Maximal Airway Response to Methacholine in Cough-Variant Asthma*

Comparison With Classic Asthma and Its Relationship to Peak Expiratory Flow Variability

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Background: In asthmatic subjects, not only airway sensitivity but maximal airway response are increased on the dose-response curve to methacholine, and peak expiratory flow (PEF) variability is closely related to airway hypersensitivity and maximal airway response.

Objective: The aims of this study were to compare the prevalence and the level of maximal response plateau between patients with cough-variant asthma (CVA) and those with classic asthma (CA), and to examine the relationship between airway hypersensitivity or maximal airway response and PEF variability in patients with CVA.

Methods: A high-dose methacholine inhalation test was performed on 83 patients with CVA and on 83 patients with CA matched for provocative concentration of methacholine causing a 20% fall in FEV₁ (PC₂₀). PEF was recorded in the morning and evening for 14 consecutive days in 78 CVA patients, and the amplitude percentage mean was used to express the diurnal PEF variation.

Results: Fifty-two CVA subjects (62.7%) but only 33 CA subjects (39.8%) showed a maximal response plateau. This difference was significant after correction by the Bonferroni method (corrected p = 0.024). Subjects in the CVA and CA groups showing a plateau had significantly different plateau levels (38.0 ± 5.9% vs 42.9 ± 3.9%, corrected p = 1.0 × 10⁻⁴). In patients with CVA, no significant relationship was found between PC₂₀ and PEF variability. However, the absence of a maximal response plateau and a higher plateau level were associated with increased PEF variability.

Conclusions: Maximal airway response may be an important confounder in the relationship between airway hypersensitivity and the clinical expression of asthma. The identification of a maximal response plateau and the level of this plateau in patients with CVA provide information relevant to PEF variability.

Key words: airway hypersensitivity; classic asthma; cough-variant asthma; maximal airway response; maximal response plateau; peak expiratory flow variability.

Abbreviations: AHR = airway hyperresponsiveness; CA = classic asthma; CVA = cough-variant asthma; PC₂₀ = provocative concentration of methacholine causing a 20% fall in FEV₁; PEF = peak expiratory flow

Most clinicians are familiar with coughing as a symptom of asthma, concomitant with wheezing and dyspnea (classic asthma [CA]). Cough, however, may be the sole presenting symptom of asthma.¹ This type of asthma, known as cough-variant asthma (CVA), was initially described as uncommon, but it now appears that CVA is one of the most common causes of a chronic cough in children² as well as in adults.³

A diagnosis of CVA is made when a chronic cough is associated with airway hyperresponsiveness (AHR) and a favorable response to asthma therapy in the absence of other discernible causes.⁴ AHR is usually defined as an increased sensitivity of the airways to inhaled histamine or methacholine.⁵ The sensitivity of the airways to these agents is commonly expressed by using the provocative concentration causing a 20% fall in FEV₁. However, there is accumulating

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**CHEST** 2005; 128:3881–3887

Manuscript received March 9, 2004; revision accepted June 27, 2005.

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evidence that AHR is a more complex functional abnormality that comprises more than just hypersensitivity.\(^6\) When exposed to high concentrations of inhaled histamine or methacholine, asthmatic patients show excessive narrowing of the airways, as reflected by an elevated or absent maximal response plateau. It can be argued that the latter is clinically a more relevant component of AHR than the former because it reflects the potential severity of airway obstruction in an individual.\(^7\)

It has been shown that the maximal response of the airways increases with increasing sensitivity to bronchoconstrictor stimuli in patients with symptomatic asthma, leading to unmeasurable or elevated plateau levels when the sensitivity is relatively high.\(^8\) However, for airway hypersensitivity in patients with CVA, the prevalence and level of maximal response plateau have been little studied. Furthermore, it is not clear whether the profile of maximal airway response in patients with CVA differs from that in patients with CA.

Another hallmark of asthma is increased spontaneous variation in airway caliber, which is commonly measured by peak expiratory flow (PEF) monitoring.\(^9,10\) Higher diurnal PEF variations have been reported in patients with CVA than in normal subjects.\(^11\) In asthmatic subjects, several studies\(^12,13\) have shown a significant direct correlation between airway sensitivity to methacholine or histamine and PEF variability. Another study\(^14\) demonstrated a greater PEF variation in asthmatic patients without a maximal response plateau than in those with a plateau. However, the relationships between PEF variation and airway hypersensitivity or maximal airway response to pharmacologic agents have not been investigated in patients with CVA.

This study was designed with two main aims. The first aim was to compare the prevalence and level of the maximal response plateau in patients with CVA with those in patients with CA. For an adequate comparison, CA patients were selected by matching the provocative concentration of methacholine causing a 20% fall in FEV\(_1\) (PC\(_{20}\)) levels with the CVA patients. The second aim was to examine the relationship between airway hypersensitivity or maximal airway response and PEF variability in patients with CVA.

**Methods and Materials**

Children with a diagnosis of CVA were enrolled in this study. Initially, the patients were referred to our clinics for a cough that had persisted for a minimum of 2 months (range, 9 weeks to 2 years). The cough was usually dry or productive with minimal amounts of clear sputum, and was mainly nocturnal. None of the patients had a history of wheezing or dyspnea, nor was any wheeze or prolonged expiratory phase detected on physical examination. At the time of diagnosis, all had a PC\(_{20}\) level < 16 mg/mL. Bronchodilators (inhaled \(\beta_2\)-agonists and/or oral theophylline) were effective against the cough, but it recurred when medication was stopped. Normal results were found for the following tests: chest radiography, spirometry, sinus radiographs, and tuberculin skin tests. No other apparent causes of cough were present.

A group of patients with CA was also recruited. These patients had a history of mild symptoms (episodic wheezing or dyspnea) within the previous year, which had been controlled by using an “as-needed” bronchodilator. Those subjects with a history of major exacerbations requiring systemic corticosteroids or near-fatal asthma were excluded. None of the patients had used inhaled or oral corticosteroids, long-acting \(\beta_2\) agonists, leukotriene antagonists, sodium cromoglycate, or nedocromil sodium in the year prior to entry into the study. Candidates were selected from the results of a methacholine inhalation test at the initial diagnostic workup, by matching PC\(_{20}\) levels with the CVA patients.

At the start of the study, these two subject groups underwent high-dose methacholine inhalation testing, and maximal airway response as well as PC\(_{20}\) were measured. Spirometric measurements were made in accordance with recommendation of the American Thoracic Society.\(^15\) The subjects had to be capable of performing pulmonary function tests in a reproducible way (ie, a difference between the two values of FEV\(_1\) < 5%) and needed to have an FEV\(_1\) of at least 70% of the predicted value.\(^16\) None of the subjects had exhibited any symptoms of upper respiratory infection or asthma exacerbation in the month prior to the study. Subjects were excluded from the study if they could not tolerate the test, or if their PC\(_{20}\) level was \(\geq\) 16 mg/mL. After the methacholine inhalation test, patients with CVA were instructed to use peak flow meters. After achieving a documented reproducibility within 20 L/min, PEF recordings were obtained. The study was approved by the Hospital Ethics Committee, and the parents of all participating children provided their informed consent.

**Methacholine Inhalation Test**

High-dose methacholine inhalation tests were carried out using a modification of the method described by Chai et al.\(^17\) Methacholine (Sigma Diagnostics; St. Louis, MO) solutions were prepared at different concentrations (0.075, 0.15, 0.3, 0.625, 1.25, 2.5, 5, 10, 25, 50, 100, 150), and 200 mg/mL in buffered saline solution (pH 7.4). Lung function was measured using a computerized spirometer (Microspiro-HI 298; Chest; Tokyo, Japan), and the largest FEV\(_1\) among triplicate values at each time was used for analysis. A Rosenthal-French dosimeter (Laboratory for Applied Immunology; Baltimore, MD), triggered by a solenoid valve set to remain open for 0.6 s, was used to generate an aerosol from a nebulizer (DeVilbiss 646; DeVilbiss Health Care; Somerset, PA), using pressurized air at 20 pounds per square inch. Each subject inhaled five inspiratory capacity breaths of buffered saline solution and increasing concentrations of methacholine at 5-min intervals. This set-up produced an output of 0.009 ± 0.0014 mL (mean ± SD) per inhalation. FEV\(_1\) was measured 60 to 90 s after inhalation at each concentration. The procedure was terminated when the FEV\(_1\) level fell below 50% of the post-saline solution value, or when a maximal response plateau had been established. This was considered to have occurred if three or more data points at the highest concentrations fell within a 5% response range.\(^8\) An additional 5 or 10 inhalations of the 200 mg/mL solution were taken if the last three data points, showing less than a 50% FEV\(_1\) fall, did not satisfy the above criteria. For safety reasons, subjects were given the opportunity to stop the challenge test if they had...
too much discomfort. Response, expressed as the percentage fall in FEV₁ from the post-saline value, was plotted against the log of the inhaled methacholine concentration. The dose-response curves were characterized by their position and maximal response, the former was expressed as PC₂₀, which was calculated by log-linear interpolation between two adjacent data points, and the latter was defined as the level of the maximal response plateau obtained by averaging the consecutive points on the plateau or as the last data point of the dose-response curve if a plateau could not be obtained.

**Measurement of PEF Variability**

Patients with CVA were asked to record PEF mornings and evenings for 14 consecutive days using a adult type peak flowmeter (Mini-Wright; Clement Clarke; London, UK). The patients were given printed instructions on the use and care of the peak flowmeter and a peak flow diary for charting. Morning values were obtained just after rising and evening values between 7 and 9 PM. On each occasion, the best of three attempts was recorded. In subjects using bronchodilators, premedication PEFs were recorded. The amplitude percentage mean ([high PEF - low PEF]/mean PEF of the day x 100) was used to summarize the diurnal PEF variation, and the mean value of this variation over the 14 days was calculated. Diary cards with values missing for > 2 of the 14 days were excluded from the analysis.

**Statistical Analysis**

The primary outcome variables were the frequency and the level of maximal response plateau. Based on data collected from previous studies,18,19 to detect a difference in the frequency of maximal response plateau between the CVA group and the CA group (65% vs 43%), with 80% power and 5% significance level, a minimum of 79 subjects per group was required. Therefore, 91 subjects per group were recruited to allow for dropouts due to intolerance of the high-dose methacholine challenge test or due to their PC₂₀ level ≥ 16 mg/mL. This sample size was also sufficient to identify a 4% difference (effect size, 0.62) in the level of maximal response plateau between the groups18,19 and also to find the correlation of PEF variability against PC₂₀ (α = 0.05, β = 80%).13 Power analyses for the other results in which the variation over the 14 days was calculated. Diary cards with values missing for > 2 of the 14 days were excluded from the analysis.

**RESULTS**

Ninety-one patients with CVA and 91 PC₂₀ matched patients with CA were enrolled. Of these, two patients with CVA and three patients with CA could not tolerate the high-dose methacholine challenge test, and three patients with CVA had a PC₂₀ value > 16 mg/mL on the test. These eight subjects and their eight matched counterparts were excluded from the comparative study. The clinical characteristics of the two studied groups are shown in Table 1. The two groups were similar in terms of age, sex ratio, serum total IgE, blood eosinophils, FEV₁, and PC₂₀.

Fifty-two patients (62.7%) with CVA and 33 patients (39.8%) with CA featured a maximal response plateau on their dose-response curves to methacholine. This difference was significant after correction by the Bonferroni method (corrected p = 0.024). Representative cases that exhibited a plateau or not are shown in Figure 1. In subjects with a plateau, a significant difference in plateau level was observed between patients with CVA (38.0 ± 5.9%) and those with CA (42.9 ± 3.9%; corrected p = 1.0 x 10⁻⁴) [Fig 2]. When the percentage decline in FEV₁ at the end of testing was taken as the maximal response in those without a plateau in order to allow comparison across all subjects, the level of the maximal airway response was found to be significantly lower in patients with CVA (43.5 ± 8.7%) than in those with CA (48.8 ± 5.5%; corrected p = 6.5 x 10⁻⁵; data not shown).

Of the 86 patients with CVA who completed the high-dose methacholine inhalation test with a PC₂₀ value < 16 mg/mL, 8 patients had inadequate or unreliable PEF recordings and were excluded from the analysis of PEF variability. The mean PEF variability in the remaining 78 CVA patients was 10.5 ± 2.9%. Regression plots of PEF variability against PC₂₀ are shown in Figure 3. No significant correlation was found between these two parameters.

Figure 4 compares PEF variability between CVA patients with and without a maximal response plateau. The former group had a significantly lower PEF variability (9.6 ± 2.5%) than the latter group (12.1 ± 2.8%; corrected p = 0.001). The relationship between maximal airway response and PEF variability was also found to be significant in both groups with and without a plateau.

**Table 1—Clinical Characteristics of the Two Asthma Groups**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CVA (n = 83)</th>
<th>CA (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>11.4 ± 2.1</td>
<td>11.7 ± 2.3</td>
</tr>
<tr>
<td>Male/female gender, No.</td>
<td>47/36</td>
<td>56/27</td>
</tr>
<tr>
<td>Serum IgE, IU/mL</td>
<td>255.0 (129.0–504.2)</td>
<td>329.1 (116.7–928.1)</td>
</tr>
<tr>
<td>Blood eosinophils, /μL</td>
<td>309 ± 136</td>
<td>348 ± 176</td>
</tr>
<tr>
<td>Methacholine inhalation test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁, % predicted</td>
<td>97.3 ± 7.5</td>
<td>96.4 ± 9.7</td>
</tr>
<tr>
<td>PC₂₀, mg/mL</td>
<td>5.19 (2.30–11.69)</td>
<td>5.12 (2.27–11.53)</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD or geometric mean (range of 1 SD) unless otherwise indicated.
variability is shown in Figure 5. Among those with a maximal response plateau, a significant correlation was found between its level and PEF variability ($r = 0.558$, corrected $p = 2.4 \times 10^{-4}$). When the subjects without a plateau were included employing the percentage fall in FEV$_1$ at the end of testing, the correlation between maximal airway response and PEF variability was also significant ($r = 0.574$; corrected $p = 3.2 \times 10^{-7}$).

Figure 1. Methacholine concentration-response curves in four subjects. Two CVA patients with (○) or without (●) a maximal response plateau, and two CA patients with (□) or without (■) a maximal response plateau are shown. The level of maximal response plateau was calculated by averaging the last three points on the plateau (○ = 41.7%, □ = 44.8%).

Figure 2. Maximal response plateau levels in patients with CVA and in patients with CA. Horizontal bars represent mean ± SD; p values are corrected by the Bonferroni method.

Figure 3. Simple regression plots of PEF variability against PC$_{20}$ in patients with CVA. No significant correlation was found between the two parameters. Open circles indicate patients with a maximal response plateau; closed circles indicate subjects with a FEV$_1$ fall > 50% without a plateau. NS = not significant.
Discussion

This study shows that levels of maximal airway response on the dose-response curves to methacholine were significantly lower in patients with CVA than in patients with CA. In patients with CVA, PEF variability was not found to correlate with PC_{20}, but the absence of a maximal response plateau or a higher level of maximal response plateau, when present, was associated with increased PEF variability.

In normal subjects, the dose-response curve achieves a plateau at mild degrees of airway narrowing, whereas in asthmatic subjects increasing doses of inhaled pharmacologic agents usually lead to progressive airway narrowing without the achievement of a plateau response. We found that patients with CVA had a higher frequency of plateau on the dose-response curves to methacholine than CA patients with a similar degree of airway sensitivity to methacholine (Bonferroni-corrected p = 0.024). It is arguable that some subjects, who showed FEV_{1} falls of > 50% without evidence of plateau, might present a plateau beyond a 50% fall. However, we do not believe that this factor has a predilection in CA. Furthermore, the level of maximal airway response was significantly lower in the CVA group than in the CA group, despite a possible underestimation of this difference due to the more frequent inclusion of subjects without a plateau in the CA group. In view of the fact that subjects with mild asthma were chosen for the CA group, in order to match the degree of airway sensitivity in the two groups and to minimize the risk inherent in producing an excessive FEV_{1} fall, it is likely that a more representative group of patients with CA would have shown a greater maximal airway response than patients with CVA.

The relatively mild degree of maximal airway response in patients with CVA, compared to that in CA patients with a similar degree of airway sensitivity to methacholine, suggests that the level of maximal airway response, rather than airway sensitivity, is the more important determinant of CA symptom occurrence. This finding also supports the hypothesis that maximal airway response is an important confounder in the relationship between airway sensitivity and the clinical expression of asthma.

Only one previous study analyzed PEF variability in patients with CVA. Tokuyama and coworkers reported that the degree of diurnal variation of PEF in children with CVA is significantly higher than that in control children and comparable with that shown by children with mild-to-moderate asthma. In our group of children with CVA, the mean PEF variation was 10.4%. This seems to be intermediate between previously reported mean values (10.6 to 22.6%) in...
children with CA\textsuperscript{13,22} and those (5.7 to 9.9\%) reported for normal children.\textsuperscript{23,24} However, it is difficult to make direct comparisons because of differences in the frequency and duration of monitoring, the formulas used to calculate variability and the timing of PEF measurements in relation to the administration of medication. Our subjects measured their PEFs twice daily for 2 weeks. It is clear that more frequent readings would have increased the diurnal PEF variation, and that a longer period would have led to more stabilized PEF variation.\textsuperscript{25} However, too frequent measurement for several weeks would have seriously hampered patient compliance.\textsuperscript{26} However, the presentation of PEF variation is a contentious issue, and different indexes of PEF variability have been proposed.\textsuperscript{27} We used amplitude percentage mean, which has been commonly used to quantify PEF diurnal variability.\textsuperscript{28} Precautions were taken not to include PEF measured within 6 h after use of inhaled \(\beta_2\)-agonist.

Thus, we can exclude the possibility that the PEF variability recorded in patients with CVA might be a consequence of \(\beta_2\)-agonist use.

Most of the published studies on the relationship between AHR and PEF variability have treated AHR as a measure of airway hypersensitivity. In asthmatic subjects, several studies\textsuperscript{12,13} have shown a significant direct correlation between airway sensitivity to methacholine or histamine and PEF variability. To the best of our knowledge, this relationship has not been investigated in CVA patients. We did not find a significant relationship between PC\textsubscript{20} and PEF variation in CVA. Similar results were found in a study\textsuperscript{29} of nonasthmatic patients with allergic rhinitis. Our results suggest that PEF variability is not a good marker of the degree of airway hypersensitivity in patients with CVA, and are consistent with previous reports\textsuperscript{29,30} that PEF variability yields information on a different physiologic component of the disease than that measured by airway sensitivity.

Data on the relationship between maximal airway response and PEF variability is limited. Prieto et al\textsuperscript{26} reported that a loss of plateau on the dose-response curve to inhaled methacholine identifies subjects with allergic rhinitis who show greater PEF variability. They also found that asthmatic patients without a plateau showed greater PEF variation than patients with a plateau.\textsuperscript{14} Our results also indicate that the absence of a maximal response plateau is associated with increased PEF variation in CVA patients. Furthermore, a significant correlation was found between the degree of maximal airway response and PEF variability, whether the subjects without a plateau were included employing the percentage fall in FEV\textsubscript{1} at the end of testing as the maximal response or not. Our results suggest that the identification of a plateau and its level in CVA patients provide relevant information on PEF variability. The discrepancy between airway hypersensitivity and maximal airway response in terms of their relationship with PEF variability strengthens the suggestion that both airway sensitivity and maximal airway response to agonist are, at least in part, the consequence of different mechanisms.\textsuperscript{6,31}

The present observations are relevant to the reasons why typical asthmatic symptoms, such as a wheeze, are absent in CVA. Previously, we reported that CVA is associated with a higher wheezing threshold (the minimal degree of airway obstruction when wheezing becomes audible) than CA.\textsuperscript{32} In addition, the relatively mild degree of maximal airway response in CVA patients vs CA patients, at a similar degree of airway sensitivity to methacholine, may explain the absence of wheeze in CVA patients. This is because maximal airway response reflects the potential degree of airways obstruction in individual patients irrespective of the level of sensitivity.\textsuperscript{7} We have reported\textsuperscript{18} that a higher level of maximal airway response is an important risk factor for the future development of CA in CVA patients. In the present study, the lack of a maximal response plateau and a higher level of the plateau, when this is present, were found to be associated with a greater diurnal PEF variation. We speculate that CVA patients who have an elevated or absent maximal response plateau have an increased risk for wheezing due to increased airflow obstruction variability, on exposure to an allergic or a nonallergic stimulus.

In conclusion, maximal airway response may be an important confounder in the relationship between airway hypersensitivity and the clinical expression of asthma. The identification of a maximal response plateau and the level of this plateau in patients with CVA provide relevant information on PEF variability.

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