

The utility of methacholine airway responsiveness measurements in evaluating anti-asthma drugs

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Background: Measurements of airway responsiveness are frequently used to evaluate anti-asthma drugs.

Objective: This study investigated the utility of methacholine airway responsiveness measurements in evaluating anti-asthma medications, both in terms of a bronchoprotective effect and the ability to attenuate allergen-induced methacholine airway hyperresponsiveness.

Methods: Methacholine airway responsiveness was measured as PC₂₀ on two occasions (separated by 35 ± 17 days, mean ± SD) in 40 subjects with mild, stable asthma. Additional subjects had PC₂₀ measurements made before and after administration of either inhaled salbutamol (200 µg) (*n* = 20) or allergen inhalation challenge (*n* = 31).

Results: The reproducibility of the methacholine PC₂₀ with this method was high (intraclass correlation coefficient = 0.94). The average shift in PC₂₀ after salbutamol was 4.11 doubling concentrations (SD = 1.08). On the basis of these results, a sample size of 12 subjects would be required to demonstrate a 1 doubling concentration difference in the bronchoprotective effect of two drugs with a 90% power. The average shift in PC₂₀ after allergen was 1.29 doubling concentrations. On the basis of these results and an estimated SD of 0.96, a sample size of 24 subjects would be required to demonstrate that a drug is effective in attenuating 50% of allergen-induced airway hyperresponsiveness with a 90% power.

Conclusion: These results confirm the high reproducibility of methacholine PC₂₀ measurements in subjects with mild, stable asthma and demonstrate its utility in evaluating the effects of anti-asthma drugs. (*J Allergy Clin Immunol* 1998; 101:342-8.)

Key words: Airway responsiveness, asthma, reproducibility, bronchoprotection, allergen

Airway responsiveness is defined as the relative ease with which airways can be made to constrict when exposed to nonsensitizing chemical or physical stimuli such as methacholine, histamine, cold air, and exercise or hyperventilation.¹ Measurements of airway responsiveness to inhaled chemical stimuli were first made in

Abbreviation used

ICC: Intraclass correlation coefficient

1941.² Since that time, the techniques have been standardized, with two similar techniques currently receiving wide use both as clinical and research tools.^{3, 4} The first of these⁴ is based on delivering doubling concentrations of methacholine or histamine over a set period of time (usually 2 minutes) until a given level of bronchoconstriction (often a 20% fall in FEV₁) is achieved. Thus the provocative concentration producing a 20% fall in FEV₁ is termed the PC₂₀. The second technique³ is based on delivering doubling doses (the actual amount is controlled rather than delivering a set concentration for a set period of time, which will not give the same dose between subjects, between which minute ventilation may differ) of histamine or methacholine until a given level of bronchoconstriction is achieved. The provocative dose responsible for the 20% fall is thus termed PD₂₀. The widespread use of these techniques is based in part on reports of their high reproducibility.^{5, 6} Generally, repeated measurements on an individual have fallen within 1.5 to 1.6 doubling doses/concentrations.^{5, 6}

The extent to which airway hyperresponsiveness in asthmatic subjects can be reversed by bronchodilator drugs is currently being used to compare the clinical effectiveness of different bronchodilator agents.⁷⁻⁹ Sample sizes in these studies range from 8 to 12. No studies have yet been performed to determine the magnitude of treatment differences that could be detected with these sample sizes.

Airway responsiveness increases in some asthmatic subjects during seasonal allergen exposure¹⁰ or after allergen inhalation challenge.¹¹ Furthermore, allergen-induced airway hyperresponsiveness has been closely linked to the development of airway inflammation.^{12, 13} On the basis of this relationship, the ability to block allergen-induced airway hyperresponsiveness has been evaluated in the investigation of several established or investigational anti-asthma drugs.^{7, 14-22} Sample size in studies of this nature typically range between 8 and 20. We have previously shown that these sample sizes are sufficient to demonstrate drug effects on the allergen-induced early and late bronchoconstrictor responses.²³ Whether this is also the case for measurements of

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TABLE I. Subject demographics

Protocol	n	Sex (F/M)	Age (yrs)	Height (cm)	Weight (kg)	FEV ₁ (ml)	FEV ₁ (% predicted)
PC ₂₀ reproducibility	40	9/31	25.5 (7.17)	178 (9.4)	75.5 (11.6)	378 (62)	86.5 (11.0)
Salbutamol protection	20	13/7	23.6 (3.7)	172 (10.3)	67.8 (12.5)	340 (70)	85.6 (7.7)
Allergen challenge	31	9/22	25.9 (7.16)	178 (10.3)	72.4 (9.73)	374 (62)	86.5 (11.1)

Values are mean (SD).

allergen-induced airway hyperresponsiveness has not been shown.

In this study we investigated the reproducibility of methacholine PC₂₀ after both bronchodilator treatment and allergen challenge and used these measurements, along with published values, to predict sample sizes necessary when studying anti-asthma drugs in these types of studies.

METHODS

Subjects

Sixty subjects with a history of mild, stable asthma were studied. All subjects used only inhaled β_2 -agonists less than twice daily to treat their asthma, and no subject had used any other anti-asthma medication for more than 1 year. There was no current exposure to allergens to which subjects were sensitized (with the exception of the house dust mite), and there had been no exacerbations of asthma or respiratory tract infections for at least 4 weeks. FEV₁ was greater than 75% of predicted normal value²⁴ in all subjects on all study days. At each visit subjects arrived in the laboratory at the same time in the morning after a period free from inhaled β_2 -agonists (≥ 8 hours), caffeine (≥ 12 hours), and any nonsteroidal antiinflammatory medication (≥ 48 hours). The study was approved by the hospital ethics committees, and each subject gave written informed consent before beginning the study.

Study design

PC₂₀ reproducibility. Forty subjects (Table I) were studied on two occasions separated by a period of 14 to 60 days (mean $\pm$ SD = 35 ± 17 days). All subjects had asthma according to accepted diagnostic guidelines²⁵ or had demonstrated a late asthmatic response (greater than 15% fall in FEV₁) after allergen inhalation challenge. At each visit, airway responsiveness to inhaled methacholine (PC₂₀) was measured according to the technique described below. At the second visit, both the subjects and the experimenter were blind to the PC₂₀ value measured at the first visit.

Change in PC₂₀ after administration of inhaled β_2 -agonist. Twenty subjects (Table I) were studied during a single laboratory visit. Initially, a methacholine challenge was administered to all subjects to determine baseline PC₂₀. Subjects were then given a 4-hour recovery period and then treated with two inhalations from a 100 μ g/puff salbutamol metered-dose inhaler (Ventolin; Glaxo, Mississauga, Canada), provided FEV₁ had returned to within 10% of baseline. A second methacholine challenge was administered 30 minutes after this inhaled β_2 -agonist treatment.

Change in PC₂₀ after allergen inhalation challenge. Thirty-one subjects (Table I) with allergen-induced asthma were subjected

to methacholine challenges before and after allergen-inhalation challenges. All of these were also included in the PC₂₀ reproducibility section of this article. All subjects were known to develop early ($\geq 15\%$ fall in FEV₁ in first 2 hours) and late ($\geq 15\%$ fall in FEV₁ between 3 and 7 hours) asthmatic responses on the basis of a screening allergen challenge performed at least 2 weeks before the study visit. The baseline methacholine challenge was administered to the subjects in the morning, 1 day before the allergen inhalation challenge. The postallergen methacholine challenge was administered in the morning 24 hours after the allergen inhalation challenge.

Procedures

Methacholine challenge. Methacholine inhalation challenges were performed as described previously.^{4, 5, 26} Aerosols of the test solutions were generated by a Wright nebulizer (output of 0.13 ml/min). The nose was clipped, and the subject breathed through a mouthpiece with resting tidal volumes for 2 minutes. After an initial control solution of isotonic saline, increasing doubling concentrations of methacholine from 0.03125 mg/ml to 256 mg/ml were inhaled for 2 minutes at intervals of 5 minutes. The response was measured by FEV₁ at 30 seconds and 90 seconds and 3 minutes and each minute thereafter if still dropping. The lowest value after each inhalation was recorded. Baseline FEV₁ was taken as being the lowest FEV₁ after saline inhalation. The methacholine inhalations were stopped when the FEV₁ had fallen by more than 20% of the baseline value, and the response was expressed as the PC₂₀. All spirometric measurements were made with a Collins 9-L water spirometer (Warren E. Collins, Braintree, Mass.).

Allergen inhalation challenge. Allergen inhalation tests were performed as previously described¹¹ by using a Wright nebulizer operated by air at 50 psi and at a flow rate to give an output of 0.13 ml/min. The nose was clipped, and aerosols were inhaled through a mouth piece during tidal breathing.

All subjects inhaled extracts of either ragweed, cat dander, or house dust mite antigen, according to their own allergies (Miles/Hollister-Stier, Mississauga, Ontario, Canada). Extracts were stored at -70°C and diluted for skin tests and allergen inhalation on the day they were to be used. The starting concentration of extract for the first allergen challenge was determined by using the results from skin prick test end-point titration with the same extract and the methacholine PC₂₀ according to the formula published by Cockcroft et al.²⁷

Each concentration of allergen was inhaled for 2 minutes, and the FEV₁ was measured 10 minutes after the inhalation. If the FEV₁ had fallen by 10% or more, the measurement was repeated at 10 minute intervals until no further fall was observed. The dose of inhaled allergen was increased in a doubling manner until the FEV₁ had decreased by at least 15%. After inhalation of the highest allergen concentration, the

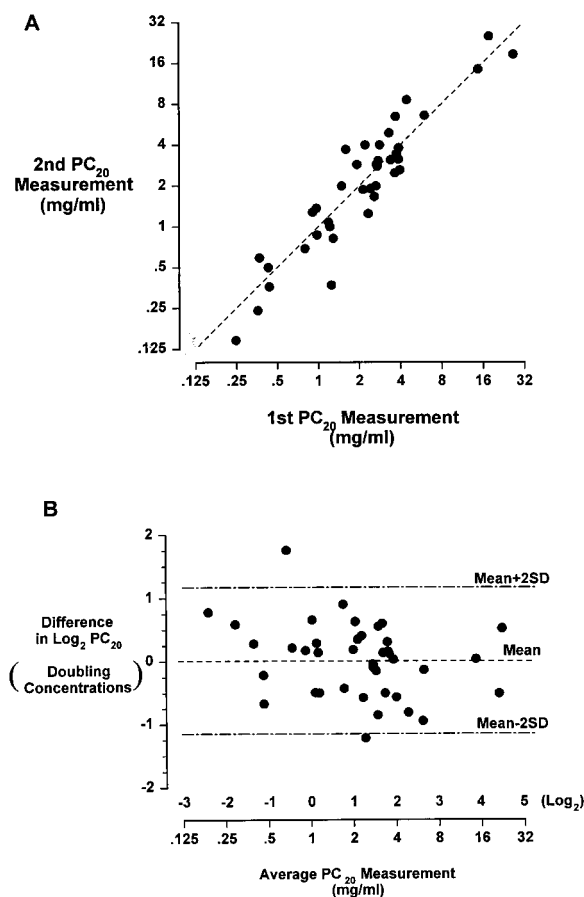


FIG. 1. Illustration of reproducibility of measurements of methacholine PC_{20} . **A**, Dashed line represents line of identity ($ICC = 0.94$). **B**, Difference between two measurements is plotted against average of two measurements.²⁹ In all but two cases, repeated measurements differed by less than 1 doubling concentration.

FEV_1 was measured at 10, 20, 30, 45, 60, 90, and 120 minutes and then at hourly intervals until 7 hours after the inhalation.

Analysis

All PC_{20} values were normalized by $\log_2$ transformation before statistical analysis. This form of presentation is believed to be more convenient than using $\log_{10}$ because the units are equivalent to methacholine doubling concentrations. Summary results are presented as geometric mean (logarithmic) and percent standard error (%SEM; equal to anti-logged standard error of the mean logged PC_{20}). Changes in PC_{20} after inhaled bronchodilator or allergen were expressed as the mean and standard deviation of the $\log_2$ values.

An index of the reproducibility of PC_{20} measurements was obtained by measuring the intraclass correlation coefficient (ICC) of the repeated measurements²⁸ and the coefficient of repeatability as suggested by Bland and Altman.²⁹ The ICC calculation results in a coefficient ranging from 1 (perfect agreement, not simply correlation) through 0 (no agreement) to -1 (perfect disagreement).

Sample size predictions were made on the basis of measured

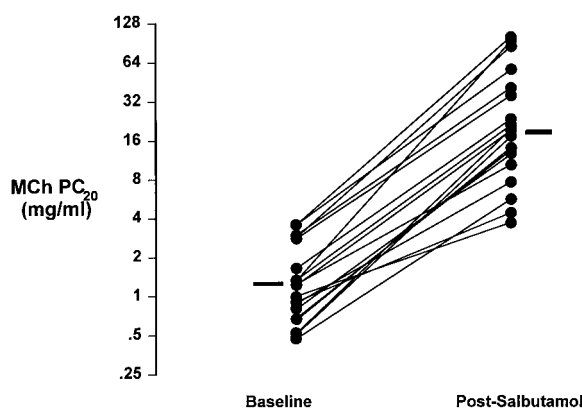


FIG. 2. Methacholine PC_{20} before and 30 minutes after 200 μ g inhaled salbutamol in 20 asthmatic subjects. *MCh*, Methacholine.

variances (SD^2) and anticipated changes in mean values by using the following equation:

$$s = \left[\frac{(Z_{1-\alpha/2} + Z_{1-\beta})\sigma}{\mu_1 - \mu_2} \right]^2$$

where s is the predicted sample size, Z is the Z score, σ is the standard deviation and $\mu_1 - \mu_2$ is the expected difference in means.³⁰ This equation is appropriate for two-tailed tests. In the case of the required sample size to demonstrate different protective effects of drugs against methacholine-induced bronchoconstriction, the variance in the subtraction of postbronchodilator PC_{20} (logged) minus baseline PC_{20} (logged) was used. In the case of the required sample size to demonstrate significant allergen-induced airway hyperresponsiveness, the standard deviation in the difference between the preallergen and postallergen PC_{20} was used. In the case of the effectiveness of anti-asthma drugs to block allergen-induced airway hyperresponsiveness, published standard deviations in the difference between treated and untreated PC_{20} shifts (i.e., preallergen minus postallergen) were used. Thus all standard deviations are based on within-subject variance, which is appropriate only for repeated measures designs (either crossover or prepost measurements). Clinical significance was set at $p < 0.05$.

RESULTS

PC_{20} reproducibility

The repeated methacholine PC_{20} results are plotted in Fig. 1, *A* and *B*. The mean PC_{20} for the first and second challenges were 2.12 mg/ml (%SEM = 1.17) and 2.08 mg/ml (%SEM = 1.19), respectively ($p > 0.05$). The ICC of these two measurements was 0.94, indicating that 94% of the total variability in the measurement was between subjects rather than within subject. The high reproducibility of the PC_{20} measurement is further illustrated in Fig. 1, *B*, which shows that in 38 subjects (95%) the difference between the two measurements is less than 1 doubling concentration (repeatability coefficient of 2 SD = 1.16 doubling concentrations). Fig. 1, *B* also illustrates that the reproducibility of the PC_{20} measurement remains constant over the range of measurements made in this study.

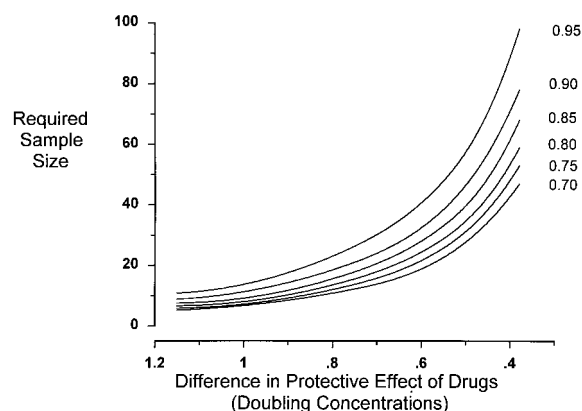


FIG. 3. Sample sizes required to demonstrate differences in bronchoprotective effects of two treatments. If differences of less than 1 doubling concentration are considered clinically nonsignificant, then sample sizes of 12 can be used with 90% power. Sample sizes were calculated assuming repeated measures design and SD of 1.08 doubling concentrations in difference of protective effect from each treatment.

Change in PC₂₀ after administration of inhaled β_2 -agonist

Methacholine PC₂₀ before and after 200 μ g salbutamol was 1.13 mg/ml (%SEM = 1.22) and 19.54 mg/ml (%SEM = 1.28), respectively ($p < 0.0001$) (Fig. 2). The mean pre- versus postsalbutamol shift in the PC₂₀ was 4.11 doubling concentrations (SD = 1.08). This SD value was used to calculate the sample sizes that would be required to demonstrate significant differences between drugs in their ability to protect against methacholine-induced bronchoconstriction (Fig. 3). Thus a sample size of 12 subjects could demonstrate a difference in protective effect of 1 doubling concentration with a power of 90%. If the difference between the drugs was only half a doubling concentration, the sample size would need to be 49 to detect a difference (90% power).

Change in PC₂₀ after allergen inhalation challenge

All subjects developed a late asthmatic response after allergen inhalation as demonstrated by a greater than 15% fall in FEV₁ between 3 and 7 hours after challenge (mean $\pm$ SD = 22.9% $\pm$ 7.41%). Methacholine PC₂₀ before and 24 hours after allergen inhalation challenge was 1.69 mg/ml (%SEM = 1.22) and 0.69 mg/ml (%SEM = 1.22), respectively ($p < 0.0001$) (Fig. 4). The mean pre- versus postallergen shift in the PC₂₀ was 1.29 doubling concentrations (SD = 0.92). These values were used to calculate the minimum sample size that would be required to demonstrate allergen-induced airway hyperresponsiveness with different levels of power (Table II). The standard deviation in the difference between treated and untreated shifts in PC₂₀ was estimated with data from 118 subjects in 10 published studies (Table III). Using this estimated standard deviation of 0.959 doubling concentrations and our measurement of an average untreated shift of 1.29 doubling concentrations, we

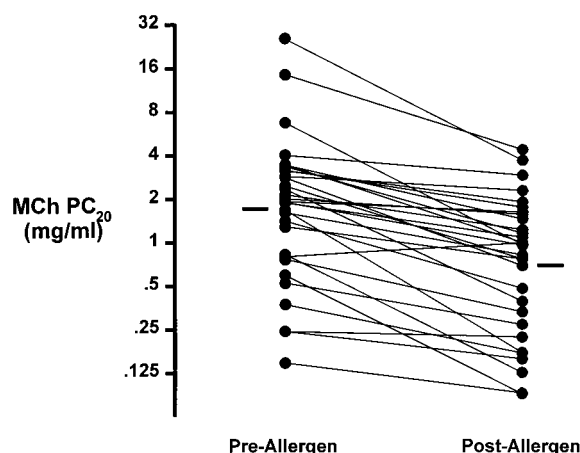


FIG. 4. Methacholine PC₂₀ before and 24 hours after allergen inhalation challenge in 31 subjects known to have allergen-induced late asthmatic responses.

TABLE II. Minimum sample size required to demonstrate significant allergen-induced airway hyperresponsiveness with different levels of power

Power	Two-tailed	One-tailed
0.95	7	6
0.9	6	5
0.85	5	4
0.8	4	4
0.75	4	3
0.7	4	3

Sample sizes were calculated assuming a repeated measures design, with an average shift in PC₂₀ of 1.29 doubling concentrations and a standard deviation of 0.92 doubling concentrations.

calculated sample sizes required to demonstrate different degrees of protection against allergen-induced airway hyperresponsiveness (Fig. 5). Thus a sample size of 24 would be required to demonstrate a 50% attenuation of allergen-induced airway hyperresponsiveness (a decrease in the shift from 1.29 to 0.645 doubling concentrations) with a power of 90%. This data could also be used to estimate the sample size required to demonstrate significant differences between drugs in their ability to protect against allergen-induced airway hyperresponsiveness. Twenty-four subjects would be required to demonstrate differences of 50% in the degree of protection (i.e., one drug blocking 75% of the response and the other blocking only 25%) with a power of 90%. With smaller differences between the drugs, greater numbers would be required, with 146 subjects being required to show a difference in protection of 20% with a power of 90%.

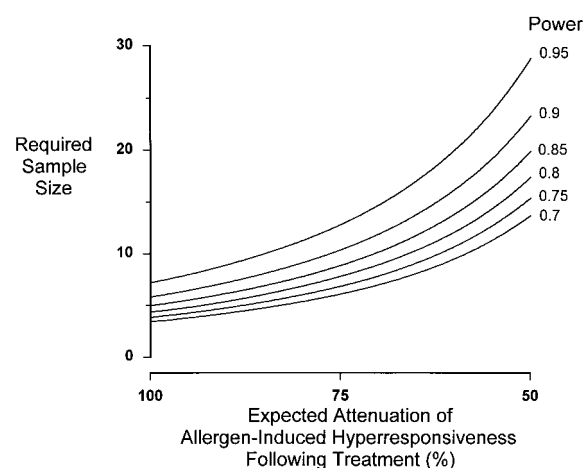
DISCUSSION

In this study we have confirmed that the measurement of methacholine airway responsiveness with the tidal

TABLE III. Published standard deviations of the difference between treated and untreated allergen-induced airway hyperresponsiveness

Authors	Treatment	Sample size	Standard deviation
Cockcroft et al. ³¹	Cromolyn sodium	9	1.217
Cockcroft et al. ³¹	Beclamethasone	9	0.892
Cockcroft et al. ¹⁵	Salbutamol	10	0.742
Crimi et al. ³²	Nedocromil	12	0.943
Freitag et al. ¹⁴	WEB2086	8	1.011
Hamilton et al. ³³	BAYx1005	7	1.310
Hendeles et al. ³⁴	Theophylline	14	0.755
Hendeles et al. ³⁴	Cromolyn sodium	14	0.908
Kirby et al. ²²	Indomethacin	7	0.366
Pavord et al. ¹⁷	PGE ₂	8	1.363
Manning et al. ³⁵	CGS13080	12	0.977
Taylor et al. ²⁰	ICI 204.219	8	0.823
Pooled estimate		118	0.959

Standard deviation in doubling concentrations of the difference between treated and untreated allergen-induced PC₂₀ shifts.

**FIG. 5.** Sample sizes required to demonstrate statistically significant attenuation of allergen-induced shifts in methacholine PC₂₀. Attenuation of 50% of shift can be demonstrated by less than 20 subjects with 90% power. Sample sizes were calculated assuming repeated measures design with average untreated shift in PC₂₀ of 1.29 doubling concentrations after allergen and SD of 0.96 doubling concentrations between treated and untreated responses.

breathing method is highly reproducible in a population of subjects with mild, stable asthma. More importantly, we have investigated the utility of this test to make comparisons of airway responsiveness. We have shown that sample sizes of less than 20 may be used to compare drugs in their ability to protect against methacholine-induced bronchoconstriction and that 25 to 30 subjects should be used to investigate the protective effects of drugs against allergen-induced shifts in methacholine responsiveness in crossover studies.

Repeated measurements of methacholine PC₂₀ resulted in an ICC of 0.94. Thus PC₂₀ measurements in repeated challenges over 2 to 8 weeks differed by less than 1 doubling concentration in 95% of cases. This finding is in close agreement with previous studies of this nature.^{5,6} Furthermore, we found no relationship be-

tween the size of the interval between the repeated tests and the absolute value of the difference in logged PC₂₀ values ($r^2 = 0.03$), suggesting that the reproducibility of the test in this population is quite stable over periods up to 8 weeks. This information is valuable in that it supports the use of single PC₂₀ measurements for clinical or research purposes. It should be noted, however, that the subjects in this study had mild, stable asthma requiring infrequent inhaled β_2 -agonists as their only treatment. It seems unlikely that subjects with unstable asthma would exhibit the same high degree of reproducibility over the time period studied here.

Although we have observed a high measurement reproducibility for the methacholine PC₂₀, we argue that this is not sufficient to infer a high degree of utility in all research applications. In studies where change in a measurement is the outcome variable, it is the variability in the change, as well as the magnitude of the change, that determine the power of the study. For this reason we have measured the variance and magnitude of the change in the PC₂₀ in two types of research studies and used these measurements to predict sample sizes required for future studies.

The first of these study types is concerned with bronchoprotection against methacholine as a means of detecting differences between two bronchodilator treatments. Studies of this nature are important for comparing different bronchodilator agents and delivery systems. Our findings indicate that the between-subject variance and the magnitude of the shift in methacholine PC₂₀ are such that a 1 doubling concentration difference in the degree of bronchoprotection could be detected by 12 subjects with a power of 90% in crossover studies. A difference of 1 doubling concentration is approximately 25% of the protection achieved with salbutamol in this study. It is important to note, however, that differences of less than this will only be detected with larger sample sizes and that differences of less than 0.5 doubling concentrations will require sample sizes of approximately 50 or more subjects or will have low power.

Therefore it is important when designing studies to determine the lowest difference in bronchoprotective effect that is deemed clinically significant and select a sample size accordingly. These guidelines can also be used to interpret published studies. For example, a recent publication has shown that there was no significant difference in the bronchoprotective effects of formoterol (12 μ g) and salmeterol (50 μ g).⁹ On the basis of the sample size of 12, the power to demonstrate any difference smaller than 0.5 doubling concentrations would have been approximately 40%. Thus interpretation of these findings depends on whether differences less than 0.5 doubling concentrations are considered clinically significant.

The second of these study types is concerned with evaluating the protective effect of treatment against allergen-induced airway hyperresponsiveness. Our findings indicate that the between-subject variance and the magnitude of the allergen-induced shift in methacholine PC₂₀ are such that sample sizes of 24 subjects are required to demonstrate that a drug can block 50% of the allergen-induced shift in methacholine PC₂₀ with a power of 90%. We have previously shown that eight subjects are sufficient to demonstrate that a drug can attenuate 50% of the late bronchoconstrictor response.²³ Thus greater numbers of subjects are required to document a given degree of protection against allergen-induced hyperresponsiveness than against allergen-induced bronchoconstriction. These findings should be kept in mind both when designing studies and also when interpreting published studies, in which sample sizes of eight or less are often used.^{7, 14, 17, 21, 22}

We have also used our data to calculate sample sizes required to compare two or more drugs in their effectiveness in attenuating allergen-induced airway hyperresponsiveness. Our findings indicate that if two treatments differed by 20% in the degree of attenuation of the PC₂₀ shift, then 146 subjects would be required to demonstrate statistical significance. Thus studies of this nature are only feasible if large sample sizes are available or when the difference between the protection afforded by the two treatments is relatively large (i.e., 50% or greater).

When estimating sample sizes, we have calculated the number of subjects required to complete each of the study types. This does not take into account subjects who fail to complete the study. If subject drop-out is expected then this should be included in sample size calculations. It should also be pointed out that the equation used to predict sample sizes is based on a Z (or normal) distribution, whereas analyses of small subject studies typically assume a T distribution. However, the errors in sample size predictions are small. It has been calculated that predicted sample sizes will be accurate to within two to three subjects when using this equation for small sample studies.³⁰ It may, however, be appropriate to add two or three subjects to the sample sizes suggested in our article. We have also assumed that studies will be subjected to two-tailed analysis. If, however, only one-

tailed analysis is required, then required sample sizes will be smaller. To calculate sample sizes for one-tailed analysis, our recommended sample sizes should be multiplied by a factor of 0.82. Finally, the sample sizes calculated here are based on measurements of within-subject standard deviation and are thus to be used in repeated measures studies, rather than parallel group studies.

In this study we have made measurements of methacholine airway responsiveness. Frequently in studies of the type addressed in this article, histamine is used to assess airway responsiveness.^{14, 15, 18, 21} Airway responsiveness measurements made with both methacholine and histamine are equally reproducible and correlate highly with each other. Although we have not formally demonstrated it, we would expect that the figures for predicting sample size in this article could be used for experiments with either histamine or methacholine.

In agreement with previous authors we have found that there is high reproducibility between repeated measures of methacholine airway responsiveness. More importantly, we have investigated the utility of this test by determining the sample sizes required for two important types of research study. Sample sizes of less than 20 may be used to compare bronchoprotective effects of different drugs, and 20 to 30 subjects are required to evaluate the protection afforded against allergen-induced airway hyperresponsiveness. Sample sizes must be selected according to the researcher's choice of clinical significance.

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