

Respiratory pathophysiologic responses

Reproducibility of allergen-induced early and late asthmatic responses

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Background: Constant-dose allergen inhalation challenges are frequently used to examine the effect of antiasthma drugs on the allergen-induced early and late asthmatic responses. The end-point measurements in such studies are the maximal early and late percent decreases in the forced expiratory volume in 1 second (FEV₁).

Objective: Our purpose was to observe the reproducibility and to determine the sample sizes required for such studies.

Methods: Twenty-eight subjects with allergen-induced early and late responses were studied with two constant-dose allergen challenges separated by 2 to 12 weeks. The early response was measured as the maximum percent decrease in FEV₁ during the first 2 hours and the late response as the maximum percent decrease in FEV₁ between 3 to 7 hours.

Results: The mean $\pm$ SEM early responses were 23.1% $\pm$ 1.0% and 24.7% $\pm$ 2.0%, whereas the mean late responses were 23.3% $\pm$ 2.0% and 24.5% $\pm$ 2.2%. Reproducibility of measurements were such that fewer than eight subjects are required, to show 50% attenuation of either the early or late response (with 90% power).

Conclusions: The method of constant-dose allergen challenge is a sensitive tool for detecting changes in early and late asthmatic responses after the use of antiasthma medication. (J ALLERGY CLIN IMMUNOL 1995;95:1191-5.)

Key words: Allergen inhalation challenge, early asthmatic response, late asthmatic response, reproducibility, sample size

Inhalation of a relevant allergen by sensitized subjects results in airway narrowing, which usually develops within 10 minutes of the inhalation, reaches a maximum within 30 minutes, and generally resolves within 1 to 3 hours. This response is called the early asthmatic response.¹ In some subjects in whom an early response develops, the airway narrowing persists or recurs after 3 to 4 hours and reaches a maximum within 8 to 12 hours. This airway narrowing may last 24 hours or more

Abbreviations used

FEV₁: Forced expiratory volume in 1 second
PD₂₀: Antigen dose causing 20% decrease in FEV₁

and is called the late asthmatic response. A further consequence of inhaling allergen is the development of airway hyperresponsiveness,² the magnitude and duration of which appear to be related to the occurrence of late responses. Allergen-induced late, but not early, responses are also associated with influx of activated inflammatory cells, predominantly eosinophils and metachromatic cells, into the airways.³

Allergen challenge has been a useful clinical model to establish the activity of antiasthma drugs.⁴⁻⁷ This is because allergen challenge allows the evaluation of acute bronchoconstriction, and the late response and allergen-induced airway hy-

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perresponsiveness. Methods used for allergen challenge have not been uniform. In 1975 Chai et al.⁸ recommended that all bronchial inhalation challenge procedures be administered according to standardized protocols. Their recommendation for allergen challenge was 10-fold increases in the inhaled antigen dose at 10- to 15-minute intervals until a 20% fall in forced expiratory volume in 1 second (FEV₁) is observed. The end-point measurement in this protocol is the antigen dose causing the 20% fall in FEV₁ (PD₂₀). This method was found to be reproducible, with no more than a 10-fold shift in PD₂₀ observed in repeated measurements from the same subjects.⁹ Although this method is frequently used today to study the effects of antiasthma drugs on the early response, it is inadequate for studies of the late response. With these recommendations the dose of allergen may vary between drug and placebo conditions, making interpretation of any changes in the late response impossible. Because of this, an alternate protocol has been developed wherein a constant dose of antigen is delivered during both drug and placebo conditions, with end-point measurements for comparison being the maximal percent decrease in FEV₁ during the early and late phases of the response. Although this method has been used successfully in the evaluation of antiasthma drugs,^{3, 10-20} the within-subject variability of repeated measurements has not been published. This information is necessary to determine reproducibility indices and to calculate sample sizes required to show significant attenuation of either response. This information is also useful in identifying appropriate uses of allergen inhalation challenge and in the planning and interpretation of studies with these challenges.

The purpose of this study was to observe the reproducibility of the early and late maximal percent change in FEV₁ after one commonly used method of allergen challenge¹ and to construct curves for sample size estimation. No attempt was made in this study to test these sample size estimates in the evaluation of any one antiasthma drug, because these studies already exist.^{3, 10-20}

METHODS

Subjects

Twenty eight subjects with a history of mild, stable asthma and allergy to an environmental antigen were studied. The subjects infrequently used only inhaled β_2 -agonists to treat their asthma, and no subject had used any other antiasthma 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 for at least 4 weeks. The baseline FEV₁ was more than 75% predicted normal value²¹ in all subjects on all study days. The study was approved by the hospital ethics committees, and each subject gave written informed consent before the beginning of the study.

Study design

Subjects were studied on two separate occasions, not less than 2 and not more than 12 weeks apart. On each occasion subjects had an allergen inhalation challenge and spirometry was monitored for 7 hours. A methacholine inhalation test was performed the day before each allergen challenge, with the protocol of Cockcroft et al.²² If the provocative concentration of methacholine required to produce a 20% decrease in FEV₁ (PC₂₀) before the second challenge differed by more than one doubling dose from that before the first challenge, then the challenge was delayed for 1 week. The concentrations of inhaled allergen were identical on the two study days.

Allergen inhalation challenge and inclusion criteria

Allergen inhalation tests were performed as previously described,¹ with a Wright nebulizer operated by oxygen at 50 psi and at a flow rate to give an output of 0.13 ml/min. The subject's nose was clipped and aerosols were inhaled through a mouthpiece during tidal breathing.

All subjects inhaled extracts of either ragweed, cat dander, or house dust mite allergen. The starting concentration of extract for the first allergen challenge was determined with the results from a skin prick test end-point titration with the same extract, and the methacholine PC₂₀, according to the formula published by Cockcroft et al.²³ The starting concentration of allergen extract for inhalation was two doubling concentrations less than that predicted to cause a 20% decrease in FEV₁ during the early response.

During the first allergen challenge, 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%. During the second challenge subjects received the same pattern of allergen inhalation that they had in the first, regardless of changes in FEV₁. During both challenges, after inhalation of the highest allergen concentration, the FEV₁ was measured at 10, 20, 30, 45, 60, 90, and 120 minutes, and then at hourly intervals until 7 hours after the inhalation. Subjects were included in the study if a decrease in FEV₁ greater than 15% was observed during the early phase (0 to 2 hours) of the first challenge and if a decrease in FEV₁ greater than 15% was observed during the late phase (3 to 7 hours) of either challenge.

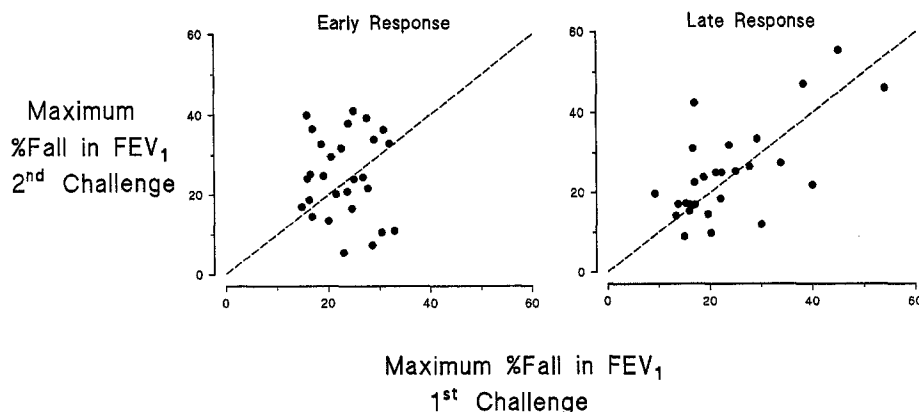


FIG. 1. Maximal early and late decreases in FEV₁ during both allergen challenges. Dashes represent lines of identity.

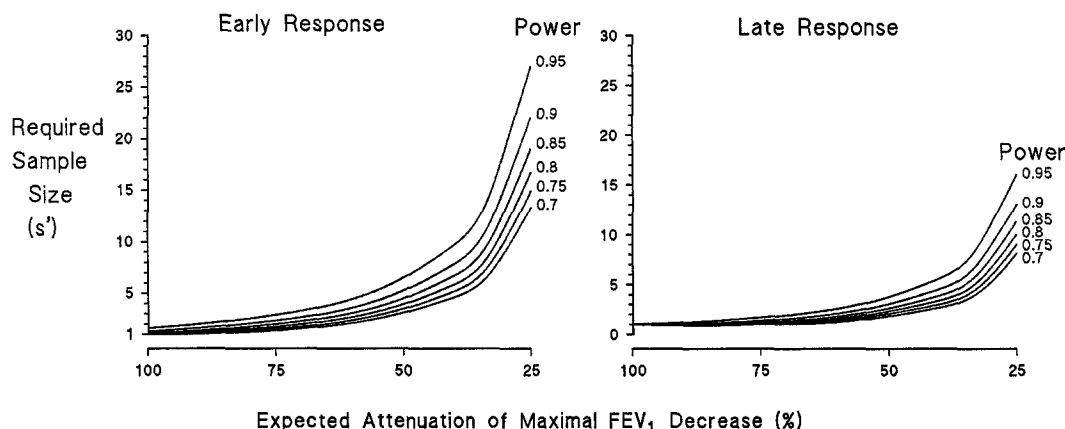


FIG. 2. Curves allowing estimation of sample sizes for given power and expected attenuation of maximal decrease in FEV₁ during early and late asthmatic response.

The allergen extracts were obtained from 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.

Analysis

Allergen-induced early-response measurements were calculated as the maximal percent decrease in FEV₁ from baseline within the first 2 hours after allergen. Similarly, late-response measurements were defined as the maximal percent decrease in FEV₁ between 3 and 7 hours after allergen. Values are expressed as means and SEM. Curves used as a guide for predicting sample sizes were constructed with within-subject variability measurements and published power functions for predicting sample size.²⁴

RESULTS

The mean $\pm$ SEM maximal early decreases in FEV₁ were $23.1\% \pm 1.0\%$ and $24.7\% \pm 2.0\%$. The maximal late decreases in FEV₁ were $23.3\% \pm 2.0\%$ and $24.5\% \pm 2.2\%$. Measurements of the maximal early and late percent decreases in FEV₁

on both study days are illustrated in Fig. 1. Deviation from the lines of equality reflect differences between measurements on the two study days. The mean difference between the early percent fall in FEV₁ during the first and second challenges was $9.54\% \pm 1.4\%$. The mean difference between the late decrease in FEV₁ during the two challenges was $6.61\% \pm 1.2\%$.

The required sample sizes needed to show a statistically significant attenuation of an expected magnitude in the early and late percent decrease in FEV₁ at a given power level are illustrated in Fig. 2. Thus if a drug was expected to produce a 50% attenuation of the maximal decrease in FEV₁ during the early response (i.e., maximum decrease reduced from 24% to 12%), then seven subjects would be needed to show significance at a power level of 0.9 in a crossover study. If the same parameters were applied to the late response, then five subjects would be needed.

DISCUSSION

This study has demonstrated that the reproducibility of the maximal percent decrease in FEV₁ during the early and late responses to inhaled allergen is such that sample sizes required to demonstrate a 50% fall in either response are small.

Predictions of sample sizes are based on the within-subject variance as a fraction of the expected change in measurement. The curves in Fig. 2 are predictions of sample size required to show expected changes based on within-subject variance measured in this study. As illustrated in this figure, only seven subjects are required, to show a 50% attenuation of the maximum early percent decrease in FEV₁ after treatment, with a power of 90%. Similar attenuation of the maximum late fall in FEV₁ may be shown with only five subjects. These findings support and are supported by published applications of the constant-dose allergen challenge, where significant drug effects on both early and late responses have been found with a variety of drugs, with sample sizes ranging from 8 to 14 subjects.^{3, 10-20}

The use of a constant-dose allergen challenge protocol such as ours, as opposed to the traditional method of measuring the PD₂₀ to allergen,⁸ is necessary when a measurement of the effects of treatment on the late response is desired. The findings in this study do not indicate that the constant dose protocol holds any advantage over the PD₂₀ approach in circumstances where only the early response is of interest.

Because of concerns about safety of allergen challenge, the protocol is designed to produce an allergen-induced decrease in FEV₁ of 15%, and once this is achieved, no further allergen is inhaled. This was successful in that all measurements during the first challenge resulted in a decrease between 14.8% and 33.0%. The range of measurements was larger during the second challenge (decrease between 5.5% and 41.1%) because subjects inhaled the same concentrations of allergen as during the first challenge regardless of FEV₁ changes. Restricting the range of measurements to such a degree makes it difficult to get a high index of reproducibility as measured by standard techniques. An intraclass correlation²⁵ of the early-response data in Fig. 1 yields a coefficient of -0.03, suggesting that the measurement has no reproducibility whatsoever. We argue that this is not the case. Rather, it is the high reproducibility of the test that results in the narrow range of measurements and prevents a high intraclass correlation

coefficient. The intraclass correlation coefficient of the late-response data in Fig. 1 is 0.67, because of the larger range of measurements.

Although we do not consider the low intraclass correlation coefficient for the early response as an indicator of poor reproducibility, it does illustrate an important point regarding utility of the constant-dose allergen challenge. Because of the relatively small range of early response measurements, it is inappropriate to use this method of challenge in studies where the magnitudes of these measurements are compared between subjects or are correlated with any other measurements made from the same subjects. This is because the test is designed to produce an early decrease in FEV₁ of slightly more than 15% in *all* subjects. Thus it would not be appropriate to correlate the magnitudes of early and late maximal decreases in FEV₁. In the current study, if the early and late maximal decreases in FEV₁ measured during the first challenge are correlated, the resulting r^2 is 0.00 ($p > 0.05$). This finding cannot be taken as an indicator that the magnitudes of early and late asthmatic responses are unrelated, because the range of early-response measurements is too small. If the same correlation is performed on measurements from the second challenge (when the range of early-response measurements was larger), the resulting r^2 is 0.19 ($p < 0.05$), suggesting that the magnitudes of the two responses may be related.

In conclusion, measuring the maximal percent decrease in FEV₁ after constant-dose allergen challenge is a sensitive tool for detecting changes in early and late asthmatic responses after treatment in a repeated-measures crossover design. However, studies where between-subject differences in magnitude of early-response measurements are considered important will require much larger sample sizes or a protocol designed to produce a larger range of early response measurements.

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