

Original article

The links between allergen skin test sensitivity, airway responsiveness and airway response to allergen

Background: The allergen-induced early asthmatic response [provocation concentration (PC)₂₀, the concentration causing a 20% forced expiratory volume in 1 s (FEV)₁ fall] depends on the level of IgE sensitivity and the degree of non-allergic airway hyperresponsiveness (AHR) and can be predicted from histamine PC₂₀ and allergen skin test endpoint.

Objectives: We examined the relationships between allergen PC₂₀, methacholine PC₂₀, and allergen skin test endpoint and assessed the accuracy of both the histamine PC₂₀-based prediction of allergen PC₂₀ (using methacholine) and a new methacholine PC₂₀-based prediction equation.

Methods: From 158 allergen challenges, the allergen PC₂₀, the methacholine PC₂₀, and the skin test endpoint were recorded and relationships between these three were sought. We compared the measured allergen PC₂₀ to that predicted from the previous histamine PC₂₀-based and the new methacholine-based formulae.

Results: In single regressions, allergen PC₂₀ correlated with both methacholine PC₂₀ ($r = 0.25$, $P = 0.0015$) and skin test endpoint ($r = 0.52$, $P < 0.00005$). The relationship was improved by multiple regression of log-allergen PC₂₀ vs. log-methacholine PC₂₀ and log-endpoint ($r = 0.61$, $P < 0.00005$). The histamine-based formula predicted allergen PC₂₀ to within 2 doubling concentrations in 80% and within 3 in 92%. The new methacholine-based formula to within 2 and 3 concentrations in 81% and 94%, respectively; only nine of 158 subjects were outside the 3 concentrations.

Conclusions: We have confirmed the dependence of the allergen-induced early asthmatic response upon the level of allergic sensitivity and the degree of AHR, the latter as assessed by methacholine challenge. The allergen PC₂₀ can be predicted to within 3 doubling concentrations in 94% of cases.

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Airway hyperresponsiveness (AHR) to direct stimuli such as histamine or methacholine is a fundamental feature of asthma (1, 2). The relationship of direct AHR to atopic allergy is complex. Allergic-type (eosinophilic) airway inflammation is at least mildly associated with the level of AHR (2, 3). AHR increases following allergen exposure following both laboratory (4) and natural (2) exposures. AHR to both histamine (5) and methacholine (6) is associated with the presence and magnitude of atopy, likely a manifestation of allergen-induced increase in AHR. However, AHR is an important determinant of the airway response to allergen.

The early asthmatic response to allergen, often expressed as the allergen PC₂₀ [provocation concentration causing a 20% forced expiratory volume in 1 s (FEV)₁ fall] is dependent upon the degree of allergen sensitization and the level of airway smooth muscle hyperresponsiveness (AHR) (7–9). We have previously documented that the allergen PC₂₀ can be predicted within 2–3 doubling concentrations, from the skin test endpoint (as a measure of magnitude of allergen sensitization) and the histamine PC₂₀ (a measurement of the AHR) (10). We have been using this histamine-based prediction equation as a guide to select a safe and efficient starting concentration for allergen inhalation challenges; however, we have substituted the methacholine PC₂₀ for the histamine PC₂₀. This retrospective review from three centres using identical methods was carried out with three aims: (i) to assess the value of methacholine PC₂₀ as a determinant of airway response to allergen; (ii) to assess how accurately the 'old'

Abbreviations: AHR, (non-allergic) airway hyperresponsiveness; EAR, (allergen-induced) early asthmatic response; FEV₁, forced expired volume in one second; PC₂₀, provocation concentration causing a 20% FEV₁ fall.

prediction equation predicted the allergen PC₂₀ in real-world research situations; and (iii) if significantly different, to develop an equation based on the methacholine PC₂₀ and the allergen skin test endpoint in a larger number of subjects.

Methods

Subjects

All subjects were adults, had asthma (11) and were moderately or highly atopic to at least one aeroallergen. Subjects had FEV₁ ≥ 60% predicted and methacholine PC₂₀ ≤ 32 mg/ml (12, 13). All subjects were participating in research studies for which ethical approval had been granted and signed informed consent obtained.

Methacholine inhalation test

The methacholine inhalation test was performed by the standardized 2-min tidal breathing technique (12, 13). Aerosols were generated continuously from a jet nebulizer (Bennett Twin in Saskatoon, Wright in Hamilton and Quebec, Canada), calibrated to an output of 0.13 ml/min. Saline was initially inhaled for 2 min followed at 5-min intervals (start of one to that of the next concentration) by doubling concentrations of methacholine. Doubling concentrations of methacholine were available from 0.03 to 32.0 mg/ml; starting concentrations were based on established guidelines (14, 15). Spirometry was initially measured in triplicate and one acceptable FEV₁ was then obtained 30 and 90 s after each inhalation. Per cent change in FEV₁ was determined from the lowest post-saline to lowest post-methacholine FEV₁ (16) and the test was continued until a 20% or greater FEV₁ fall was obtained; PC₂₀ was calculated by an algebraic formula (17).

Allergen skin test endpoint

Allergen skin test endpoint was performed using aqueous extracts of the same allergen preparation to be used for inhalation. Duplicate skin-prick tests were performed with doubling dilutions of allergen from 1 : 4 to 1 : 4096 (or more dilute in occasional circumstances). The wheal diameter was measured in two perpendicular directions at 10 min and the mean wheal diameter determined for each dilution. The skin test endpoint was defined as the threshold concentration producing a 2-mm wheal.

Allergen inhalation tests

Allergen challenges were performed using the 2-min tidal breathing technique analogous to the methacholine challenge (10). Aerosols were inhaled for 2 min from a Wright nebulizer with an output 0.13 ml/min. The specific allergens used are summarized in Table 1 and included mite, pollen, or animal in all but one subject (*Alternaria*). We used the best-quality allergens available in several different units and strengths from several Canadian or American suppliers over the years. Diluent was inhaled on three occasions at 12–13-min intervals on a control day, usually the day before allergen inhalation, to assure FEV₁ stability and no irritant response. On the allergen-challenge day, allergen PC₂₀ was predicted by substituting methacholine PC₂₀ in the histamine-based equation (10). For safety purposes, the first concentration of allergen to be inhaled was usually 4 concentrations below the predicted. Doubling concentrations of allergen were

inhaled for 2 min and duplicate FEV₁ measurements were made at 10 min post-inhalation. The next concentration was administered if there was <15% FEV₁ fall. After a threshold 15–20% FEV₁ fall, spirometry was monitored every 10 min for an hour and the early asthmatic response determined as the maximum per cent fall in FEV₁.

Study design

Allergen challenge research studies from July 1992 to spring 2003 from the three institutions were reviewed retrospectively. Data were extracted for individuals from a non-medicated (or a placebo-medicated) allergen challenge where all three parameters were available. The methacholine PC₂₀ and the allergen skin test endpoint were performed the day before the allergen challenge in most cases. For subjects who had participated in multiple investigations, the first data retrieved were utilized to avoid any possibility of a selection bias.

Analysis

Logarithmic transformations were used for allergen PC₂₀, methacholine PC₂₀, and allergen skin test endpoint. We looked at both a *clinical allergen PC₂₀* defined as the concentration of allergen inhaled causing a 20% or greater FEV₁ fall (18 or 19% fall in eight individuals). We also determined the allergen PC₂₀ calculated from the per cent fall in FEV₁ at the last concentration (18). As the results were virtually identical, and as the clinical allergen PC₂₀ is a practical endpoint, we only report the data on the clinical PC₂₀. Analyses were performed using single and multiple linear regression of the log-transformed data by a computerized statistical

Table 1. Demographics

	Saskatoon	Quebec	Hamilton	Total
<i>n</i>	39	28	91	158
Gender (M/F)	18/21	12/16	37/54	67/91
Age [years; (SE)]	23.6 (1.1)	26.9 (0.9)	25.6 (0.9)	25.4 (0.6)
Height [cm; (SE)]	170 (1.6)	170 (1.6)	172 (1.1)	171 (0.8)
FEV ₁ [l; (SE)]	3.48 (0.14)	3.47 (0.12)	3.51 (0.08)	3.50 (0.06)
% predicted (SE)	82.8 (2.5)	86.3 (2.6)	84.8 (1.4)	84.6 (1.1)
Methacholine PC ₂₀ [mg/ml; geometric mean (95% CI)]	0.78 (0.54–1.1)	1.4 (0.90–2.0)	2.2 (1.7–2.9)	1.6 (1.3–1.9)
Allergen				
Mite				
<i>Dermatophagoides pteronyssinus</i>	0	7	41	48
<i>D. farinae</i>	0	1	5	6
Pollen				
Ragweed	0	3	17	20
Birch	3	2	0	5
Mixed grass	9	2	7	18
Mixed tree	0	0	2	2
Mixed weed	4	0	0	4
Animals				
Cat	9	13	18	40
Horse	11	0	0	11
Dog	1	0	0	1
Rabbit	1	0	0	1
Guinea pig	1	0	0	1
Mould				
<i>Alternaria</i>	0	0	1	1

program (STATISTIX for Windows; Analytical Software, Tallahassee, FL, USA).

Results

We identified 158 subjects who met the enrollment criteria. There were 39 from Saskatoon, 28 from Quebec City and 91 from Hamilton. The demographics are outlined in Table 1. The mean early asthmatic response in these 158 subjects was 27.1 ± 0.65 (SE) % FEV₁ fall.

We initially analysed the various pairs of data with single linear regressions. The allergen PC₂₀ correlated with the methacholine PC₂₀ ($r = 0.25$, $P = 0.0015$); the allergen PC₂₀ correlated with the allergen skin test endpoint ($r = 0.52$, $P < 0.00005$); and there was no correlation between the methacholine PC₂₀ and the allergen skin test endpoint ($r = 0.08$, $P = 0.15$). A multiple linear regression of log-allergen PC₂₀ vs. log-methacholine PC₂₀ and log-allergen skin test endpoint improved the statistical significance and revealed the following regression formula:

$$\begin{aligned} \log \text{allergen PC}_{20} &= 0.41 \log \text{methacholine PC}_{20} + 0.50 \\ &\log \text{skin test endpoint} - 0.42 \quad (r = 0.61, P < 0.00005) \end{aligned}$$

The regression coefficients for log-methacholine PC₂₀ ($t = 4.92$) and log-skin test endpoint ($t = 8.79$) were both highly significant ($P < 0.00005$).

We then assessed the accuracy of predicting the allergen PC₂₀ by the previous histamine PC₂₀-based formula (10) (substituting methacholine PC₂₀ for histamine PC₂₀) and the new regression formula. These data are summarized in Fig. 1. The old formula predicted the clinical allergen PC₂₀ to within ± 2 concentrations in 80% of the subjects and to within ± 3 concentrations in 92% of the subjects (Fig. 1). The new regression equation predicted the clinical allergen PC₂₀ to within ± 2 concentrations in 81% and to within ± 3 concentrations in 94%, i.e. all but nine of the subjects (Fig. 1).

Discussion

These data confirm that the early asthmatic response to allergen is dependent upon the degree of nonallergic airway hyperresponsiveness and the magnitude of the allergic hypersensitivity. Although hypothesized on basic principles many years ago (19, 20) and previously demonstrated with histamine and exercise (7–10), this is the first documentation of this relationship using methacholine. These data also indicate that with knowledge of methacholine PC₂₀ and allergen skin test endpoint that it is possible to predict the allergen PC₂₀ to within three doubling dilutions in the majority of instances; the new methacholine-based formula performs slightly better than the old histamine-based formula.

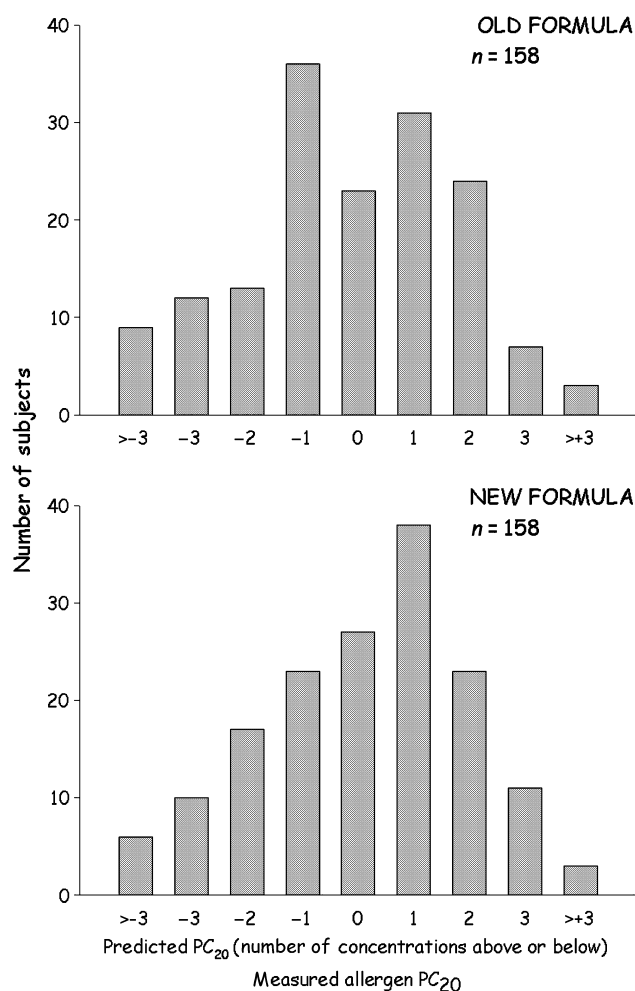


Figure 1. Under- and over-prediction of clinical allergen PC₂₀ by the two formulae. The top graph shows the performance of the histamine-based formula [based on the previous study of Cockcroft et al. (10)] and the bottom graph shows the methacholine-based regression from the present study. Negative values indicate a prediction that is more dilute than the measured PC₂₀.

As outlined previously (10), these results have relevance in several areas. First, these data support the concept of the mechanism of the early asthmatic response to allergen as being dependent upon the degree of allergy and an independent effect of the *shock organ* (19), namely the degree of AHR as assessed by airway response to methacholine (current study) or histamine (10). Secondly, they can be of value in safely determining a starting inhalation concentration for allergen and, thus, avoiding overly long challenge protocols. Finally, one can argue that if it is possible to predict the airway response to allergen with even the fair accuracy found in this study, that allergen challenge studies to determine the *clinical relevance* of allergen would be indicated infrequently if ever.

The methacholine PC₂₀ (21) and the allergen PC₂₀ (22) are reasonably reproducible, certainly to within better than ± 2 doubling concentrations, perhaps better than that in well-trained research subjects. The reproducibility of the allergen skin test endpoint has not been documented to our knowledge. It is, however, our observation in at least some subjects that it can be difficult to precisely identify the threshold concentration causing the 2-mm wheal as several concentrations may occasionally cause similar-sized small wheals. It is therefore likely that the precision of the allergen skin test endpoint and its reproducibility are not as good as the PC₂₀ values. Additionally, there is the very real possibility of an inter-performer variability based on the mechanics of the inter-cutaneous maneuver. Consequently, we would

hypothesize that the weak link in the prediction equations is the determination of the allergen skin test endpoint.

Despite the uncertainties surrounding the intra- and inter-observer reproducibility of the allergen skin test endpoint, these two simple measurements, namely the skin test endpoint and the methacholine PC₂₀, allow a fair prediction of allergen PC₂₀. This is of value in determining starting allergen concentrations for allergen-inhalation protocols.

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References

- Hargreave FE, Ryan G, Thomson NC, O'Byrne PM, Latimer K, Juniper EF et al. Bronchial responsiveness to histamine or methacholine: measurement and clinical significance. *J Allergy Clin Immunol* 1981;**68**:347–355.
- Brusasco V, Crimi E. Methacholine provocation test for diagnosis of allergic respiratory diseases. *Allergy* 2001;**56**:1114–1120.
- Kirby JG, Hargreave FE, Gleich GJ, O'Byrne PM. Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am Rev Respir Dis* 1987;**136**:379–383.
- Cockcroft DW, Ruffin RE, Dolovich J, Hargreave FE. Allergen-induced increase in nonallergic bronchial reactivity. *Clin Allergy* 1977;**7**:503–513.
- Cockcroft DW, Murdock KY, Berscheid BA. Relationship between atopy and bronchial responsiveness to histamine in a random population. *Ann Allergy* 1984;**53**:26–29.
- Fowler SJ, Lipworth BJ. Relationship of skin-prick reactivity to aeroallergens and hyperresponsiveness to challenges with methacholine and adenosine monophosphate. *Allergy* 2003;**58**:46–52.
- Killian D, Cockcroft DW, Hargreave FE, Dolovich J. Factors in allergen-induced asthma: relevance of the intensity of the airways allergic reaction and non-specific bronchial reactivity. *Clin Allergy* 1976;**6**:219–225.
- Hill DJ, Shelton MJ, Hosking CS. Predicting the results of allergen bronchial challenge by simple clinical methods. *Clin Allergy* 1982;**12**:295–301.
- Cockcroft DW, Ruffin RE, Frith PA, Cartier A, Juniper EF, Dolovich J et al. Determinants of allergen-induced asthma: dose of allergen, circulating IgE antibody concentration and bronchial responsiveness to inhaled histamine. *Am Rev Respir Dis* 1979;**120**:1053–1058.
- Cockcroft DW, Murdock KY, Kirby J, Hargreave FE. Prediction of airway responsiveness to allergen from skin sensitivity to allergen and airway responsiveness to histamine. *Am Rev Respir Dis* 1987;**135**:264–267.
- ATS. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987;**136**:225–244.
- Cockcroft DW, Killian DN, Mellon JJA, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. *Clin Allergy* 1977;**7**:235–243.
- Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin C et al. Guidelines for methacholine and exercise challenge testing-1999. *Am J Respir Crit Care Med* 2000;**161**:309–329.
- Juniper EF, Cockcroft DW, Hargreave FE. Histamine and Methacholine Inhalation Tests: Tidal Breathing Method – Laboratory Procedure and Standardisation, 2nd edn. Lund: AB Draco, 1994.
- Cockcroft DW, Marciniuk DD, Hurst TS, Cotton DJ, Laframboise KF, McNab BD et al. Methacholine challenge: test-shortening procedures. *Chest* 2001;**120**:1857–1860.
- Davis BE, Cockcroft DW. Calculation of provocation concentration causing a 20% fall in FEV₁: comparison of lowest vs. highest post-challenge FEV₁. *Chest* 2000;**117**:881–883.
- Cockcroft DW, Murdock KY, Mink JT. Determination of histamine PC₂₀: comparison of linear and logarithmic interpolation. *Chest* 1983;**84**:505–506.
- Jokic R, Davis EE, Cockcroft DW. Methacholine PC₂₀ extrapolation. *Chest* 1998;**114**:1796–1797.
- Tiffeneau R. Hypersensibilite cholinergo-histaminique pulmonaire de l'asthmatique. *Acta Allergologica* 1958;(Suppl 5):187–221.
- Zuidema P. Value of inhalation tests in bronchial asthma. 2nd International Symposium – Tuberculosis, Climate, Asthma, Chronic Bronchitis, Davos 1967. *Respiration* 1969;(Suppl 26):141–150.
- Juniper EF, Frith PA, Dunnett C, Cockcroft DW, Hargreave FE. Reproducibility and comparison of responses to inhaled histamine and methacholine. *Thorax* 1978;**33**:705–710.
- Inman MD, Watson R, Cockcroft DW, Wong BJ, Hargreave FE, O'Byrne PM. Reproducibility of allergen-induced early and late asthmatic responses. *J Allergy Clin Immunol* 1995;**95**:1191–1195.