore, the PC₂₀ was extrapolated.²² Since the screening PC₂₀ was greater than 4 mg/ml in those two patients (4.7 mg/ml each) and did not strictly meet the inclusion criteria of 4 mg/ml or less, the University of Florida IRB granted an exception to include them in the study. Thus, 12 patients were randomized. Of these patients, in two who received the 12-μg dose of formoterol on the first randomization study day, the FEV₁ did not decrease sufficiently to allow determination of PC₂₀, so both of these patients were withdrawn since it was unlikely that a PC₂₀ could be measured after the higher dose. In four patients, PC₂₀ could not be measured after the 24-μg dose, so a censored PC₂₀ of 64 mg/ml was assigned. Thus, 10 patients (aged 21–58 yrs) completed the study and were included in the analysis (Figure 1).

At the time of study enrollment, all patients required asthma drugs: four used a combination product containing fluticasone and salmeterol, two used fluticasone alone, and one used budesonide DPI; all patients used an albuterol MDI as needed (Table 1). The mean ± SD baseline FEV₁ was 2.6 ± 0.8 L (79.5 ± 6.8% predicted), and the baseline geometric mean PC₂₀ was 1.1 mg/ml (95% confidence interval [CI] 0.56–2.2 mg/ml; Table 1).

Bronchodilator Response

Seven patients demonstrated an increase in FEV₁ of 12% or greater after 12 μg of formoterol, and five patients demonstrated this level of response after 24 μg (Table 2). There was no significant dose-response relationship; the mean ± SD increase in FEV₁ was 14 ± 5% after 12 μg and 14 ± 8% after 24 μg (Figure 2).

Methacholine Response

The PC₂₀ was increased from the screening value in nine patients after 12 μg and in all patients after 24 μg (Table 3). There was a significant dose-response relationship; the geometric mean was 7 mg/ml (95% CI 2–22 mg/ml) after 12 μg and 16 mg/ml (95% CI 5–45 mg/ml) after 24 μg (p<0.001; Figure 3). The PC₂₀ ratio after 24 μg compared with after 12 μg was increased in all but one patient (patient no. 29), with a geometric mean of 2.2-fold (95% CI 1.6–3.0-fold; Table 3). When compared with the screening values, the PC₂₀ increased 6-fold (95% CI 2–16-fold) after 12 μg and 14-fold (95% CI 6–33-fold) after 24 μg (Table 3). Only one patient (patient no. 36) did not demonstrate an increase in PC₂₀ from the screening visit after 12 μg, but this patient did demonstrate a 3.5-fold increase from screening after 24 μg.

None of the patients required prednisone, an emergency department visit, or hospitalization for asthma during the study, and no adverse events were reported.

Discussion

The results of this study demonstrated a highly significant dose-response relationship for formoterol-induced inhibition of methacholine challenge. The geometric mean PC₂₀ increased an average of 2.2-fold between 12 and 24 μg (p<0.001), with only 10 patients completing the study. This indicates that methacholine challenge methods that have been used successfully to assess equivalence of short-acting β₂-adrenergic receptor agonist (SABA) formulations¹² can be applied to evaluate bioequivalence of LABA formulations.

The statistical power of a bioassay is a function of the ratio of within-patient variability (s), estimated from the root mean squared error of the analysis of variance of log₁₀ PC₂₀, and

slope of the dose-response curve (b), estimated by the difference in $\log_{10}$ PC₂₀ divided by the difference in $\log_{10}$ formoterol dose.²³ The lower the s:b ratio, the greater the statistical power and the steeper the dose-response curve relative to the response variability. In our study, the s:b ratio was 0.116. This value is considerably lower than in previous studies of albuterol where s:b ratios as high as 1.0 or more have been observed (see Figure 2A in reference 12).

With use of the slope and within-patient variability in our study, generation of simulated data sets suggests that a sample size of 28 patients would be required to determine a relative potency of 0.95 of test and reference products with a 90% CI of 0.80–1.25 (i.e., FDA requirement for bioequivalence³). Several measures were taken to decrease the within-patient variability in PC₂₀ in our study. These included selecting patients with high reactivity to methacholine who could perform reliable and reproducible spirometry, stabilizing the baseline FEV₁ before administration of saline, excluding patients with greater than 10% decrease in FEV₁ after administration of saline, having the methacholine dilutions prepared by the investigational pharmacy service and freezing the samples until use, and selecting the highest FEV₁ after each methacholine concentration rather than the lowest. In addition, the respiratory therapist who performed the challenges was certified in performing spirometry and had extensive training and experience in performing challenges. Without attention to such details, within-patient variability will be much larger and, thus, a larger sample will be required. For example, if the within-patient variability is increased 3-fold, simulations suggest a sample of 75 patients would be required for the same slope, effect size, and CI as in this study. However, the above calculations are based on the Finney straight line analysis with potency on the dose axis, not the response axis.²³ This assumes a linear dose-response curve. Hence, lack of linearity would limit the reliability of this power computation. Nevertheless, the data from this study are linear when the screening visit PC₂₀ is taken as a zero dose.

In contrast, the dose-response relationship for bronchodilatation was not significant. After both doses, the mean increase in FEV₁ was 14%. This is consistent with previous studies indicating that bronchodilatation often does not distinguish between doses in daytime studies of patients with stable asthma.^{9, 10–13, 24} Patients were included in this study only if their FEV₁ was at least 70% predicted at the screening visit. Thus, on average, the response was on the upper portion of the dose-response curve. That is, the dose producing 50% of the maximum effect (ED₅₀) for bronchodilatation would be expected to be much higher than the ED₅₀ for methacholine.²⁵

No significant correlation was found between FEV₁ or PC₂₀ measured during the screening visit and postdose PC₂₀ ratio for 24 µg:12 µg, suggesting that these screening tests may not adequately predict response. Of interest, visual inspection of Figure 3 suggests that there may have been two distinct groups of responders: those who had a large increase in PC₂₀ with both doses (upper group) and those who had a much smaller response (lower group). One possible explanation for the different groups is chance alone, whereas another is downregulation of β -receptors (e.g., tachyphylaxis) from regular use of LABAs or frequent use of as-needed albuterol.²⁶ Unfortunately, we did not capture the frequency of use of as-needed albuterol. We did, however, identify four patients at screening who used a combination inhaled corticosteroid-LABA product before the study. In spite of observing the ATS recommended washout period of 36 hours for the LABA,¹⁸ three of these four patients had a postdose PC₂₀ ratio for 24 µg:12 µg of less than 2.0, indicating a blunted dose-response relationship. Tachyphylaxis has been reported after discontinuing a LABA,²⁷ but it is unclear how long it lasts and, therefore, how long the washout period should be.

Although our results indicate that the methods in this study can be used, without modification, to assess equivalence of drug delivery to the lung by different inhaled formoterol formulations, we did learn several lessons that could prove useful in the design of subsequent SABA and LABA bioequivalence studies. First, we propose that the maximum methacholine concentration should be increased to 128 mg/ml to be able to determine PC₂₀ in patients with a greater bronchoprotective effect than 64 mg/ml. This would minimize “top-censoring” of the data. Concentrations as high as 256 mg/ml have been used safely.²⁸ Second, to reduce possible tachyphylaxis of bronchoprotective effects of β_2 -agonists, LABAs should be withheld for a longer period than recommended by ATS (e.g., 3 wks) and patients who require SABAs more frequently than 3 times/week should be excluded. Third, an additional screening day should be added to perform methacholine challenge after the highest dose of reference product that will be used in the study to exclude patients who do not demonstrate a bronchoprotective effect (i.e., a PC₂₀ 4-fold higher than after the screening PC₂₀ measured in absence of β_2 -agonist effect). Such additional screening also will identify the occasional patient in whom a PC₂₀ cannot be determined after the higher reference dose at 128 mg/ml of methacholine.

Increasing the screening PC₂₀ from 4 mg/ml or less to 8 mg/ml or less is likely to decrease screening failures without adversely affecting the outcome. In a subsequent study where this was done, 3 of 21 randomized patients with a PC₂₀ greater than 4 mg/ml but less than or equal to 8 mg/ml would have been unnecessarily excluded (unpublished data, L. Hendeles, 2011). Using the tidal breathing method is another method of decreasing the number of screening failures. One group of investigators found that 25% of patients would have a PC₂₀ greater than 8.0 mg/ml by dosimeter when their PC₂₀ was less than 8.0 mg/ml by the tidal breathing method.²⁹ In addition, tachyphylaxis from frequent use of an SABA may be circumvented by substituting ipratropium HFA MDI for as-needed bronchodilatation, but the onset of action is slower³⁰ and it does not block exercise-induced bronchospasm.³¹ Tiotropium could be substituted for LABAs, but this drug has an effect on methacholine PC₂₀ for 72 hours,³² which makes its use less practical than simply excluding patients who require combination therapy with an inhaled corticosteroid and LABA.

These recommendations modestly increase the complexity of the study and will increase the number of screening failures, but since this is a clinical bioassay, the extra efforts are likely to enrich the study sample with patients most likely to yield a steep dose-response curve and to decrease within-patient variability.

An additional modification may be needed for future LABA bioequivalence studies. It is likely that FDA and other regulatory agencies will require a second methacholine challenge, 8–12 hours after dosing, to confirm that the duration of action of the test product is similar to the reference product. This second challenge should be added to subsequent studies. In fact, in one recent study of an experimental once-daily LABA, the authors performed repeat challenges at 4, 8, 24, and 32 hours after each dose.²⁸

Conclusion

The results of this study indicate that methacholine bronchoprovocation can successfully be used to assess bioequivalence of pulmonary delivery of formoterol by different DPIs or MDI formulations. Incorporating recommendations from lessons learned from this study may further increase the statistical power of this method. It is likely that this methodology will be useful for other LABAs, such as salmeterol, but sample sizes will be different if either within-patient variability or the slope of the dose-response curve differs substantially from those observed in this study.

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Figure 1. Schematic of the patient selection process. FEV₁ = forced expiratory volume in 1 second.

Figure 2.

Bronchodilator response measured as percent increase in forced expiratory volume in 1 second (FEV₁) 1 hour after administration of single doses of formoterol 12 µg and 24 µg, on separate days, at the same time of day, in the 10 patients who completed the study. No significant dose-response relationship was noted; the mean $\pm$ SD increase in FEV₁ was $14 \pm 5\%$ after the 12-µg dose and $14 \pm 8\%$ after the 24-µg dose ($p > 0.05$).

Figure 3.

Provocational concentration of methacholine required to decrease the forced expiratory volume in 1 second by 20% (PC₂₀) measured 1 hour after administration of single doses of formoterol 12 µg and 24 µg, on separate days, at the same time of day, in 10 patients who completed the study. A significant dose-response relationship was noted; the geometric mean (95% confidence interval) was 7 mg/ml (2–22 mg/ml) after the 12-µg dose and 16 mg/ml (5–45 mg/ml) after the 24-µg dose ($p < 0.001$).

Table 1Demographics, FEV₁, and Methacholine PC₂₀ During the Screening Visit for the 10 Study Patients

Patient No.	Sex/Age (yrs)	FEV ₁ , L (% predicted)	PC ₂₀ (mg/ml)	Previous Therapy
3	M/29	4.07 (89)	0.61	Fluticasone-salmeterol, albuterol p.r.n.
7	F/26	2.40 (82)	0.62	Albuterol p.r.n.
12	F/52	1.85 (75)	0.42	Fluticasone, albuterol p.r.n.
13	M/25	3.17 (78)	4.70	Fluticasone-salmeterol, albuterol p.r.n.
20	F/29	2.79 (90)	0.61	Albuterol p.r.n.
21	F/54	1.91 (78)	1.38	Fluticasone-salmeterol, albuterol p.r.n.
29	F/49	1.89 (69)	0.34	Fluticasone-salmeterol, albuterol p.r.n.
30	M/25	3.35 (84)	2.27	Budesonide, albuterol p.r.n.
31	F/58	1.71 (78)	4.70	Fluticasone, albuterol p.r.n.
36	M/21	3.08 (72)	1.24	Albuterol p.r.n.
Mean ± SD	36.8 ± 14.5	2.6 ± 0.8 (79.5 ± 6.8)		
Geometric mean			1.1 (95% CI 0.56–2.2)	

FEV₁ = forced expiratory volume in 1 second; PC₂₀ = provocative concentration of methacholine required to decrease FEV₁ by 20%; CI = confidence interval.

Table 2
Bronchodilator Response After the 12- and 24- μ g Doses in the 10 Study Patients

Patient No.	12- μ g Dose			24- μ g Dose		
	Baseline FEV ₁ , L (%) predicted)	Postdose FEV ₁ , L (%) predicted)	Postdose % Increase in FEV ₁	Baseline FEV ₁ , L (%) predicted)	Postdose FEV ₁ , L (%) predicted)	Postdose % Increase in FEV ₁
3	3.97 (87)	4.74 (104)	19	4.22 (92)	4.71 (103)	12
7	2.73 (93)	3.13 (106)	15	2.69 (90)	3.25 (109)	21
12	1.72 (70)	1.94 (78)	13	1.91 (77)	2.10 (85)	10
13	3.23 (79)	3.59 (88)	11	3.17 (78)	3.58 (88)	13
20	2.51 (80)	2.98 (95)	19	2.74 (88)	3.05 (98)	11
21	1.90 (78)	2.09 (86)	10	1.89 (76)	1.94 (80)	3
29	2.08 (76)	2.13 (78)	2	2.06 (75)	2.25 (82)	9
30	3.28 (82)	3.74 (93)	14	3.40 (85)	3.71 (93)	9
31	1.55 (71)	1.88 (86)	21	1.55 (71)	1.83 (84)	18
36	2.58 (60)	2.97 (69)	15	2.75 (64)	3.66 (85)	33
Mean $\pm$ SD						
	2.55 $\pm$ 0.77 (78 $\pm$ 9)	2.92 $\pm$ 0.93 (88 $\pm$ 12)	14 $\pm$ 5	2.64 $\pm$ 0.82 (80 $\pm$ 9)	3.01 $\pm$ 0.95 (91 $\pm$ 10)	14 $\pm$ 8 ^a

FEV₁ = forced expiratory volume in 1 second.

^a p>0.05 compared with the 12- μ g dose.

Table 3

Response to Methacholine Challenge 1 Hour After Dosing in the 10 Study Patients

Patient No.	PC ₂₀ (mg/ml)		Fold Change in PC ₂₀ from Screening			
	12 µg	24 µg	PC ₂₀ Ratio, 24 µg:12 µg	12 µg	24 µg	
3	1.29	2.17	1.68	2.10	3.60	
7	27.75	64.00	2.30	44.80	103.00	
12	2.03	7.71	3.80	4.80	18.30	
13	43.30	64.00	1.48	9.21	13.62	
20	34.80	52.50	1.50	57.10	86.10	
21	3.19	8.40	2.60	2.30	6.10	
29	1.47	1.57	1.07	4.30	4.60	
30	30.75	64.00	2.08	13.50	28.20	
31	18.00	64.00	3.60	3.80	13.62	
36	1.24	4.30	3.46	1.00	3.50	
Geometric Mean (95% confidence interval)						
	7 (2-22)	16 (5-45) ^a	2.2 (1.6-3.0)	6 (2-16)	14 (6-33)	

PC₂₀ = provocative concentration of methacholine required to decrease forced expiratory volume in 1 second by 20%.^a p 0.0001.