Difference Between Dosimeter and Tidal Breathing Methacholine Challenge*

Contributions of Dose and Deep Inspiration Bronchoprotection

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Background: Two bronchoprovocation methods are widely used. Compared to the tidal breathing method, the dosimeter method delivers approximately half the dose and involves five deep inhalations. Both the lower dose and the bronchoprotective deep inhalations contribute to the lesser airway response of the dosimeter.

Objective: To determine the relative role of dose and deep inspiration in the difference between the two methods.

Methods: Subjects with asthma (n = 24) underwent three methacholine challenges: a dosimeter challenge, a 2-min tidal breathing challenge (twice the dose), and a modified 2-min tidal breathing challenge (twice the dose plus five deep inhalations).

Results: The dosimeter method produced a nonsignificantly lower response than the modified tidal breathing method (p = 0.14). Both deep inhalation methods produced significantly less response than did the standard tidal breathing method (p = 0.011). In the 12 subjects with the most mild airway hyperresponsiveness (AHR), the differences between the deep inhalation method and the tidal breathing method were greater (p = 0.007). By contrast, deep inhalations produced no effect in the 12 subjects with greater AHR; the two tidal breathing methods produced identical results, while the dosimeter produced less response than either (p = 0.033). Six current asthmatics with mild airway responsiveness (tidal breathing method) had negative dosimeter methacholine challenge results.

Conclusions: In subjects with moderate airway responsiveness, the difference between the methods is due to the difference in dose, whereas in subjects with mild AHR, deep inhalations had a large effect overwhelming the dose effect and producing false-negative methacholine challenge results in 25% of the subjects.

Key words: airway responsiveness; deep inspiration; dosimeter; methacholine; tidal breathing method

Abbreviations: AHR = airway hyperresponsiveness; ANOVA = analysis of variance; ATS = American Thoracic Society; PC_{20} = provocation concentration of methacholine producing a 20% fall in FEV_{1}; T_i/T_{tot} = duty cycle; TLC = total lung capacity

Methacholine inhalation challenges are widely used to measure airway hyperresponsiveness (AHR) both in research and clinical settings. Current guidelines from the American Thoracic Society (ATS)\textsuperscript{1} outline two methods for performing methacholine challenges: the five-breath dosimeter method, and the 2-min tidal breathing method. The 2-min tidal breathing method\textsuperscript{2} delivers approximately twice the volume (90 µL vs 45 µL) at each methacholine concentration, compared to the five-breath dosimeter method; this is due primarily to differences in the method of inhalation. The dosimeter method requires five inhalations to total lung capacity (TLC) with a 5-s breathhold at TLC on five occasions, whereas subjects performing a tidal breathing method take no deep inhalations until the first spirometric measurement of response. We have demonstrated that the dosimeter provocation concentration of methacholine producing a 20% FEV\textsubscript{1} fall (PC\textsubscript{20}) was nearly twice that of the tidal breathing
PC\textsubscript{20}. The difference in PC\textsubscript{20} between the two methods in subjects with mild AHR (tidal breathing PC\textsubscript{20} > 1 mg/mL) was larger than those with moderate-to-severe AHR (PC\textsubscript{20} < 1 mg/mL) [3.2-fold vs 1.6-fold].\textsuperscript{3} Several studies\textsuperscript{4–6} have investigated the effect of deep inhalations on methacholine-induced bronchoconstriction. These studies\textsuperscript{4–6} have shown that deep inspirations provide marked bronchoprotection in normal subjects and some subjects with mild AHR, whereas there was little or no effect at preventing bronchoconstriction in subjects with asthma, \textit{i.e.}, those with more significant AHR. In the present study, we attempted to determine the relative roles of the dose difference and the deep inhalations both of which should contribute to the dosimeter method producing less response, \textit{i.e.}, a higher PC\textsubscript{20}.

\section*{Materials and Methods}

\subsection*{Subjects}

Subjects with asthma were recruited from the University of Saskatchewan student population and from the Royal University Hospital respiratory clinic. Inclusion criteria included a diagnosis of current asthma, a tidal breathing PC\textsubscript{20} \textleq 16 mg/mL, FEV\textsubscript{1} > 65\% of predicted, and no respiratory tract infection or allergen exposure for \approx 4 weeks. The study was approved by the University of Saskatchewan Ethics Review Board, and signed consent was obtained.

\subsection*{Study Design}

Subjects performed methacholine challenges in the laboratory on 3 separate days at the same time of day, at least 24-h apart, and all within a 2-week period. The three methacholine challenges were performed in random order and included a standard five-breath dosimeter challenge, a standard 2-min tidal breathing challenge, and a modified 2-min tidal breathing challenge incorporating five deep inhalations with breathhold (Fig 1). Inhaled salbutamol (\(n = 18\)) was withheld for \approx 8 h prior to challenge, inhaled formoterol (\(n = 1\)) was withheld for 36 h, and inhaled corticosteroids (\(n = 4\)) were maintained at the same dose. Eight subjects (four patients with mild AHR and four patients with moderate AHR) performed a supplemental study to measure the duty cycle (\(T\textsubscript{i}/T\textsubscript{tot}\)) during a period of standard 2-min tidal breathing and during tidal breathing modified to include five deep inhalations.

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\section*{Five-Breath Dosimeter Methacholine Challenge}

The standard five-breath dosimeter method was performed as outlined by the ATS.\textsuperscript{1} We used a dosimeter (Koko; Pulmonary Data Services; Doylestown, PA) and a nebulizer (DeVilbiss 646; Sunrise Medical HHG; Somerset, PA) calibrated to deliver 9 \(\mu\)L per breath activation. Complete spirometry was done in triplicate initially. Subjects performed five slow inspiratory capacity inhalations from functional residual capacity to TLC followed by a 5-s breathhold at TLC. In order to match the timing with the modified tidal breathing method (see below), the stopwatch was started at the beginning of each cycle; inhalations were commenced at 20, 40, 60, 80 and 100 s; and spirometry was performed at 150 s and 210 s (approximately 35 to 40 s and 95 to 100 s after the completion of the final breathhold). The next cycle was started precisely at 5 min. Sterile isotonic saline solution was followed by doubling concentrations of methacholine available from 0.03 to 64 mg/mL. We approximated 128 mg/mL in some subjects by doubling the number of inhalations of the 64 mg/mL concentration. Change in FEV\textsubscript{1} was calculated from the lowest post-saline solution FEV\textsubscript{1} to the lowest post-methacholine FEV\textsubscript{1},\textsuperscript{7} and the challenge was continued until the FEV\textsubscript{1} had fallen by \approx 17\% or the top concentration had been administered. PC\textsubscript{20} was then interpolated\textsuperscript{8} or extrapolated\textsuperscript{9} from the log dose vs response curve by algebraic equations.

\section*{Tidal Breathing Methacholine Method}

The tidal breathing methacholine challenge was done using a jet nebulizer (Bennett Twin; Puritan Bennett Corporation; Carlsbad, CA) calibrated to deliver 0.13 mL/min. The patients wore a nose clip, and the aerosols were directed to the mouth via a loose-fitting facemask. Spirometry was initially measured in triplicate and was repeated at 150 and 210 s after the commencement of each inhalation (\(i.e.\), 30 s and 90 s after completion). Other features including the available concentrations, the starting concentrations, the timing between doses, and the calculation of the PC\textsubscript{20} was identical to the dosimeter method.

\section*{Modified Tidal Breathing Methacholine Challenge}

The modified tidal breathing method utilized the same equipment as the standard tidal breathing method. During the 2 min of tidal breathing, subjects were requested to take a slow inspiratory capacity inhalation starting 20, 40, 60, 80, and 100 s into the 2-minute period of tidal breathing. Subjects were instructed to hold their breath for five seconds at TLC.

\section*{Duty Cycle}

Eight of the subjects returned to the lab to perform two 2-min volume vs time spirometric traces. The first was a period of quiet tidal breathing, the second a period of tidal breathing incorporating the five deep inhalations to TLC and breathhold as in the modified tidal breathing method. The \(T\textsubscript{i}/T\textsubscript{tot}\) was calculated manually.

\section*{Analysis}

PC\textsubscript{20} values were log transformed and analyzed by two-way (method, subject) analysis of variance (ANOVA) using a computerized program (STATISTIX for Windows; Analytical Software; Tallahassee, FL). When the ANOVA was significant, pairwise comparison of means was done using the least-squares difference method. The population was dichotomized and the analyses
repeated in the 12 subjects with the mildest AHR and the 12 subjects with the more severe AHR. Ti/Ti\textsubscript{tot}s were compared using the paired t test.

**Results**

The 24 subjects (13 men and 11 women) were 27 ± 10 (mean ± SD) years of age and 68.3 ± 3.4 inches in height. The FEV\textsubscript{1} was 3.66 ± 0.90 L or 91 ± 14% of predicted. All subjects completed the investigation without adverse events.

The results are summarized in Figure 2, which shows the geometric mean PC\textsubscript{20} for the three methacholine methods in the whole population as well as the 12 subjects with milder AHR (tidal breathing PC\textsubscript{20} ≥ 2 mg/mL) and the 12 subjects with moderate AHR (tidal breathing PC\textsubscript{20} ≤ 2 mg/mL). The overall analysis revealed a highly significant ANOVA (p = 0.0005, n = 24). The dosimeter PC\textsubscript{20} (5.2 mg/mL) and the modified tidal breathing PC\textsubscript{20} (3.6 mg/mL) were not significantly different (p = 0.14), and both were significantly larger than the standard tidal breathing PC\textsubscript{20} (2.0 mg/mL, p = 0.011). In the 12 subjects with milder AHR (PC\textsubscript{20} > 2 mg/mL), these differences appeared greater (ANOVA, p = 0.0019); the dosimeter PC\textsubscript{20} (24 mg/mL) and the modified tidal breathing PC\textsubscript{20} (17 mg/mL) were not significantly different (p > 0.20), and both were larger than the standard tidal breathing PC\textsubscript{20} (5.1 mL) [p = 0.007]. By contrast, in the 12 subjects with greater AHR (PC\textsubscript{20} ≤ 2 mg/mL; ANOVA, p = 0.04), the standard tidal breathing PC\textsubscript{20} and modified tidal breathing PC\textsubscript{20} (0.76 mg/mL and 0.78 mg/mL, respectively) were not significantly different, while the dosimeter PC\textsubscript{20} (1.1 mg/mL) was significantly higher than either (p = 0.033). Six of our subjects with current symptomatic asthma and a positive tidal breathing methacholine challenge result had negative dosimeter challenge results (PC\textsubscript{20} values from 28 to > 128 mg/mL).

The Ti/Ti\textsubscript{tot}s for the two 2-min maneuvers were 0.36 and 0.33 for the tidal breathing and modified breathing methods, respectively. These were neither clinically nor statistically (p > 0.05) significantly different.

**Discussion**

These data confirm our previous observation that the dosimeter PC\textsubscript{20} is larger than the tidal breathing PC\textsubscript{20}.\textsuperscript{3} In the current study, the differences were greater perhaps because of a heavier weighting toward milder AHR, a larger percentage of subjects with false-negative dosimeter challenge results (25%
vs 8%), and because we measured PC$_{20}$ in the nonresponders up to 128 mg/mL. The difference between the two methods was greater in the subjects with mild AHR, and this appeared to be due, to a large extent, to the bronchoprotective effect of the deep inspiration. In contrast, in subjects with more significant AHR (PC$_{20}$ < 2 mg/mL), the difference between the two methods was less than one doubling concentration and was entirely due to dose since the deep inhalation did not appear to influence the tidal breathing method. Similar observations were made by Bennett and Davies, who observed that, in subjects who had PC$_{20}$ < 2 mg/mL, the tidal breathing method produced a lower PC$_{20}$ than the dosimeter. However, the differences disappeared when corrected for the dose delivered.

The important observation from this study is the profound inhibitory effect of the five deep inhalations/breathholds on the airway response to methacholine in clinical asthmatics with mild AHR. This effect was so marked that the dose difference, also present in the subjects, was overwhelmed and was no longer statistically significant.

The small subinvestigation comparing T$_i$/T$_{TOT}$ during the modified tidal breathing method and the standard 2-min tidal breathing method allows for differentiation between dose delivered and bronchoprotection from deep inspiration as contributors to differences in PC$_{20}$. We observed no change in T$_i$/T$_{TOT}$, which suggests that any differences in PC$_{20}$ cannot be explained by changes in dose.

There is a strong correlation between clinical
severity of asthma and degree of airway responsiveness. In addition, it has been suggested that AHR might be caused by a loss of the bronchodilating/bronchoprotecting effect of deep inspiration in subjects with asthma. The reasons for the loss of this bronchoprotection in hyperresponsive individuals has been extensively studied, and several theories have emerged. First, it has been suggested that in asthmatics, airway smooth muscle exists in a frozen state with an increased number of latch bridges between actin and myosin, rendering the muscle stiffer and resistant to stretch. In addition, chronic smooth-muscle stimulation in asthmatics may lead to the polymerization of both actin and myosin, leading to a decreased need for the airway muscle to dissolve its contractile apparatus and increase the number of contractile units in series. Thirdly, Wang and Pare have suggested that the asthmatic environment can lead to a conversion of the normal multi-unit airway smooth muscle into single-unit airway smooth muscle that would be able to generate action potentials rather than the graded depolarization seen in multunit smooth muscle; this may lead to a myogenic response to stretch. Finally, an increase in the amount of myosin light-chain kinase, in asthmatic airway smooth muscle, could lead to an increase in the velocity of renarrowing of the airways.

There may be other mechanisms contributing to the differential response between the two methods and indeed difference between studies. These include differences in the technical aspects of the nebulizers used, differences in aerosol deposition and retention between methods, and possibly the small difference in the time between completion of inhalation and the first spirogram. The two previous studies comparing the dosimeter and tidal breathing method both used Wright nebulizers for the tidal breathing method. Some Wright nebulizers underestimate the response. The similarity of response between the two methods as suggested by the ATS, despite the dose difference, was hypothesized to be due to superior deposition and retention of aerosol with the dosimeter method. Indeed, in our subjects who did not exhibit the bronchoprotective effect of the deep inhalation (ie, those with PC20 < 2 mg/mL), the differential response was less than would be expected considering the dose difference indicating that deposition and retention may be greater for the dosimeter method but not quite enough to overcome the dose difference. In an effort to standardize the timing of the deep inhalations between the dosimeter and the modified tidal breathing method, we inadvertently caused the first FEV1 in the dosimeter method to be 5 s or 10 s later than the usual (ie, 35 to 40 s after completion of the last inhalation vs 30 s by the standard method). It is unlikely that this 5-s or 10-s difference had any effect. The effect of delaying the first FEV1 maneuver is in the direction of enhancing the response and, thus, in the opposite direction to the differences that we observed.

Our study suggests that subjects with mild, well-controlled asthma and mild airway hyperresponsiveness behave more like normal subjects with regard to the bronchoprotective effect of a deep inhalation. The majority of subjects who would be candidates for a diagnostic methacholine challenge, ie, those with symptoms and normal resting lung function, will have airway responsiveness in the borderline-to-mild range. It is these very subjects in whom the dosimeter method inhibits the response; in this study, 6 of the 12 subjects with tidal breathing PC20 between 2 mg/mL and 16 mg/mL had a false-negative dosimeter methacholine challenge result. Even if the dosimeter cut points were shifted upward, ie, to 32 mg/mL, as we previously suggested, there were still 5 of the 12 subjects with false-negative methacholine challenge results. This is an extremely important observation clinically since the major strengths of the direct bronchoprovocation challenges (histamine, methacholine) is the very high sensitivity. The high sensitivity equates to very few false-negative challenge results in subjects with current symptomatic asthma. The data from our current study as well as the recent comparison study document that the commonly used dosimeter method with its five inhalations to TLC and five 5-s breathholds results in a potentially marked reduction in the sensitivity of the challenge leading to potential for misdiagnosis. Alternative methacholine challenge methods that do not involve deep inhalations would include the 2-min tidal breathing technique, or a modified dosimeter challenge with submaximal inhalations that has previously been shown not to inhibit the airway response to methacholine.

In conclusion, these data confirm that the dosimeter methacholine challenge produces less response than the tidal breathing method. This is due in small part to the 50%-smaller dose administered at each concentration and in large part to the bronchoprotective effect of the five deep inhalations, which are an integral part of the method. The importance of the bronchoprotective effect of the deep inhalations was confirmed using a modified tidal breathing method that incorporated five deep inhalations. The bronchoprotective effect of the deep inhalations was limited to subjects with the milder airway responsiveness; in this study, subjects with tidal breathing PC20 values between 2 mg/mL and 16 mg/mL. Unfortunately, this is the range of results expected for many subjects undergoing diagnostic methacho-
line challenges. In this study, 25% of the entire population, and 50% of those whose tidal breathing PC_{20} values were between 2 mg/mL and 16 mg/mL exhibited false-negative methacholine challenges with the dosimeter method. We would strongly suggest that the dosimeter method be performed with submaximal inhalations.

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