

An In Vitro Study to Investigate the Use of a Breath-Actuated, Small-Volume, Pneumatic Nebulizer for the Delivery of Methacholine Chloride Bronchoprovocation Agent

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BACKGROUND: Current American Thoracic Society and American Association for Respiratory Care guidelines for the delivery of aerosol agents such as methacholine chloride (MC) for bronchoprovocation testing require the use of pneumatic jet nebulizers that have well-defined droplet size and mass output. A recently developed disposable, breath-actuated nebulizer (AeroEclipse) may offer bronchoprovocation testers an alternative to existing devices. **METHODS:** We studied the performance of 5 AeroEclipse nebulizers with regard to mass of MC delivered with various MC solution concentrations and numbers of inhalations, using a model of adult tidal breathing. Each nebulizer was operated with compressed air (8 L/min at 50 psig) and an initial fill of 2 mL. MC solutions with mass concentrations of 0.25, 0.98, 3.85, and 15.70 mg/mL were tested. The total mass of MC delivered was determined after 5, 10, and 15 complete breathing cycles, by assaying the MC collected on a filter placed at the nebulizer mouthpiece. The aerosol droplet size distribution, fine droplet fraction (FDF) (percentage of droplets < 4.8 μ m diameter), and fine droplet mass (FDM) (mass of droplets < 4.8 μ m diameter) were determined by laser diffractometry, using physiologically normal saline as a surrogate for MC solution. **RESULTS:** The mean \pm SD FDM collected in 5 breathing cycles was 654 ± 29 μ g with the 15.70 mg/mL solution, 158 ± 9 μ g with the 3.85 mg/mL solution, 37 ± 3 μ g with the 0.98 mg/mL solution, and 7 ± 2 μ g with the 0.25 mg/mL solution. FDM showed a linear correlation ($r^2 = 0.9999$) with MC concentration, within the range studied. FDM also showed a linear correlation ($r^2 = 0.999$) with the number of breathing cycles. For instance, with the 15.70 mg/mL solution, FDM was 654 ± 29 μ g with 5 breathing cycles, $1,228 \pm 92$ μ g with 10 breathing cycles, and $1,876 \pm 132$ μ g with 15 breathing cycles. **CONCLUSIONS:** Although the bronchoprovocation test procedure had to be slightly modified from the guidelines to accommodate the operation of the AeroEclipse's breath-actuation feature, our measurements indicate that a predictable dose of MC, within the useful range for bronchoprovocation testing, can be delivered to an adult patient breathing tidally. The green indicator on the AeroEclipse could be used to coach the patient to inhale for a specific period, thereby controlling MC delivery per breathing cycle. *Key words:* methacholine chloride, bronchoprovocation, AeroEclipse, dosimeter, aerosol, nebulizer. [Respir Care 2003;48(1):46–51]

Introduction

Challenging the airways of patients who have potential or actual hyperresponsiveness, using a bronchoprovoca-

tion agent such as methacholine chloride (MC), is widely practiced as part of the process towards reaching a diagnosis of asthma.¹ Although several different MC dose protocols have been developed,² guidelines recently published by both the American Thoracic Society (ATS)³ and the American Association for Respiratory Care⁴ describe current practices. In particular, the ATS guidelines define in detail a 2-min tidal breathing protocol and a 5-breath dosimeter protocol, which formed the basis of our study design herein.

Both the 2-min tidal breathing protocol and the 5-breath dosimeter protocols describe the use of pneumatic nebulizers for delivery of the challenge agent. The 2-min tidal breathing protocol specifies using a nebulizer capable of delivering an aerosol with a fixed and well-defined droplet size distribution and a mass median aerodynamic diameter

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between 1.0 and 3.6 μm , with the flow adjusted to provide an output of 0.13 ± 0.013 mL/min. Although specific nebulizer brands are identified, other designs may be used as long as the basic performance specifications are met.

The 5-breath dosimeter protocol specifies a nebulizer capable of delivering 9.0 ± 0.9 μL per actuation in 0.6 s of the inhalation portion of each breathing cycle. That type of nebulizer is referred to as a dosimeter because it delivers a defined volume (and mass) of challenge agent for a specified time per inhalation. There is therefore a choice between the use of a standard nebulizer having well-defined droplet size and liquid delivery characteristics and a dosimetric nebulizer, the volume delivery of which is specified for a given duration, depending on the protocol followed.

Each nebulizer type has advantages and disadvantages. The simple jet nebulizers used for the 2-min tidal breathing protocol are easy to obtain, but the performance of the various models differs markedly,⁵ and the reproducibility from one unit to another of the same type can be poor with some of the disposable designs.⁶ In general, non-dosimetric nebulizers suffer the drawback that they continue to nebulize during exhalation, so measures must be taken to prevent fugitive MC emissions from being inhaled by the person administering the test. Such protection measures include a low resistance filter to capture aerosol in the patient's exhaled breath.³ Dosimetric nebulizers afford greater control of fugitive emissions when they are operated during the inhalation portion of each breathing cycle. Dosimetric nebulizers require some form of triggering to synchronize dose delivery with the onset of inhalation,³ although one group has reported that exact timing of the dose delivery did not affect the response to MC.⁷ Significant inter-nebulizer differences have also been reported for at least one type of dosimeter.⁸

We studied a recently developed, disposable, breath-actuated, pneumatic nebulizer (AeroEclipse, Monaghan Medical, Plattsburgh, New York) as a candidate device to deliver MC aerosol. Although it can be used to deliver aerosol for a given period to a tidally breathing patient, this device also has the characteristics of a dosimeter, in that aerosol production is initiated shortly after the onset of inhalation, when sufficient flow is generated to operate a mechanism so that solution is drawn up from the reservoir through the nebulization nozzle. Aerosol generation continues only during inhalation, so release of MC droplets during exhalation is greatly reduced.

Methods

MC powder (Provocholine, Methapharm, Brantford, Ontario, Canada) was stored in its original sealed container until required. A stock solution that contained 15.70 mg/mL MC (nominally 16 mg/mL), which is the maximum concentration recommended for both the 2-min tidal breathing

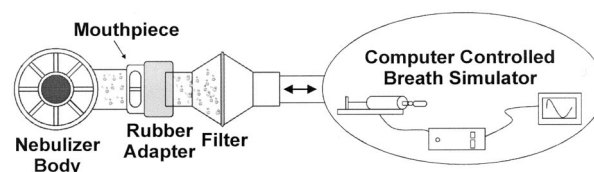


Fig. 1. Breathing simulator setup for determining the mass of methacholine chloride collected during a given number of breathing cycles. Aerosol from the AeroEclipse nebulizer was collected on the filter placed at the nebulizer mouthpiece.

and 5-breath dosimeter protocol, was initially made by dissolving sufficient powder in physiologically normal saline (0.9% weight/volume aqueous NaCl), in accordance with the recommendation in the ATS guidelines.³ This solution was stored at 4° C when not in use, and all measurements were made within a 1-week period, thereby minimizing the risk of degradation.⁹ Further dilutions to make solutions containing nominally 4.0, 1.0, and 0.25% weight/volume MC, in accordance with the shortened dose regimen in the ATS guidelines, were made by diluting a portion of the stock solution with saline immediately before use. A solution containing the lowest recommended MC concentration (0.0625% weight/volume) was also made, but the mass of MC collected from the nebulizer in the performance measurements with that solution was below the limit of detection of the high-performance liquid chromatography assay method (Star HPLC System, Varian Associates, Walnut Creek, California).

Each AeroEclipse nebulizer ($n = 5$, 1 measurement per device) was operated with compressed air at 50 psig and 8 L/min, at ambient temperature of $25 \pm 2^\circ\text{C}$ and relative humidity of $50 \pm 5\%$. Prior to the start of each measurement, 2.0 mL of solution was placed in the reservoir. A breathing simulator (Compas, PARI GmbH, Starnberg, Germany) simulated adult tidal breathing (Fig. 1) with tidal volume of 600 mL, breathing frequency of 10 cycles/min, inspiratory time of 2 s, and inspiratory time/expiratory time ratio of 1:2, selected based on previous experience with this nebulizer.¹⁰ An absolute aerosol electret filter (Model 303, Marquest Medical Products, Englewood, Colorado) was placed at the nebulizer mouthpiece to capture the aerosol produced after 5, 10, or 15 complete breathing cycles, representing the total mass (TM) of MC delivered to the patient's lips. These measurements were repeated with each MC solution concentration.

A laser diffractometer (Mastersizer X, Malvern Instruments, Worcestershire, United Kingdom) equipped with a 100-mm range lens (measurement range 0.2–180 μm diameter) was used to determine the aerosol droplet size distribution. Each nebulizer (5 nebulizers, 3 measurements/device) was operated with the same driving gas conditions as were used for the breathing simulator tests, but the nebulizer reservoir was filled with 2 mL of normal saline

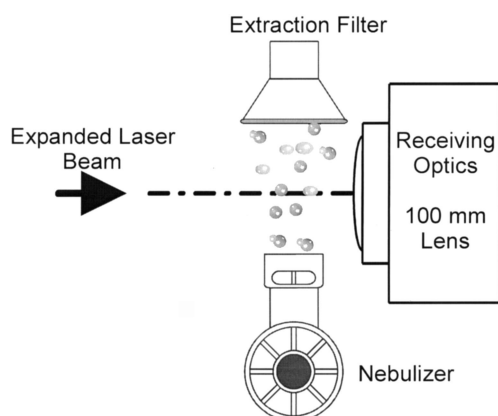


Fig. 2. Laser diffraction setup for determining the aerosol droplet size distribution from the AeroEclipse nebulizer. The aerosol plume was captured by a vacuum system (extraction filter) to prevent recirculation of droplets in the measurement zone.

instead of MC solution. The practice of using an inert surrogate solution for diffraction conforms to the recommendations of a recently published standard for nebulizers.¹¹ Testing with the inert solution avoids releasing MC aerosol into the ambient air during the diffraction (the aerosol is unconfined as it travels through the diffraction laser beam) (Fig. 2). The nebulizer mouthpiece was placed 1–2 cm in front of the detector lens and 1 cm from the edge of the laser beam, to avoid bias due to vignetting.¹² The AeroEclipse's breath-actuation feature was temporarily disabled by depressing the nebulizer's manual control button so that it operated continuously. The aerosol flowed across the path of the laser beam and was captured by a vacuum system, thereby avoiding recirculation of droplets within the measurement zone. This arrangement avoided the need to confine the aerosol in a tube, which would have decreased the accuracy of the measurement because the tube windows necessary for passage of the laser beam would have been subject to fouling by aerosol droplets.¹¹ The droplet size distribution measurements were used to determine the percentage of droplets $< 4.8 \mu\text{m}$ (the fine droplet fraction or FDF) and the mass of those droplets (the fine droplet mass or FDM). Studies of bronchodilator delivery indicate that droplets larger than $4.8 \mu\text{m}$ are unlikely to penetrate to the deep lung.¹³ The FDF and FDM were determined for each MC concentration for 5 (FDM₅), 10 (FDM₁₀), and 15 (FDM₁₅) inhalations, in accordance with the expression:

$$\text{FDM}_{5/10/15} = \text{TM}_{5/10/15} \cdot \text{FDF}$$

The relationships of FDM to MC concentration and to the number of inhalations at different MC concentrations were analyzed (using Sigmaplot software, SPSS, Chicago, Illinois) by least-squares regression. The correlation coefficient

Table 1. Fine Droplet Mass of Methacholine Chloride Aerosol from The AeroEclipse Breath-Actuated Nebulizer*

MC Concentration (mg/mL)		FDM ₅ μg	FDM ₁₀ μg	FDM ₁₅ μg
Actual	Nominal			
15.70	16	654 ± 29	$1,228 \pm 92$	$1,876 \pm 132$
3.85	4.0	158 ± 9	304 ± 22	459 ± 49
0.98	1.0	37 ± 3	65 ± 5	111 ± 6
0.25	0.25	7 ± 2	15 ± 2	25 ± 2

Fine droplet mass (FDM) = mass of droplets $< 4.8 \mu\text{m}$ diameter

*Based on breathing simulator measurements of total mass and determination of fine droplet fraction (percentage of droplets $< 4.8 \mu\text{m}$ diameter) by laser diffraction

MC = methacholine chloride

FDM₅ = FDM collected over 5 breathing cycles

FDM₁₀ = FDM collected over 10 breathing cycles

FDM₁₅ = FDM collected over 15 breathing cycles

Values are mean \pm SD

coefficient (r^2) indicates the goodness-of-fit to a linear relationship (an r^2 of 1.0 represents a perfectly linear relationship).

Results

Table 1 summarizes the mean \pm SD values of FDM₅, FDM₁₀, and FDM₁₅ for each concentration of MC solution. These results were obtained on the basis that, for this group of nebulizers, FDE, expressed as a percentage of the total aerosol sampled by the laser diffraction, was $81.0 \pm 1.5\%$. A representative aerosol droplet size distribution from the AeroEclipse nebulizers (Fig. 3) shows that the mass median aerodynamic diameter was $2.8 \pm 0.1 \mu\text{m}$, which is well within the 1.0 – $3.6 \mu\text{m}$ range given in the ATS guidelines for the 2-min tidal breathing protocol. Particle size variations within that range do not influence the measurement of airway hyperresponsiveness.¹⁴ FDM was linear with respect to MC concentration, based on 5-breathing-cycle data (Fig. 4), with $r^2 > 0.9999$. FDM was also linear with respect to the number of breathing cycles at each MC concentration (Fig. 5), with $r^2 \geq 0.981$.

Discussion

Predictability of delivery of a bronchoprovocation agent such as MC is important for the assessment of airway hyperresponsiveness by challenge testing a patient with progressively higher doses. The liquid volume delivery rate from the nebulizer/dosimeter (expressed either in mL/min or as mL/s-during-each-inhalation) determines the mass of agent that is likely to be inhaled during a challenge test, assuming that the mass concentration of MC is stable and precisely known.⁹ The range of variability specified by the ATS guidelines is $\pm 10\%$ for both the 2-min tidal breathing and 5-breath dosimeter protocol.³ In the

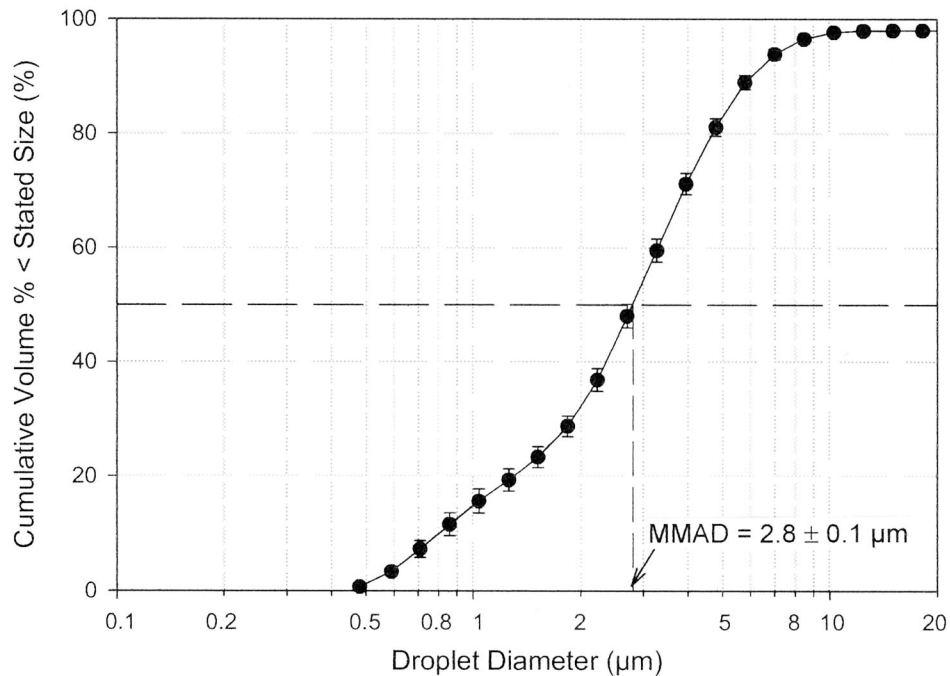


Fig. 3. Representative droplet size distribution data from laser diffractometry of aerosols from 5 AeroEclipse nebulizers. The mass median aerodynamic diameter (MMAD) is the size that corresponds to 50% cumulative volume (ie, 50% of the particles by weight will be smaller than the MMAD).

present study we determined FDM as a more direct measure of MC delivery than liquid feed rate, since it is not necessary to allow for evaporation, which can result in the

measurement of less than the actual liquid volume delivery from the nebulizer, if the surrounding atmosphere is sub-saturated with water vapor. In addition, the contribution of

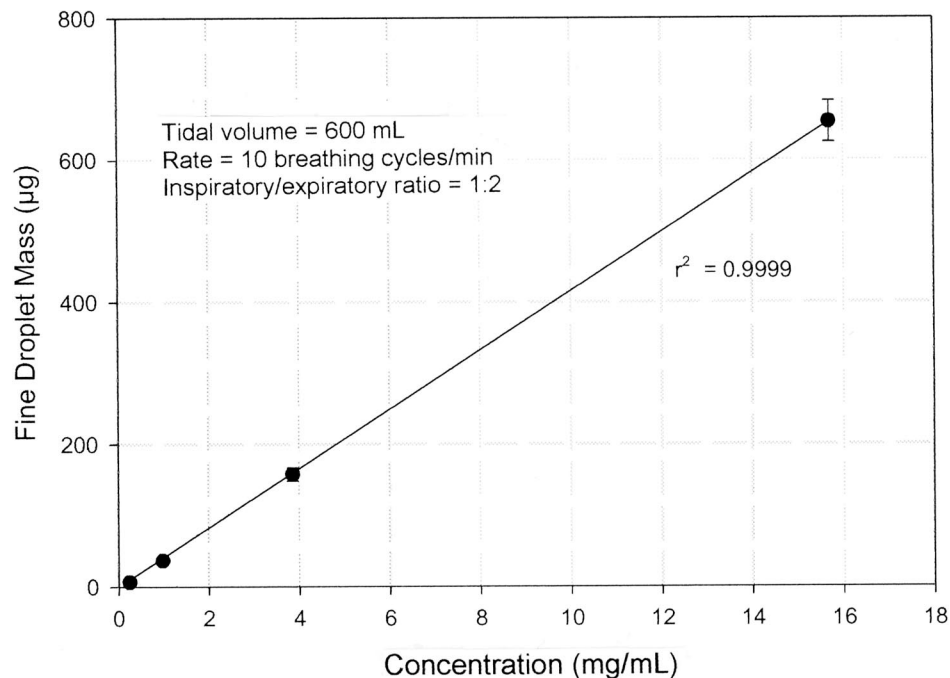


Fig. 4. Fine droplet mass (mass of droplets < 4.8 μm diameter) versus methacholine chloride solution concentration.

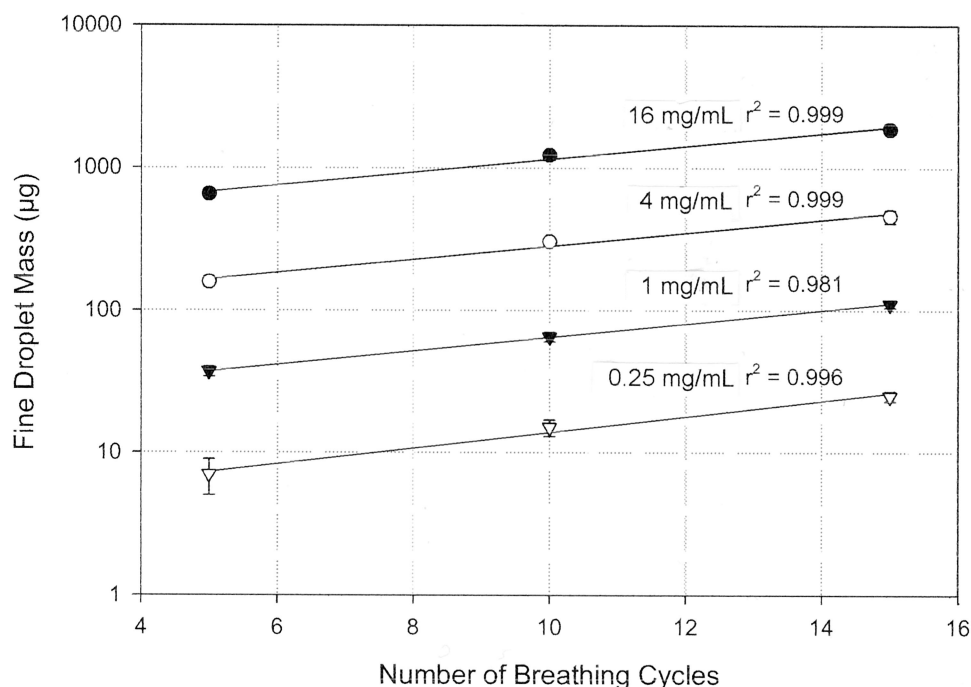


Fig. 5. Fine droplet mass (mass of droplets < 4.8 μm diameter) versus number of breathing cycles. Nominal solution concentrations are shown.

droplets larger than 4.8 μm , which are unlikely to reach the lower airways, was removed by the choice of FDM rather than TM as the performance metric. This boundary is close to the 5.0 μm upper limit adopted by Zanen et al in their study of bronchodilator aerosol efficacy as a function of particle size.¹³ On this basis, and taking the data obtained with the highest MC concentration (15.70 mg/mL), in which the contribution to uncertainty from the MC assay was smallest, the variability in FDM (based on the magnitude of the coefficient of variation) after 5, 10, or 15 breathing cycles was $\leq 7.5\%$. As expected, the coefficient of variation increased at lower MC concentrations because of the greater contribution to uncertainty from the assay. However, it was close to 10% for all except the measurement of FDM₅ with the most dilute solution (0.25 mg/mL), which was close to the lower limit of detection of the MC assay. Incidentally, the liquid feed rate estimated from TM₅ measured with the 15.70 mg/mL solution (and assuming that evaporation of water was negligible) was close to 0.11 mL/min, which is only slightly lower than the 0.13 mL/min rate specified for the 2-min tidal breathing protocol.

Both the ATS protocols for bronchoprovocation challenge testing are based on assessing the spirometry response (change in forced expiratory volume in the first second [FEV₁] is the primary measure) to increasing the mass concentration of bronchoprovocation agent added in solution form to the nebulizer, in a series of well-defined step changes. It therefore follows that the mass output of

agent from the nebulizer must also increase in direct proportion to the change in solution concentration placed in the reservoir. Our data confirm that the AeroEclipse nebulizer provides a highly linear increase in FDM as a function of MC mass concentration, within the range investigated. Such behavior is expected, because there is no mechanism to segregate the MC from the homogeneous solution during nebulization, in contrast with the more complicated behavior of suspension formulations.¹⁵

Although we were unable to detect collected MC at the lowest concentration (0.0625 mg/mL) recommended for the abbreviated protocol in the ATS guidelines,³ the strong linearity of the FDM/concentration relationship probably also applies to the lowest concentration, since solute-concentration-related factors (changes in surface tension and viscosity, which can affect droplet formation) are probably most important at high concentrations.¹⁶ In any case, based on a retrospective analysis of data from 1,000 subjects undergoing bronchoprovocation testing, Cockcroft et al recently argued for shortening the 2-min tidal breathing protocol by starting with an MC concentration between 0.125 and 2.0 mg/mL, depending on FEV₁ and asthma medication profile,¹⁷ so testing with very dilute solutions might be obviated.

An important advantage of a breath-actuated nebulizer is the avoidance of aerosol production during exhalation. Aerosol produced during exhalation is mostly wasted, and aerosol released to the ambient air poses a risk to attending clinicians. Although we did not specifically examine waste

aerosol production in this study, unpublished measurements made with bronchodilator (albuterol) solutions using the AeroEclipse nebulizer during late-stage development indicate that < 3% of the active compound placed in the nebulizer reservoir was not inhaled or retained within the device, compared to 30% for a comparable non-breath-actuated nebulizer used without an exhalation filter.

The degree of FDM linearity that we achieved with a number of breathing cycles at each MC concentration using the AeroEclipse nebulizer also offers the prospect that this device could be operated as a dosimeter by using the position of the green indicator button (which is retracted during inhalation) on top of the nebulizer to coach the patient to inhale for a given period during each breathing cycle. Although the dosimeter protocol in the ATS guidelines³ is based on 5 inhalations, our data indicate that by increasing the number of inhalations in steps, from 5 to 10 and then to 15, it should be possible to progressively increment the dose of MC delivered, in direct proportion to the number of inhalations. It follows that the number of MC solution concentrations required could therefore be reduced. We did not explore the delivery of MC with this nebulizer beyond 15 breathing cycles (1.5 min total elapsed time), as it would probably be impractical to expect a patient to be able to exercise control of inhalation behavior beyond that limit. We also recognize that increasing the number of breathing cycles per exposure beyond the standard 5 inhalations may introduce pharmacokinetic considerations (beyond the scope of this study) that could impact the proportionality between mass of challenge agent delivered and physiological response.

Conclusions

Although the procedures we used in our study were slightly modified from the protocols in the ATS guidelines, to accommodate the AeroEclipse's breath-actuation feature, we demonstrated that a predictable dose of fine-droplet MC aerosol can be delivered to an in vitro model of a tidally-breathing adult. Our droplet size measurements indicate that the aerosol from the AeroEclipse met the ATS guidelines for the 2-min tidal breathing protocol. The AeroEclipse may also be used as a dosimeter in a modified version of the 5-breath dosimeter protocol, by virtue of the ability to control the duration of each inhalation, using the nebulizer's green indicator to coach the patient. The linear relationship between FDM and the number of inhalations for a given MC solution concentration also offers the opportunity to reduce the number of solution concentrations required for a complete bronchoprovocation challenge. By simply increasing the number of inhalations from 5 (in steps of 5) to as many as 15 inhalations, the challenge could be conducted using

fewer solution concentrations. Our in vitro study was designed to demonstrate the AeroEclipse's potential for delivering bronchoprovocation agent solutions. The clinical application of these findings remains to be explored.

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