Development of a Methacholine Challenge Method to Minimize Methacholine Waste*

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Background: The standard 2-min tidal breathing methacholine challenge utilizes 3 mL to produce an output of 0.26 mL per 2 min, resulting in a substantial amount of methacholine being discarded.

Objective: To develop a method with reduced methacholine waste and to compare it to the standard method.

Methods: Twelve subjects with mild, well-controlled asthma volunteered for this investigation. They underwent three methacholine challenges in random order. The first challenge was the conventional 2-min tidal breathing method using 3 mL of doubling concentrations inhaled for 2 min at 5-min intervals. The first modification utilized 1.5 mL of quadrupling concentrations inhaled for 1 min and then 2 min, keeping the time interval constant at 3 min between completion of one inhalation and commencement of the next inhalation. The second modification utilized 1.5 mL of eightfold concentration step-ups inhaled for 30 s, 60 s, and 120 s with a time interval of 3 min between completion of one inhalation and commencement of the next inhalation. For each method, the provocative concentration of methacholine causing a 20% fall in FEV1 (PC20) was calculated based on a 2-min equivalent-dose inhalation.

Results: There was no significant difference in the geometric mean PC20 (1.5 mg/mL, 1.6 mg/mL, and 1.6 mg/mL for the three methods, respectively; p = 0.47). The quadrupling concentration method was preferred because it was less subject to error than the other modification.

Conclusion: The amount of methacholine discarded during a methacholine challenge can be reduced by two thirds by decreasing the volume from 3 to 1.5 mL, and by using quadrupling concentrations inhaled either with quadrupling-dose step-ups, or with doubling-dose step-ups by using sequential 1-min and 2-min inhalations.

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Key words: airway responsiveness; asthma; methacholine; provocative concentration causing a 20% fall in FEV1

Abbreviations: CI = confidence interval; PC20 = provocative concentration of methacholine causing a 20% fall in FEV1

The standardized 2-min tidal breathing methacholine inhalation challenge is widely used.1,2 The standard method requires 3 mL of each concentration nebulized once for 2 min at 0.13 mL/min (total, 0.26 mL); the remainder of the methacholine solution, approximately 90%, is discarded. The purpose of the current investigation was to investigate and compare two modifications that would reduce the amount of discarded methacholine.

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MATERIALS AND METHODS

Subjects

Twelve subjects with mild, well-controlled asthma were selected. Anthropometric data are listed in Table 1. All subjects had an FEV₁ ≥ 70% of predicted and a provocative concentration of methacholine causing a 20% fall in FEV₁ (PC₂₀) ≤ 16 mg/mL. The investigation was done at a time when symptoms were stable, allergen exposure was not present for ≥ 4 weeks, and there was no history of a respiratory tract infection for ≥ 4 weeks. Inhaled corticosteroids (n = 4) were continued in the same dose. Approval was granted by the University of Saskatchewan Ethics Committee, and signed informed consent was obtained.

Standard Methacholine Method

The standard 2-min methacholine method was performed as previously outlined.2,3 The inhalations are commenced with normal saline solution followed by doubling concentrations of methacholine available from 0.03 to 16 mg/mL. Starting concentrations of methacholine were adjusted based on guidelines2,3 and knowledge of subject’s previous PC₂₀ value. In the standard method, 3 mL of saline solution and of each solution were nebulized with a Bennett Twin jet nebulizer (Puritan-Bennett Corporation; Carlsbad, CA) calibrated to deliver 0.13 mL/min. The nose was clipped and aerosols were inhaled via a facemask with 2 min of tidal breathing. Full spirometry was measured in triplicate prior to the commencement of the test. Following the saline solution and each concentration, FEV₁ (without a full FVC maneuver) was repeated once at 30 s and 90 s. The time interval between inhalations was 5 min from the commencement of one inhalation to the commencement of the next inhalation, between inhalations was 5 min from the commencement of one maneuver) was repeated once at 30 s and 90 s. The time interval between the completion of one inhalation and the commencement of the next inhalation. The percentage fall in FEV₁ was calculated from the lowest post-saline solution FEV₁ to the lowest postmethacholine FEV₁ from the log dose vs response curve using an algebraic formula.4,5

Modified Method 1

The first modification involved reducing the volume by one half and eliminating every second concentration (ie, using four-fold dilutions up to 8 mg/mL). The test was carried out in the same fashion with saline solution followed by doubling amounts of methacholine inhaled by tidal breathing. The volume used in the nebulizer was 1.5 mL. In order to reproduce the doubling-dose protocol, each quadrupling concentration was inhaled for 60 s and 120 s sequentially prior to discarding. After some deliberation, we elected to keep the time interval between the completion of one inhalation and the commencement of the next inhalation constant at 3 min. Measurement of the response and calculation of the PC₂₀ was identical to the standard method. For calculation of the PC₂₀, the methacholine dose was prorated to a 2-min inhalation, ie, 8 mg/mL inhaled for 1 min was deemed to be equivalent to 4 mg/mL inhaled for 2 min as had been previously shown for both histamine and methacholine.6,7 The volume reduction reduces the wasted methacholine by 50%, and the elimination of every second concentration (4 mg/mL, 1 mg/mL, 0.25 mg/mL, etc) results in a further one-third reduction in waste; this reduces the amount of methacholine required for a challenge by two thirds (67%).

Modified Method 2

The second modification was done using 1.5 mL of solutions and an eightfold step-up in concentrations. The methacholine solutions were inhaled for 30 s, 60 s, and 120 s with a 3-min interval between the completion of one inhalation and the commencement of the next inhalation. The measurement of the response and calculations of the PC₂₀ (once again prorated to a 2-min inhalation) was done as per the other method. This method results in a 50% reduction in wasted methacholine by the volume change and a further 3/7 reduction by the concentration changes, resulting in 71% less wastage.

Study Design

Subjects attended the laboratory on 3 days at the same time of day within a 10-day period. Intermediate acting inhaled β₂-agonists were withheld for ≥ 6 h, anticholinergic bronchodilators were not used, and inhaled corticosteroids (n = 4) were continued in the same dose. Subjects rested in the laboratory for 15 min prior to commencing the methacholine challenge. The identical nebulizer (of two) was used for each challenge in an individual; these nebulizers were calibrated with 1.5 mL of saline solution and gave identical output at the same flow as with 3 mL. The starting methacholine dose was identical for all three challenges within an individual. The order of the three challenges was randomized in blocks of six.

Analysis

The PC₂₀ values were analyzed by log transformation with a two-way analysis of variance (subject, method). The study had > 95% power to detect a one-half concentration difference in PC₂₀.

RESULTS

All subjects completed the study with no adverse events. There was no difference in PC₂₀ values for the three methods (p = 0.47). The geometric mean PC₂₀ values were 1.5 mg/mL (mean log ± SEM, 0.17 ± 0.17; 95% confidence interval [CI], −0.20 to 0.54) for the standard method, 1.6 mg/mL (mean log ± SEM, 0.22 ± 0.16; 95% CI, −0.13 to 0.56) for modified method 1, and 1.6 mg/mL (mean

Table 1—Baseline Data*

<table>
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<th>Subject No.</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Height, inches</th>
<th>FEV₁, L</th>
<th>%</th>
<th>PC₂₀, mg/mL</th>
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*ICS = inhaled corticosteroids.
log ± SEM, 0.22 ± 0.18; 95% CI, −0.17 to 0.60) for modified method 2. The individual data points are shown in Figure 1. There was no sequence effect (1.7 mg/mL, 1.5 mg/mL, and 1.6 mg/mL, respectively, for the three methods; \( p = 0.49 \)).

**DISCUSSION**

These data indicate that the amount of discarded methacholine can be reduced easily and by an amount of two thirds without affecting the test result. Of the two modifications tested here, we prefer the first modification, namely the modification involving quadrupling concentrations inhaled sequentially for 1 min and 2 min to produce the doubling-dose protocol. In fact, American Thoracic Society recommendations have suggested the quadrupling dose protocol could be considered optional for the five counted breath technique; however, since the two methods recommended are comparable in all respects, a quadrupling methacholine step-up can equally be applied to the 2-min tidal breathing method, saving not only methacholine but time. A couple of minor issues made the second modification (eightfold concentrations inhaled for 30 s, 60 s, and 120 s) less desirable for routine clinical use. Importantly, the three different inhalation times provide a template for confusion and error. In at least an occasional subject, the nebulizer seemed to be malfunctioning as it got toward the end of the third nebulization, suggesting there was not quite enough liquid in the nebulizer, particularly if it was not held absolutely vertical.

Our theoretical concerns regarding these modifications, namely the reduced volume and the repeated nebulizations, were regarding evaporation. A considerable portion of the measured so-called nebulizer output from a jet nebulizer is actually evaporation. The evaporative losses from the solution in the nebulizer result in a progressive increase in concentration of the agent(s) remaining in the nebulizer. For this reason, we (and others) have recommended that the volume used in the nebulizer be kept constant and that solutions be discarded after a single use. Evaporative effects leading to increased methacholine concentration in the nebulizer would be exaggerated by both reduction of the volume used and by the repeated nebulizations. We were thus concerned that the modified methods might demonstrate slight increases in effect (ie, reductions in PC\textsubscript{20}). No such changes were seen with these modifications.

The only nebulizer studied was the Bennett Twin jet nebulizer that we currently use both clinically and in research. These results, however, should be applicable to other nebulizers, including the Wright nebulizer (Roxon Medi-tech; Montreal, QC, Canada), provided they can be adequately and reproduc-

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/20384/ on 06/26/2017)
ibly calibrated at the lower volume. We were able to show in two of our Wright nebulizers that identical outputs could be obtained at the same flow rates using either 1.5 mL or 3 mL.

In summary, the amount of methacholine discarded during a methacholine challenge can be reduced by a factor of two thirds by utilizing half the volume (1.5 mL vs 3 mL) and quadrupling rather than doubling concentrations. A doubling-dose protocol can be obtained by inhaling quadrupling concentrations sequentially for 1 min and then 2 min or, as suggested by the American Thoracic Society, a quadrupling-dose step-up can be safely used.

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