Aerosol Profile Extracted from Spacers as a Determinant of Actual Dose

Sylvia Verbanck,[1,3] Chris Vervaet,[2] Daniël Schuermans,[1] and Walter Vincken[1]

Received May 11, 2004; accepted September 3, 2004

Purpose. We propose a novel method to evaluate the efficacy of a pressurized metered dose inhaler (pMDI) in combination with a spacer, by not only considering the total dose extractable from the spacer but also the dependence of dose on the volume available for aerosol inhalation.

Methods. We studied volume-dependence of aerosol concentration during extraction from two commonly used plastic spacers (150 ml AerochamberPlus; 750 ml Volumatic) after a single puff of a 100 μg salbutamol pMDI (HFA-Ventolin), using laser photometric measurements.

Results. After a delay of 1s in each spacer, the aerosol peak dose for AerochamberPlus was 2-fold that for Volumatic ($p < 0.001$), with the peak appearing well within the first 0.5 L even for the largest spacer. The opposite dose relationship is reached when considering total cumulative dose, which was 2-fold higher for Volumatic than for AerochamberPlus ($p < 0.001$); >95% of total cumulative dose was extracted well within 3 L for the largest spacer. The 2-fold cumulative dose relationship was confirmed by chemical assay on an absolute filter [AerochamberPlus: 21.4 ± 3.2 (SD) μg; Volumatic: 43.8 ± 9.1 (SD) μg].

Conclusions. Actual aerosol dose available to patients during inhalation via spacers can only be done on the basis of a quantification of aerosol peak dose and cumulative dose as a function of extracted volume.

KEY WORDS: aerosol; dose; metered dose inhalers; spacers.

INTRODUCTION

Spacers are an elegant means of slowing down an aerosol puff fired from pressurized metered dose inhalers (pMDI). Spacer shape and dimensions have previously been tailored to chloroflurocarbon-pMDI plume dimensions, and it has been suggested that for the hydrofluorolkane-pMDI with lower exit velocity (1), smaller spacers could suffice. On the one hand, one could expect that less aerosol dose will exit from a small-sized spacer because more aerosol gets impacted on the spacer's walls upon firing the pMDI. On the other hand, a large-sized spacer dilutes the aerosol more, decreasing its concentration and necessitating a larger volume to get all of the aerosol dose on board. That is why we considered that volume-dependence of aerosol profile as it gets extracted from any pMDI-plus-spacer combination would be a crucial determinant of actual aerosol dose delivered during an inhalation phase.

[1] Respiratory Division, Academic Hospital, Vrije Universiteit Brussel, 1090 Brussels, Belgium.

[2] Laboratory of Pharmaceutical Technology, Universiteit Gent, 9000 Ghent, Belgium.

[3] To whom correspondence should be addressed. (e-mail: sylvia.verbanck @ az.vub.ac.be)

With respect to the inhalation phase, two aerosol dose issues are of particular importance. First, a patient's inhalation capacity must be sufficient to inhale the volume over which the aerosol is spread. Second, even if an equivalent cumulative dose can be achieved by a full inhalation from two different sized spacers, one spacer may produce a high initial aerosol concentration followed by virtually aerosol-free air, whereas another spacer may produce a lower initial peak but a more evenly distributed aerosol over the entire inspiration. This will affect the lung depths to which aerosols are being targeted, and consequently their therapeutic effect. Indeed, an aerosol confined to a small volume inhaled early in inspiration will be pushed deep into the lungs, while an aerosol inhaled toward the end of the inspiration phase is expected to be delivered to more proximal airways (2). Another aspect concerns the regional distribution of an aerosol as it gets inhaled early in inspiration, when potentially some airways may still be closed (3), depending on initial lung volume. Thus, in order to be able to assess the deposition of therapeutic aerosols and its dependence on the inhalation maneuver, it is critical to be aware of the concentration profile of the aerosol as it gets aspired from a spacer. Such data are unavailable in the literature, which merely reports overall aerosol doses cumulatively extracted out of spacers, with conflicting outcomes (e.g., Refs. 4 and 5).

In the current *in vitro* study, we monitor salbutamol aerosol dose as a function of volume as it gets aspired from two such common spacers as the large-sized Volumatic and the small-sized AerochamberPlus to illustrate how the aerosol profile is crucially determinant of the actual aerosol dose that is being delivered to a patient during an inhalation from a spacer.

MATERIALS AND METHODS

The setup is schematically represented in Fig. 1 and is a concatenation of a vacuum source for aspiration, an absolute glass fiber filter (Pall Corporation, Ann Arbor, MI, USA) suitable for subsequent HPLC analysis, a laser photometer (PARI, Starnberg, Germany) for in-line aerosol monitoring, a 90° United States Pharmacopeia (USP) induction port (Copley Scientific, Therwil, Switzerland) to simulate aerosol loss in the throat, and a computer-controlled three-way valve leading to either air or to the spacer under study with the pMDI mounted on it. The spacer was sealed on all sides in order to allow leak-free flow measurement through the spacer + pMDI system. In order to minimize electrostatic charge, the plastic spacers were coated with an anionic household detergent according to instructions (6,7) and drip dried on the day preceding the test day. Two types of spacers were studied: Volumatic (GSK, Uxbridge, UK) and AerochamberPlus (Trudell Medical, Ontario, Canada). The pMDI of choice was HFA-Ventolin (GSK) with a nominal 200 doses of 100 μg of salbutamol in an aerosol formulation with a nominal mass median aerodynamic diameter (MMAD) between 2 and 3μm (personal communication from GSK).

Each test consisted of firing a 100 μg salbutamol dose into the spacer, observing a preset aerosol residence time in the spacer (varying between 1 and 60 s), and having the aerosol aspired from the spacer for 30sec at a slow constant flow of 250 ml/s. During this time, the photometer recorded the

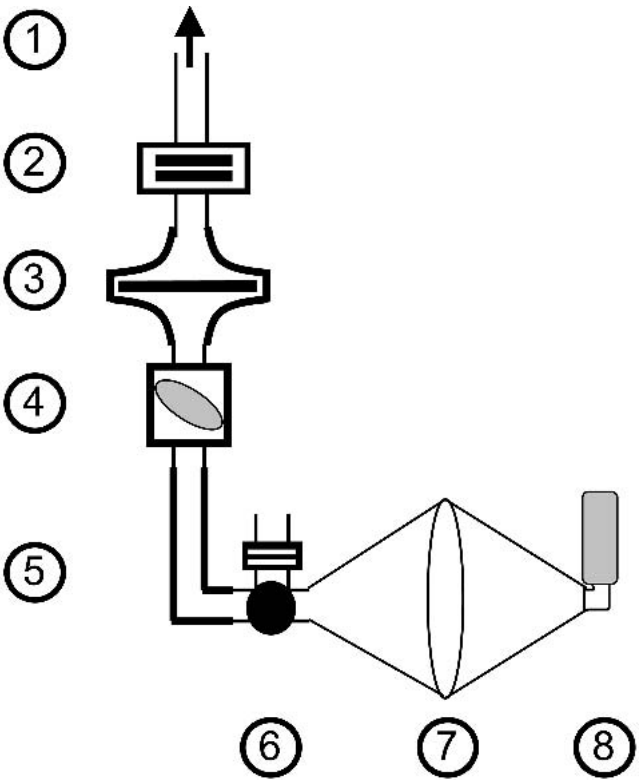

Fig. 1. Schematic representation of the setup used for testing the various pMDI plus spacer combinations: ① vacuum suction source; ② pneumatchograph; ③ glass fiber absolute filter; ④ laser scatter photometer; ⑤ USP 90° induction port; ⑥ time-controlled valve leading to spacer or to filtered air; ⑧ spacer under study; ③ pMDI.

aerosols in-line at a data acquisition frequency of 10 Hz. The photometer signal depends on aerosol particle diameter squared and particle number, and is expressed in arbitrary units. Hence, all photometer derived parameters are in arbitrary units, but are directly comparable amongst each other because all measurements were performed at a fixed photometer sensitivity which could accommodate the range of peak heights out of AerochamberPlus and Volumatic. After each test, the glass fiber filter was removed for subsequent HPLC analysis.

A test sequence for any given spacer comprised of 25 tests with variable spacer residence times (i.e., time spent in the spacer by the aerosol before it gets aspired from the spacer). Each test sequence for any given spacer followed a fixed sequence of 5 consecutive blocs of 5 spacer residence times (1, 5, 15, 30, 60 s; 1, 5, 15, 30, 60 s; etc.). This was done to anticipate a potential cumulative effect of changes in electrostatic charge conditions as the puffs were sequentially being fired into the coated spacer, by verifying that the 5 measurements for one given spacer residence time (e.g., 1 s) did not show a trend between the 1st, 6th, 11th, 16th, and 21st puff of each test sequence. Ten pairs of test sequences were performed on AerochamberPlus and Volumatic (in random order). For each pair of 25 measurements, one new HFA-Ventolin pMDI was used. Any new pMDI was shaken, and 10 puffs were fired to waste. Throughout each test sequence, the pMDI was shaken in between tests, and 1 puff was fired to waste before starting the next test, in order to be sure of a homogeneous mix in the pMDI metering chamber (8). Hence each pair of test sequences on one AerochamberPlus and one Volumatic consumed the 108 first puffs from a 200 dose pMDI.

For HPLC analysis (Merck Hitachi, Darmstadt, Germany), each filter (n = 500) was transferred to a glass bottle, 25 ml water was added and after dissolution of the salbutamol particles collected on the filter, drug concentrations were determined using the analysis method described by Clarke *et al.* (9). For HPLC analysis of salbutamol, a mobile phase consisting of 0.1% ammonium acetate solution/methanol (30/70, v/v) was used in combination with a LiChroCART 125-4 column containing 5 μm RP-C18 Lichrospher 100-particles (Merck). The analysis method was validated within the dose range of 2.5 to 100 μg salbutamol/filter, the drug recovery from the filter was >97%, and the coefficient of variation of the measurements was between 0.9 and 2.7% over the entire dose range. After each test sequence of 25 tests, the aerosol from the spacer was rinsed with 25ml of methanol to dissolve the salbutamol fraction impacted on and sedimented in the spacer and after dilution the samples were analyzed by HPLC to determine the cumulative amount of salbutamol.

Statistical Analysis

Using Statistica 5.1 (StatSoft, Tulsa, OK, USA), two-way ANOVA tests were used to detect differences in photometer peak, photometer area under the curve, and cumulative HPLC dose, considering spacer residence time and spacer type as two independent factors. Bonferroni adjustment was used to test for post-hoc differences with a significance level set at p = 0.05.

RESULTS

With each test sequence, five photometer traces are obtained for any given spacer residence time. There were no trends between the first and the last (5th) bloc of 1, 5, 15, 30, 60 s residence time measurements in each spacer measurement sequence. Therefore, the five measurements obtained for any given residence time for any given spacer were pooled for subsequent quantitative analysis. In Fig. 2, a typical average of five photometer traces is depicted from one test sequence in an AerochamberPlus and a Volumatic, for a spacer residence time of 1 s. Figure 2A shows instantaneous photometer signal, as a surrogate for aerosol concentration, and Fig. 2B shows the corresponding area under the curve, as a surrogate for cumulative aerosol dose. All curves were expressed as a function of aspired volume, and the onset was arbitrarily shifted to 200 ml for clarity of representation. The photometer curves in Fig. 2 were truncated at 5000 ml (during a 30 s aspiration, a cumulative volume of 7500 ml is aspired from each spacer), so that the characteristics of the aerosol profiles coming out of either spacer could be best appreciated. In Fig. 2A, the AerochamberPlus curve showed a higher and narrower peak than the Volumatic curve, with volumetric width at half peak height of 160 ml (AerochamberPlus) and 590 ml (Volumatic) in the example of Fig. 2A. As can be best appreciated from Fig. 2B, all aerosols were cleared out of both spacers well within 4 L. In either case, the aerosol peak occurred well within the first 500 ml of aspired volume, and peak height (representing peak aerosol dose) in AerochamberPlus and Volumatic respectively averaged 4.25 and 2.16

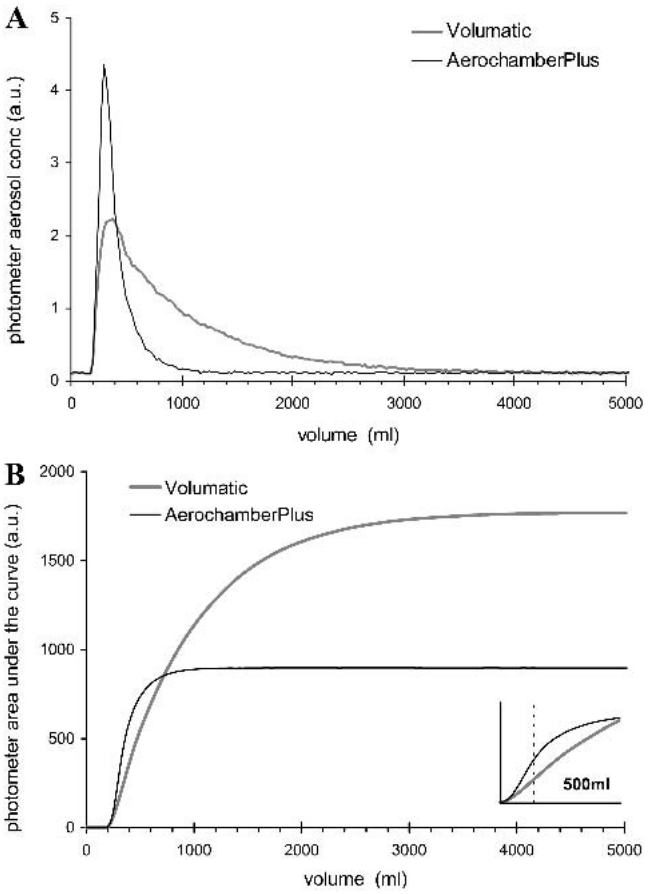

Fig. 2. Photometer traces as a function of extracted volume, obtained when an aerosol got extracted at 250 ml/s from a Volumatic (gray line; average of 5 curves) and an AerochamberPlus (black line; average of 5 curves), after 1 s spacer residence time. a.u.= arbitrary units. Panel A: instantaneous photometer signal as a function of volume. Panel B: area under the photometer curve in panel A as a function of volume. Inset in panel B: the first 500ml after aerosol onset (corresponding to the main curves between 200-700ml) with an indication of the average peak location (dotted vertical line).

arbitrary units (after subtraction of photometer background signal). The inset of Fig. 2B, where the first 500 ml of aspired aerosol is represented, indicates that in this example, cumulative dose is larger for the smaller spacer until approximately 500 ml of aspired aerosol, beyond which cumulative aerosol dose becomes larger for the Volumatic than for the AerochamberPlus. In the example of Fig. 2B, total area under the curve (representing total cumulative dose after 5 L) in AerochamberPlus and Volumatic respectively averaged 897 and 1768 arbitrary units.

Figure 3 shows the quantitative data for aerosol peak and total area under the curve, obtained for the 10 pairs of spacers tested, for the 5 different spacer residence times (n = 5 per spacer). Note that the variability contained in the standard deviation bars of Fig. 3 comprises the variability induced both by the pMDI canisters and the spacers. In a pair-wise comparison between any two spacers tested with the same pMDI, the difference between AerochamberPlus and Volumatic was significant for all residence times (all were $p = 0.02$ or less). For a 1 s spacer residence time, Fig. 3A shows a 2.00-fold greater peak for AerochamberPlus [3.82 ± 1.02 (SD) au] than for Volumatic [1.91 ± 0.30 (SD) a.u.], and Fig. 3B shows a 2.03-fold greater total area under the curve for Volumatic [1462 ± 213 (SD) a.u.] than for AerochamberPlus [719 ± 189 (SD) a.u.]. The validity of the total area under the curve from the photometer measurement with respect to actual total salbutamol dose delivered to the absolute filter can be observed by the striking resemblance between Fig. 3B and the HPLC data in Fig. 4. Figure 4 shows that the total aerosol dose extracted from a nominal 100 μg puff via spacer and a USP induction port averaged 21.37 ± 3.22 (SD) μg with an AerochamberPlus and 43.76 ± 9.13 (SD) μg with a Volumatic® (for a spacer residence time of 1s). This 2.05-fold absolute dose difference in favor of the Volumatic (Fig. 4) compares extremely well with the 2.03-fold dose relation as predicted from the total area under the curves obtained with the photometer (Fig. 3B). In Fig. 5, the total area under the curve from the photometer is actually plotted vs. the HPLC determined total salbutamol dose, where the lower and higher cumulative doses respectively correspond to the AerochamberPlus and Volumatic data. Considering all data points in Fig. 5, which covers the entire dose range, the resulting regression R is 0.91.

From the HPLC sabutamol dose data, we also computed for each spacer and for each residence time (n = 5 per spacer) the coefficient of variation. These coefficients of variation (averaged over 10 test sequences in each spacer) showed no dependence on spacer type, nor on spacer residence time, and averaged 17.4%. We also assessed the salbutamol dose that was left behind in the spacer after each test sequence of 25 puffs, by rinsing the spacer at the end of each test sequence, for post-hoc HPLC analysis. Averaged over all 25 puffs, collected with different spacer residence times between 1 and 60 s, the absolute dose collected from the spacer walls per 100 μg puff were significantly greater in the AerochamberPlus [64.8 ± 12.7 (SD) μg] than in the Volumatic [49.8 ± 11.5 (SD) μg]. When adding up the aerosol collected on all the filters after each test, and from the spacer over the course of a 25 test sequence, it could be estimated that ~20% of the total aerosol dose got lost in the actuator, the valves (e.g., the spacer valve), the USP induction port, photometer and connector tubing (22% for Aerochamber and 19% for Volumatic).

DISCUSSION

This study presents a novel and relatively easy way of characterizing therapeutic aerosol delivery from two common spacers, which should help decide which spacer type is best suited to meet specific therapeutic needs. A crucial element in this investigation is the volume dependence of aerosol dosage as it gets aspired from the spacer. This study shows how a considerable aerosol dosage advantage can be gained with a small-sized spacer such as the AerochamberPlus in cases where the aerosol concentrated in the first few hundred milliliters of the inhalation would optimize therapeutic targeting of that drug (Fig. 3A). If, by contrast, a more homogeneous distribution of the aerosol over the inhalation phase is expected to provide greater therapeutic efficacy, there is a clear dosage advantage with a large spacer such as the Volumatic (Fig. 3B). In the latter case, a double salbutamol dose can be reached with the Volumatic® vs. the AerochamberPlus (Fig. 4), provided that the patient is able to take a full inhalation of the order of a few liters. Even if a patient can only take the

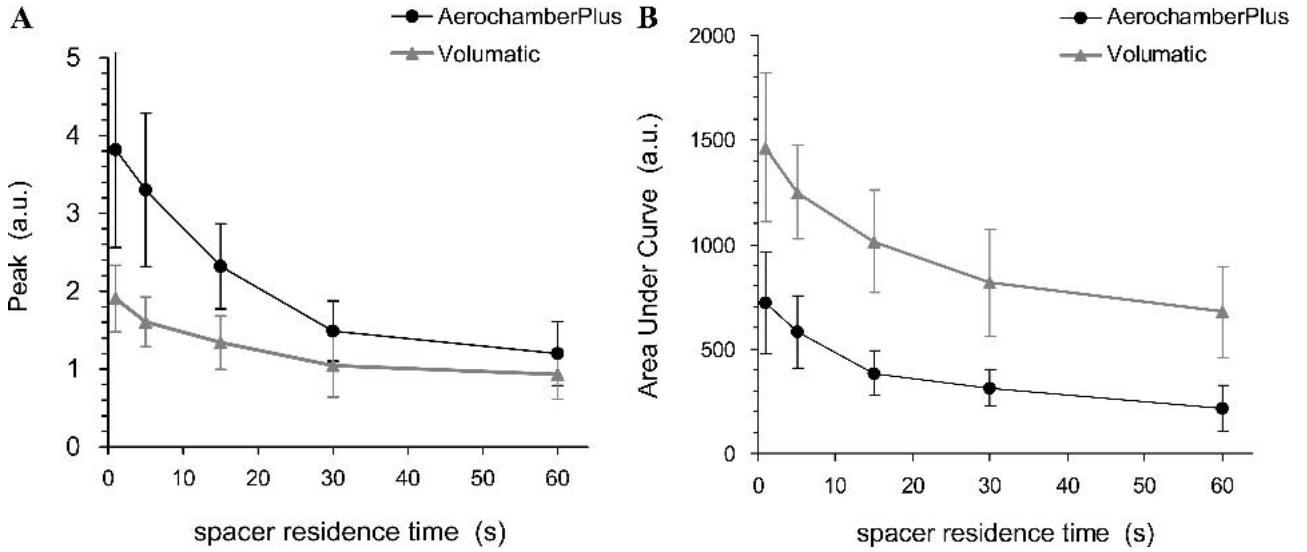

Fig. 3. Aerosol peak (panel A) and total area under the curve (panel B) derived from photometer traces obtained from AerochamberPlus (black circles) and Volumatic (gray triangles), as a function of spacer residence time. Results are presented as mean ± SD; a.u. = arbitrary units. For all spacer residence times, AerochamberPlus and Volumatic peaks were significantly different from each other: 1 s ($p < 0.001$), 5 s ($p < 0.001$), 15 s ($p < 0.001$), 30 s ($p = 0.004$), 60 s ($p = 0.02$). For all spacer residence times, AerochamberPlus and Volumatic total areas under the curve were significantly different from each other ($p < 0.001$ for 1 s, 5 s, 15 s, 30 s, 60 s).

full aerosol dose from the large spacer in subsequent 0.5 L inhalations, it is still expected to produce a delivered dose advantage over the small-sized spacer (for which the entire dosage will be almost cleared in one inhalation). Indeed, considering a tidal breathing cycle of 5 s, the aerosol dose remaining in the spacer which is available for the next inhalation, only decreases by ~20% during the first 5 s and even less thereafter (Fig. 4). Finally, it is noted that the inverse relationship between spacer peak aerosol dose (Fig. 3A) and spacer cumulative aerosol dose (Fig. 3B) holds even when the aerosol has resided in the spacer for up to 60 s.

In the literature, relatively few studies exist comparing HFA-propelled pMDI salbutamol formulations in combination with spacers, despite their widespread recommendation and use. In addition, these studies are restricted to reporting total cumulative emitted doses. Mitchell *et al.* (4) have observed that the dose advantage of a large-sized over a small-sized spacer which did exist with CFC-Ventolin (10), was

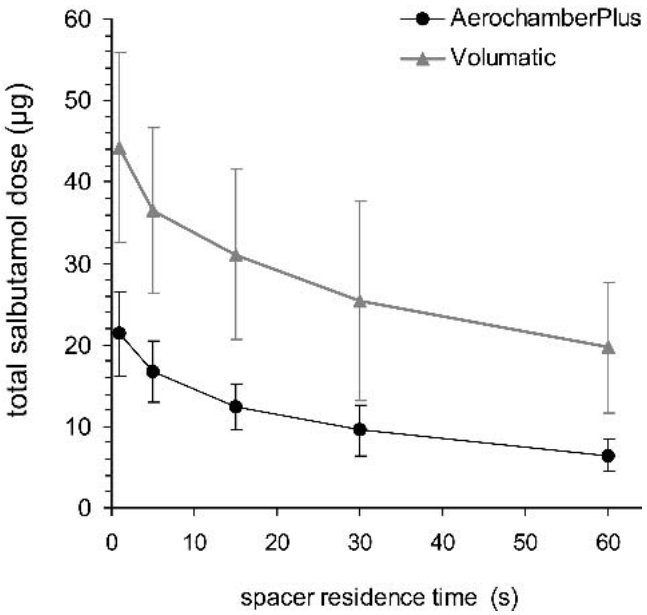

Fig. 4. HPLC determined total salbutamol dose collected on an absolute filter after extracting an aerosol from AerochamberPlus (black circles) and Volumatic (gray triangles), as a function of spacer residence time. Results are presented as mean ± SD. For all spacer residence times, AerochamberPlus and Volumatic salbutamol doses were significantly different from each other ($p < 0.001$ for 1 s, 5 s, 15 s, 30 s, 60 s).

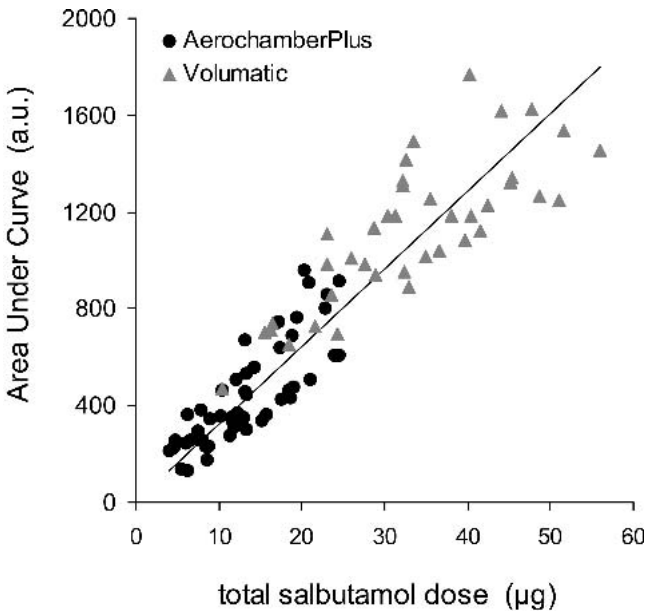

Fig. 5. Scatter plot of photometer-determined total area under the curve (AUC) vs. HPLC-determined total salbutamol dose in the respective dose ranges corresponding to AerochamberPlus (black solid circles) and Volumatic (gray triangles); the regression line through all data points corresponds to $R = 0.91$; a.u. = arbitrary units.

abolished when using HFA-Aeromir. Indeed, the HFA-Aeromir was found to produce similar <6.8 μm particle recoveries (in the range 60–70% of actuated dose) whether extracted from the 145 ml Aerochamber or the 750ml Volumatic (4). By contrast, Wildhaber *et al.* (5) found a higher <6.8 μm particle recovery from a 750 ml Nebuchamber (72% of actuated dose) than from a 165 ml Aerochamber MV (60% of actuated dose). Our HFA-Ventolin data, showing a cumulative dose for the large spacer which exceeded that of the small volume spacer (Fig. 3B, Fig. 4), agree with the findings of Wildhaber *et al.* (5). More importantly, our data on volume-dependence of aerosol dose also provide an explanation for another experimental observation in that same study (5): the dose advantage of the larger spacer over the smaller spacer was reversed in a setting of ventilator breathing when small inhaled volumes were being simulated. In that particular arrangement of the spacers in a pediatric ventilator circuit, it is highly likely that only the first part of the aerosol dose out of each spacer was effectively taken in, in which case our peak dose data (Fig. 3A) indeed predict a larger dose from the smaller vs. the larger spacer.

The much lower absolute aerosol recoveries obtained in our study vs. those reported in both Mitchell *et al.* (4) and Wildhaber *et al.* (5) are probably due to the respective methodologies. The loss of approximately 20 μg in our experimental setup downstream of the spacers, which included a valve and an in-line photometer for aerosol monitoring (rather than direct connection to an impactor), certainly is one important contributor. Also, in the two above reports (4,5), the pMDI was fired into the spacer while the aerosol was being continuously aspired, while we deliberately inserted a 1 s pause between pMDI firing and the aspiration from the spacer (this was done not only for the sake of standardization, but also in view of the patient coordination issue). The continuous aspiration as the pMDI was being fired (4,5) must have also influenced the plume geometry in both spacers, probably limiting its radial expansion toward the spacer walls. This would not only tend to favor overall aerosol output from either spacer in those previous studies, but also decrease the difference between aerosol output from small and large volume spacers. The respective aerosol deposits in the small and large spacer in Mitchell *et al.* (4) were not reported, but in Wildhaber *et al.* (5) deposition in the Nebuhaler was markedly smaller than in the Aerochamber (20.2% vs. 34.5%). This compares well with the lower aerosol deposits in the large (50%) vs. the small volume (65%) spacer in the present work (although our aerosol deposits are obviously much higher because they result from an accumulation of all the different spacer residence times ranging 1–60 s).

We certainly agree with the contention by Dompeling *et al.* (11) that aerosol dose results obtained by direct aspiration of an aerosol from the spacers into an impactor device at relatively high constant flows may not be representative of aerosol doses delivered during physiologic breathing. These authors showed that the bronchodilating effect in the children under study was similar for HFA-Ventolin delivered via either Volumatic or Aerochamber; unfortunately no inspiratory volumes were reported. Figure 2B clearly indicates that for an inspired volume around 0.5 L (not taking into account dead spaces), the aerosol doses coming out of either spacer may well have been very similar, which could explain a similar bronchodilating effect. The present work presents a different way of looking at aerosol dose as it gets inhaled by the patient, and should encourage future *in vivo* studies to report breathing patterns (volume, flow) during aerosol delivery.

Critique of the Method

A laser photometer was used to obtain a measure of aerosol dose that could be continuously monitored as a function of time, which is impossible with 'gold standard' aerosol measurement devices such as an impactor. It could be argued that the obtained photometer traces are affected by both changes in particle number concentration and changes in particle size distribution. First, it is unlikely that any significant aerosol size changes occur while the aerosol is being aspired from either spacer. Second, if the higher peak photometer signal in the Aerochamber vs. the Volumatic is not due to a higher aerosol number concentration, this would need to be due to a greater proportion of larger particles in the smaller Aerochamber, which is improbable. Even if there were a minor size shift toward the larger particles (implying greater dose), these particles would still need to be small enough to negotiate the USP induction port (Fig. 1) and to fulfill the manufacturor claim that HFA-Ventolin has a MMAD ranging 2–3 μm. Finally, the photometer optically detects the entire aerosol and cannot distinguish between the active product salbutamol and additives. Hence HPLC was performed to confirm the equivalence between photometer cumulative dose and salbutamol dose (Fig. 5). For all the above reasons, we are confident that the obtained photometer traces adequately reflect volume-dependent salbutamol aerosol dose as emitted from the respective spacers under study.

We cannot exclude the possibility of residual electrostatic charge on the detergent-coated spacer walls in our study, despite following instructions (7). This could have explained the observed aerosol decline for spacer residence times <15 s (Fig. 4), which is greater than that expected by gravitational sedimentation of 2–3 μm particle aerosols. Indeed, a simple computation by use of Eq. (3) in Heyder *et al.* (12) indicates that the aerosol loss between 30 and 60 s in Fig. 4 for the Aerochamber (horizontal cylinder of 4cm diameter) corresponds the theoretical sedimentation loss of a 2.9 μm particle. For shorter spacer residence times, other mechanisms of passive aerosol fallout are probably at work since typical half-lives of pMDI budesonide in a metal 250 ml Nebuchamber amount to only ~30 s with little influence of whether only small particles (<4.7 μm) or total dose was considered (13,14). O'Callaghan *et al.* (15) observed half-lives of less than 20 s for <5 μm sodium cromoglycate particles with a pMDI from a Volumatic coated with antistatic lining. The only study that does not observe a significant decrease in < 6.8 μm salbutamol particle aerosols after a 20 s residence time in a Nebuchamber is the one by Wildhaber *et al.* (10), even though one would expect some degree of aerosol fallout for such a particle range and time interval. From Fig. 4, it can be seen that the 50% reduction in HFA-Ventolin dose from Aerochamber and Volumatic range between 20 and 50 s, which is of the order of half-lives previously reported by others (12,13,14).

In conclusion, we studied relative efficacy of aerosol delivery via two commonly used spacers with a commonly used HFA driven salbutamol formulation. Although we agree that these results cannot be directly extrapolated to any other for-

mulation, the current study is meant to point out that cumulative dose certainly is not the only determinant of effective aerosol delivery. Volume-dependence of aerosol extraction from any given spacer can reverse dose relationships between any two spacers, depending on the efficacy of the aerosol during different portions of the inhalation phase. The distribution of the aerosol over the inhaled breath will be determinant of the lung deposition patterns of therapeutic aerosols, which can be predicted by aerosol lung models. These considerations will be of utmost importance when instructing coordination of pMDI and spacer to patients, and even more so in a setting where a spacer is incorporated in a ventilator circuit. In a similar fashion that pMDI manufacturers report aerosol size characteristics, it would be extremely useful if manufacturers of spacers reported typical peak and cumulative aerosol doses, and the respective volumes over which these can be extracted from the spacers.

ACKNOWLEDGMENTS

We are indebted to Jolyon Mitchell, Frans De Jongh and Pieter Zanen for their insightful comments on some preliminary data in the preparatory phase of this study. This study was funded by the Fund for Scientific Research – Flanders.

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