A Short Protocol for Methacholine Provocation Testing Adapted to the Rosenthal-Chai Dosimeter Technique*

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Background: The purpose of this study was to develop a rapid and safe methacholine provocation protocol equivalent to the standard dosimeter technique.

Methods: The rapid protocol comprised a short and a long subprotocol. The challenge was started with one of these subprotocols according to the subject’s answers to a questionnaire and baseline lung function. If FEV₁ dropped by 10% during the short subprotocol, the test was continued with the long subprotocol. The concentrations of methacholine and numbers of inhalations were chosen to match the concentrations of the standard method as closely as possible. To verify the protocol, we compared both methods in 38 subjects with asthma and 10 control subjects.

Results: The provocative concentrations of methacholine (PC₂₀FEV₁) obtained with the standard method and the rapid method were within one doubling concentration in 38 of 40 subjects. None of the subjects who were normoreactive according to the standard method (PC₂₀FEV₁ > 8 mg/mL) responded in the rapid protocol. The standard method required, on average (±SD), 34±11 min; the rapid method required 15±3 min.

Conclusions: The rapid provocation protocol is equivalent to the standard method, without loss in precision and safety, but with considerable saving in time. Therefore, it appears to be particularly suited for studies that require comparability with provocative concentrations obtained with the Rosenthal-Chai dosimeter method.

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Key words: bronchial hyperreactivity; bronchial provocation tests; methacholine

Abbreviations: cbu=cumulative breath unit; ECRHS=European Community Respiratory Health Survey; PC₂₀FEV₁ = provocative concentration of histamine or methacholine causing a 20% fall in FEV₁

Several methods are used for the assessment of airway responsiveness.¹ One well-accepted and validated procedure was originally described by Chai and coworkers² in 1975. The variability of this method in quantifying provocative concentrations of histamine or methacholine causing a 20% fall in FEV₁ (PC₂₀FEV₁) can be estimated as less than one doubling concentration.³⁻⁴ The disadvantage of this—and other well-documented methods—is the amount of time required, which limits its applicability.

To overcome this problem, several rapid challenge protocols have been developed. Many of them, however, lack comparability with each other. One rapid protocol has been described to be equivalent to the Rosenthal-Chai dosimeter method.⁵ This protocol, however, led to marked airway obstruction in some subjects and involved single inhalations of methacholine, which represents a potential source of variability in comparison to multiple inhalations.

The aim of our study was to develop a rapid and safe provocation protocol that yielded values for PC₂₀FEV₁ equivalent to those obtained with the standard protocol, without loss of accuracy or safety. For this purpose, we compared the results of both methods in patients with and without airway hyperresponsiveness to methacholine.

Materials and Methods

Patients

We studied 38 patients with bronchial asthma (17 men, 21 women; mean±SD age, 39±13 years; FEV₁, 97.8±19.2 [range, 53 to 143] percent of predicted) and, for control purposes, 10 subjects with no diagnosis of asthma (six men, four women; mean±SD age, 40±15 years, FEV₁, 104.6±20.1 [range, 72 to

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137] percent of predicted), including three patients with chronic bronchitis. All patients with asthma and two of the subjects with bronchitis showed airway hyperresponsiveness defined as a PC<sub>20</sub>FEV<sub>1</sub> ≥8 mg/mL methacholine. Predicted values were taken from the European Respiratory Society recommendations. In all patients, medication with inhaled β<sub>2</sub>-adrenoceptor agonists (n=32), inhaled corticosteroids (n=27), oral steroids (n=15), theophylline (n=11), and disodium cromoglycate (n=3) remained unchanged during the course of the study. Treatment with β<sub>2</sub>-adrenoceptor agonists was withheld 6 h prior to each challenge, whereas treatment with other medications was continued. The study was approved by the Ethics Committee of the Chamber of Physicians of Schleswig-Holstein.

**The Standard Provocation Protocol**

This protocol essentially followed the recommendations of Chai et al. After inhalation of isotonic saline solution, the patients inhaled doubling concentrations of methacholine chloride (0.1, 0.2, 0.5, 1, 2, 4, and 8 mg/mL). We selected 8 mg/mL as the final concentration because it is the threshold concentration indicating normal airway responsiveness. Each concentration was administered in five consecutive slow and deep inhalations from functional residual capacity to near total lung capacity. The aerosol was generated by 0.6 s nebulization at the beginning of each inspiration by a nebulizer (De Vilbiss No. 646; De Vilbiss Co., Somerset, Pa), with an output of 12.4 μg of saline solution per actuation. The same nebulizer, with fixed straw, was used in all challenges and, after purging, refilled with 2 mL of the appropriate solution. Spirometry was performed 2 min after the fifth inhalation. The challenge was stopped when FEV<sub>1</sub> had dropped by ≥20% from baseline. We computed provocative concentrations of methacholine, PC<sub>20</sub>FEV<sub>1</sub>, by linear interpolation from plots of FEV<sub>1</sub> vs the logarithm of concentration. At the end of the challenge, patients received 200 μg of salbutamol from a metered-dose inhaler to relieve airway obstruction.

**The Rapid Provocation Protocol**

Before the challenge, the subjects answered four written questions. (1) Have you had wheezing or whistling in your chest at any time in the last 12 months when you did not have a cold, and have you been at all breathless at this time? (2) Have you had an attack of shortness of breath that came on during the day when you were at rest at any time in the last 12 months? (3) Have you been awakened by an attack of coughing at any time in the last 12 months? (4) Do you have cough on most days for as much as 3 months each year. Positive answers to these questions were assigned scores of 1, 1, 1, and -1, respectively. If baseline FEV<sub>1</sub> was <90% of predicted, a score of 1 was added to the sum of scores derived from the questions.

The newly developed protocol included two subprotocols (Table 1). Subjects started with the long subprotocol if their score was ≥2 as they were judged more likely to be hyperresponsive. Subjects were assigned to the short subprotocol if their score was <2. When FEV<sub>1</sub> had fallen below 90% of baseline values during provocation, the challenge was continued with the long protocol to avoid extreme responses and to measure the provocative concentration with higher precision. This approach was taken from the European Community Respiratory Health Survey (ECRHS), with appropriate modifications. The scores were chosen to maximize the likelihood for detecting a high degree of airway hyperresponsiveness before the challenge. They were derived from an analysis of a subset of the Hamburg population within the ECRHS study.

The short subprotocol included fourfold increments of methacholine concentrations; the long subprotocol included twofold increments (Table 1). Before methacholine, isotonic saline solution was administered in four inhalations. Similar to the standard protocol, the aerosol was generated for 0.6 s at the beginning of slow and deep inspirations from residual lung volume to near total lung capacity. For the different solutions of methacholine chloride (Table 1), we used five different De Vilbiss No. 646 nebulizers with fixed straw, with outputs varying between 12.5 and 13.9 μg per nebulization. Spirometry was performed 1 min after inhalation two times and was repeated until values were reproducible within 100 mL or 5%, whichever was greater. The challenge was stopped when FEV<sub>1</sub> had decreased by at least 20%. Provocative concentrations were derived as described above, and 200 μg salbutamol was given after each test.

The patients were challenged using the standard protocol and the rapid protocol on 2 consecutive days, at the same time of the day.

**Table 1—The Short Provocation Protocol**

<table>
<thead>
<tr>
<th>Step</th>
<th>Concentration, mg/mL</th>
<th>No. of Breaths</th>
<th>Concentration Equivalent to 5 Breaths, mg/mL</th>
<th>Cumulative Dose cbu&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Subprotocol</th>
<th>Subprotocol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Short</td>
<td>Long</td>
<td>Short</td>
<td>Long</td>
<td>Short</td>
</tr>
<tr>
<td>1</td>
<td>0.104</td>
<td>–</td>
<td>2</td>
<td>0.125</td>
<td>0.04</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>0.104</td>
<td>6</td>
<td>4</td>
<td>0.125</td>
<td>0.08</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>0.42</td>
<td>–</td>
<td>2</td>
<td>0.125</td>
<td>0.17</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>0.42</td>
<td>6</td>
<td>4</td>
<td>0.125</td>
<td>0.34</td>
<td>3.14</td>
</tr>
<tr>
<td>5</td>
<td>1.67</td>
<td>–</td>
<td>2</td>
<td>0.125</td>
<td>0.67</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>1.67</td>
<td>6</td>
<td>4</td>
<td>0.125</td>
<td>1.34</td>
<td>13.16</td>
</tr>
<tr>
<td>7</td>
<td>6.67</td>
<td>–</td>
<td>2</td>
<td>0.125</td>
<td>2.67</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
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<td>6</td>
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<td>0.125</td>
<td>5.34</td>
<td>53.18</td>
</tr>
<tr>
<td>9</td>
<td>26.67</td>
<td>2</td>
<td>2</td>
<td>0.125</td>
<td>10.67</td>
<td>106.52</td>
</tr>
</tbody>
</table>

<sup>a</sup>Concentration of methacholine that would be required in the standard protocol using five inhalations to administer the same dose of methacholine as in the rapid protocol using a different number of inhalations. These concentrations were used for the computation of PC<sub>20</sub>FEV<sub>1</sub> in the rapid protocol.

<sup>b</sup>Cumulative breath units as defined in reference 2. One cbu is equivalent to one inhalation of a solution of the concentration 1 mg/mL. To obtain the total cumulative dose administered, cbu values have to be multiplied by the output of the nebulizer per actuation.
Results

In 29 subjects, challenges were started with the short subprotocol and in 19 subjects challenges were started with the long subprotocol; among the latter, 11 tests were switched to the long subprotocol. Forty subjects showed PC_{20}FEV_{1} ≤ 8 mg/mL in the standard protocol, with a geometric mean (geometric SD) PC_{20}FEV_{1} of 1.21 (1.9) mg/mL. All of them also showed PC_{20}FEV_{1} ≤ 8 mg/mL in the rapid protocol, with a mean of 1.34 (2.2) mg/mL. Eight subjects demonstrated PC_{20}FEV_{1} > 8 mg/mL in both protocols. PC_{20}FEV_{1} of the rapid procedure differed from PC_{20}FEV_{1} of the standard procedure by ≤ 0.5 and > 1 doubling concentrations in 21 (52.5%) and two (5%) patients, respectively (Fig 1).

We computed the linear correlation coefficient (r = 0.95, p < 0.001) and the linear regression line between log PC_{20}FEV_{1} of the two protocols. Slope and intercept of this line indicated that it was not significantly different (p > 0.05 each) from the line of identity, with slope one and intercept zero.

The mean ± SD maximum percent fall in FEV_{1} from baseline was 27.4 ± 8.5% for the standard method and 26.4 ± 6.6% for the rapid method. In 29 of 40 patients, the fall in FEV_{1} with the rapid protocol was ≥ 20 and ≤ 30%; in nine patients > 30 and ≤ 40%; and in two patients > 40 and ≤ 48%.

The mean ± SD time required was 34 ± 11 (range, 19 to 55) min for the standard method and 15 ± 3 (range, 9 to 25) min for the rapid method. The long subprotocol took 14.7 ± 4.2 min, the short subprotocol took 14.0 ± 2.5 min, and the tests that were started with the short and continued with the long subprotocol took 15.7 ± 2.5 min. In these patients, the standard protocol required 28.1 ± 8.1 min, 33.3 ± 8.8 min, and 39.5 ± 13.7 min, respectively.

Discussion

Our data demonstrate that the rapid provocation protocol is a safe procedure that yields provocative concentrations equivalent to those obtained with the well-known standard protocol as described by Chai and coworkers. However, it requires only about half as much time.

A multitude of provocation protocols has been described in the past 2 decades, and various protocols were reported to shorten the procedure. There are several measures to abbreviate a challenge protocol: (1) by decreasing the time between inhalations, (2) by using fourfold or higher increments in concentrations, and (3) by choosing a higher starting concentration. These approaches, however, can result in a loss of precision or in an increased risk of an unnecessarily high degree of airway obstruction. In a previous study, the protocol, which was an abbreviated version of the standard procedure, led to a fall in FEV_{1} of > 40% in 38.5% of patients as compared with 5% of patients in our study. Furthermore, as judged from that study, 9 of 13 subjects showed a difference of > 1 doubling dose between the short and the standard protocol.

Our protocol was designed following the approach chosen in the ECRHS study. We adopted the idea of assigning patients to a long or a short subprotocol according to their answers to selected questions on respiratory symptoms and baseline lung function as well as the idea of switching to the long protocol during the challenge if FEV_{1} decreased by ≥ 10%. Essentially, we aimed to reduce the likelihood that a subject would respond at the lowest two doses of the short subprotocol. This was sufficient for a maximum of safety and accuracy in the whole protocol, because the challenge was eventually switched to the long subprotocol. The negative score for question 4, which may appear surprising, was helpful in predicting just those subjects who were only mildly hyperresponsive. By this approach, we avoided severe airway responses, particularly at the beginning of the test, maintained the precision of PC_{20}FEV_{1} determination, and simultaneously reduced the number of subjects who were assigned to the long subprotocol.

The concentrations of methacholine were chosen to match those described for the standard procedure as closely as possible (Table 1) and to reproduce the threshold concentration of 8 mg/mL, which represents the upper limit of airway hyperresponsiveness. Each concentration of methacholine was...

Figure 1. Provocative concentrations causing a 20% fall in FEV_{1} (PC_{20}FEV_{1}) obtained with the rapid provocation protocol vs provocative concentrations measured with the standard provocation protocol. The straight lines indicate the line of identity and deviations of one doubling concentration from the line of identity.
administered in at least two inhalations to reduce the potential variation in the deposited dose that would result from single inhalations.

We developed our rapid protocol as adapted to the dosimeter technique published by Chai and coworkers,2 because this procedure is widely used for clinical and scientific purposes. The protocols used by different groups may deviate in minor aspects from the original protocol with respect to the concentrations chosen and the data evaluation. For example, we decided not to compute cumulative breath units (cbu) as described by Chai et al2 but to express the results directly in terms of provocative concentrations, PC<sub>20</sub>FEV<sub>1</sub>. The reason was that provocative concentrations appeared to be more easily interpreted than the cbu. However, to facilitate the evaluation in terms of cumulative doses, we included the equivalent cbu values in Table 1. It may be noteworthy that our protocol was designed to achieve a close correspondence between provocative concentrations but not cumulative doses. Probably owing to some cumulative effect of methacholine and the different sequences of concentrations in the standard and the rapid protocol, cumulative provocative dosages differed by >1 doubling dose in 4 of 40 patients compared to 2 of 40 patients for PC<sub>20</sub>FEV<sub>1</sub>. Our method may yield results that are also comparable to those of other provocation methods.5,7,14,16 It has already been shown that results of the Rosenthal-Chai protocol2 are comparable to those obtained with continuous generation of an aerosol and tidal breathing.7,8,15,17

To test the applicability and variability of the rapid procedure under the conditions of clinical practice, we studied patients with asthma and patients without asthma who were not trained in performing bronchial challenges. Potential sources of variation were reduced by maintaining regular treatment with medication and by performing the tests on two consecutive days at the same time of day.3

In summary, our data demonstrated that the rapid provocation protocol produces results that are equivalent to those of a widely used standardized dosimeter technique but requires only about 50% of the time. Therefore, the rapid method could be appropriate in clinical studies that require comparability with values obtained with the Rosenthal-Chai dosimeter method.

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REFERENCES