Standardization of inhalation provocation tests: Influence of nebulizer output, particle size, and method of inhalation

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Standardization of inhalation tests requires a knowledge of factors that will affect the response. We measured the output and particle size of six types of nebulizers used for inhalation tests. Output varied considerably between nebulizers of different types (0.12 to 1.59 ml/min) and to a lesser extent between nebulizers of the same type. Particle size varied between 0.8 and 5.2 μm aerodynamic mass median diameter (AMMD). The influence of these two properties on bronchial response to inhaled methacholine was examined. Nebulizer output but not particle size (between 1.3 and 3.6 μm AMMD) altered the response. We also examined the effect of change in inspiratory time during inhalation from residual volume to total lung capacity on lung deposition of radiolabeled aerosol and on the provocative concentration of histamine required to reduce the 1-sec forced expiratory volume (FEV_1) by 20% (PC_{20}). A reduction in inspiratory time from 8 to 2 sec resulted in a lower total lung dose, relatively more aerosol deposited in central airways, and a higher PC_{20}. The results emphasize the importance of keeping nebulizer output and pattern of breathing constant when performing inhalation provocation tests if consistent results are to be obtained.

Results of inhalation provocation tests are quantitative and are influenced by a number of technical and nontechnical factors. The technical factors include the methods of aerosol generation and inhalation,[1] of preparation and storage of test solutions, of measurement of the response, and of expression of results.[2] Nontechnical factors include subject characteristics such as baseline airway caliber,[3] medications,[4] and environmental factors such as time of day,[5] respiratory infection,[6] and exposure to allergen.[7] A knowledge of how these factors influence the response to inhaled aerosols is essential so that the tests can be standardized, thereby giving reproducible results that are comparable from time to time and from laboratory to laboratory.

The methods of aerosol generation and inhalation vary from one laboratory to another. Different nebulizers are used to produce aerosol that is generated continuously or intermittently and is inhaled by tidal breathing, inspiratory capacity, or vital capacity breaths. Aspects of the methods that might influence the response include nebulizer output, aerosol particle size, continuous or intermittent nebulization, lung volume at the start of inhalation, inspiratory time, inspiratory flow rate, and inspired volume.

In this study we examined (1) the nebulizer output and particle size of seven types of nebulizers that have been commonly used for inhalation tests, (2) the lung deposition of radiolabeled aerosol when generated continuously and inhaled over different inspiratory times (different flow rates) from residual volume to total lung capacity, and (3) the influence of variation in nebulizer output and particle size on the response to inhaled methacholine and of inspiratory flow rate on the response to inhaled histamine.

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METHODS

Measurement of nebulizer output and particle size

Output and particle size of one ultrasonic (Monaghan No. 670) and six types of air-driven nebulizers (one Bennett Twin, three DeVilbiss 40, two DeVilbiss 42, three DeVilbiss 646, one Vaponefrin, and three Wright) were examined.

Output was determined as follows. Five milliliters of normal saline were placed in each nebulizer; the setting on the Monaghan was 3, and the vent on each DeVilbiss model was open. Nebulizers were operated by air from a compressed-air cylinder (50 psi) at flow rates of 8 L/min for the Monaghan and 4, 5, 6, 7, 8, and 9 L/min for each of the others. Flow rates were measured by a calibrated flow meter (Peritron). Each nebulizer was operated for 2 min and the output was determined by measuring the change in weight using a Mettler balance. The mean of five determinations was recorded.

Particle size was measured by a low-flow-rate, seven-stage cascade impactor.[8] A trace amount of 99m technetium pertechnetate ($^{99m}TcO_4$) in normal saline was placed in each nebulizer. Nebulizers were operated as for measurement of output but only at the flow rate recommended by the manufacturer. Aerosol was sampled at ambient temperature and humidity. The radioactivity deposited on each stage was counted and from this the aerodynamic mass median diameter (AMMD) and the geometric standard deviation ($\bar{g}$) of the particles were determined from the cumulative distribution curve.

Influence of breathing pattern on deposition of aerosol

Five healthy nonsmoking adults inhaled an aerosol of normal saline and $^{99m}TcO_4$ on 5 consecutive days. On each of the first 4 days the aerosol was produced by a Monaghan 670 ultrasonic nebulizer and was inhaled at hourly intervals by two fast inspirations from residual volume (RV) to total lung capacity (TLC) (inspiratory time 2 sec), by two slow inspirations from RV to TLC (inspiratory time 8 sec) and by tidal breathing for 2 min. The subjects were trained to perform the vital capacity maneuvers while watching a second timer and told to breath normally for tidal breathing. Lung volumes and inspiratory flow rate were not measured. On the fifth day the aerosol was produced by a Wright nebulizer and inhaled by the same three methods. The Monaghan was operated at an airflow of 8 L/min (output 1.59 ml/min; particle size AMMD 4.3 μm, $\bar{g}$ 2.1) and the Wright at an airflow of 7 L/min (output 0.13 ml/min; particle size AMMD 1.32 μm, $\bar{g}$ 2.11). Measurement of the dose and distribution of radiolabeled aerosol in the right lung was carried out by a previously described method.[1, 9] Immediately before and after inhalation of the radiotracer solution, the subject was seated in front of an Anger scintillation camera and the radioactivity over the right lung was measured for 1 min. The topographic distribution of aerosol in the right lung was determined in two zones: (1) a crescentic area 5 cm wide adjacent to the lung hilus (inner zone) and (2) a crescentic area 2.5 cm wide inside the edge of the lung (outer zone). The lung edge was delineated by performing, on another day, a ^{127}Xe equilibration on each subject with the technique described by Newhouse et al.[10] The total and zonal lung radioactivity counts were corrected for decay and residual background radioactivity in the lung by subtraction and then converted to a volume of aerosol by applying the appropriate conversion and calibration factors.[1] The radiation exposure for a complete study, calculated by the Monte Carlo technique,[11] was 54 millirads to the lung and 12 millirads to the whole body.

Effect of nebulizer output, particle size, and technique of inhalation on airway responsiveness to inhaled methacholine or histamine

Twelve adults with asthma attending the Regional Chest and Allergy Unit at St. Joseph's Hospital participated in the study. All had episodic dyspnea with wheezing and an increase in bronchial responsiveness to inhaled histamine. At the time of study their symptoms were well controlled and their forced expired volume in 1 sec (FEV_1) was greater then 70% of predicted and did not vary by more than 10% on each study day. There was no history of respiratory tract infection or allergen exposure for 6 wk prior to the study. Aerosol bronchodilators were withheld for 8 hr before each test; corticosteroids were continued in their usual dosage. Subjects had no features of other respiratory disease and none was a smoker.

Histamine and methacholine inhalation tests were carried out by a method similar to that described by Cockcroft et al.[12] and Juniper et al.[13] The only difference was in the method of aerosol generation and inhalation, as indicated below. In each test an aerosol of saline was inhaled first and followed at 5-min intervals by histamine acid phosphate or methacholine in twofold increasing concentrations from 0.03 to 16 mg/ml. The response was measured by change in FEV_1. Inhalations were discontinued when there was a fall in FEV_1 of greater than 20%. The result was expressed as the provocative concentration causing a 20% fall in FEV_1 (PC_{20}), and was obtained from the log dose-response curve by linear interpolation of the last two points.

The effects of nebulizer output and aerosol particle size on the PC_{20} methacholine were studied in eight subjects. Three nebulizers were used and there were 4 consecutive study days: inhalations were by tidal breathing. On day 1 and day 4 reproducibility of response was determined with a Wright nebulizer and aerosol inhalation for 2 min (air flow rate 7 L/min; total output 0.26 ml/2 min; particle size AMMD 1.32 μm, $\bar{g}$ 2.11). Subjects were included only if the difference in PC_{20} on these two days was less than twofold; the mean was used for statistical analysis. On day 2 aerosol was produced by a DeVilbiss 40 nebulizer and inhaled over 2 min (air flow rate 6 L/min; total output 0.76 ml/2 min; particle size AMMD 3.5 μm, $\bar{g}$ 3.0). On day 3 aerosol was generated by a Bennett Twin nebulizer and was inhaled for 70 sec (air flow rate 7 L/min; total output 0.26 ml/70 sec; particle size AMMD 3.6 μm, $\bar{g}$ 3.47). The effect of nebulizer output on PC_{20} was determined by comparing

TABLE I. Nebulizer output and particle size

	Operating flow rate (L/min)	Nebulizer output (ml/min) (mean ± 1 SD)	Aerosol particle size	
			AMMD (μm)	$\bar{g}$
Wright				
A*	7	0.130 ± 0.006	1.32	2.11
B	7	0.121 ± 0.006	0.75	1.20
C	7	0.134 ± 0.005	1.48	2.34
DeVilbiss 40				
A	6	0.378 ± 0.015	1.85	2.50
B*	6	0.378 ± 0.012	3.50	3.00
C	6	0.384 ± 0.012	1.50	2.70
DeVilbiss 42				
A*	6	0.299 ± 0.011	4.40	3.28
B	6	0.290 ± 0.016	2.90	2.24
DeVilbiss 646				
A	6	0.313 ± 0.009	2.70	2.87
B	6	0.247 ± 0.021	2.75	2.39
C	6	0.282 ± 0.017	2.37	2.95
Bennett Twin	7	0.222 ± 0.005	3.60	3.47
Vaponefrin	6	0.246 ± 0.006	5.20	3.59
Monaghan 670	8	1.59 ± 0.189	4.30	2.10

AMMD = aerodynamic mass median diameter; $\bar{g}$ = geometric standard deviation.
*Nebulizer used for inhalation tests.

TABLE II. Mean dose and site of deposition of aerosol in right lung

	Total lung dose (μl) (mean ± 1 SD)		Distribution of lung dose (% total) (mean ± 1 SD)			
			Monaghan ultrasonic		Wright	
	Monaghan ultrasonic	Wright	Inner zone	Outer zone	Inner zone	Outer zone
Tidal breathing (2 min)	68.9 ± 41.3	6.9 ± 3.4	46.4 ± 3.0	21.6 ± 2.4	38.9 ± 5.8	24.9 ± 6.8
Slow VC (2 breaths)	13.7 ± 10.2	2.1 ± 0.4	48.2 ± 7.5	20.7 ± 4.8	41.9 ± 5.9	26.0 ± 4.0
Fast VC (2 breaths)	2.5 ± 1.7	0.9 ± 0.4	57.4 ± 8.7	17.2 ± 5.2	48.2 ± 10.8	21.3 ± 7.2

the results from days 2 and 3 and of particle size by comparing day 3 with the mean result from days 1 and 4.

The effect of varying inspiratory flow rate on the PC_{20} histamine was studied in six subjects. There were 5 consecutive study days. On the first and last days the Wright nebulizer was used as described above. On the other days the aerosols were generated by a DeVilbiss 42 nebulizer (air flow rate 6 L/min; output 0.299 ml/min; particle size AMMD 4.4 μm, $\bar{g}$ 3.28) and were inhaled on different days in random order by tidal breathing for 2 min, five low-flow-rate (inspiratory time 8 sec) vital capacity (VC) breaths and five high-flow-rate (inspiratory time 2 sec) VC breaths.

The investigation was approved by the hospital Research Committee, and written informed consent was obtained for all procedures.

Analysis

Mean results were compared using analysis of variance and multiple comparisons made by the Neumann-Kuels multiple-range test.[14] Logarithmic transformation of PC_{20} was used for statistical calculations.

RESULTS

The aerosol output and particle size given by different nebulizers when operated at the flow rate recommended by the manufacturer and at different flow rates are shown in Table I and Fig. 1. Nebulizers of different models and different nebulizers of the same model produced different outputs and particle size; output varied with flow rate. The variability of output

was greater for the ultrasonic nebulizer (coefficient of variation 13.8%) than for the air-driven nebulizers (coefficient of variation 2.3% to 8.3%).

The effect of the method of inhalation on the dose and site of deposition of aerosol within the right lung is shown in Table II. For all methods of inhalation the Monaghan ultrasonic nebulizer (output 1.59 ml/min) deposited a greater lung dose than the Wright nebulizer (output 0.13 ml/min) ($p < 0.05$). Fast (2-sec) VC inhalations resulted in a lower lung dose ($p < 0.02$), relatively more aerosol in the inner zone ($p < 0.04$), and less aerosol in the outer zone ($p < 0.05$) than slow (8-sec) VC inhalations for both nebulizers. The distribution of lung dose produced by tidal breathing and slow VC breaths was not significantly different ($p > 0.1$). For all methods of inhalation the Wright nebulizer (AMMD 1.32 μm) produced more peripheral deposition of aerosol than the Monaghan (AMMD 4.3 μm) although these differences were not statistically significant.

The influence of output and particle size on response to methacholine and the influence of the time of inhalation on response to histamine is shown in Table III. A 2.9-fold greater output of aerosol (DeVilbiss 40, 0.76 ml, compared with Bennett Twin, 0.26 ml) produced a 3.4-fold lower PC_{20} methacholine ($p < 0.001$) (Table III). A 2.7-fold difference in particle size (Bennett Twin AMMD 3.6 μm compared with Wright AMMD 1.32 μm) did not significantly alter PC_{20} methacholine. A decrease in the time of inspiration of VC breaths from 8 to 2 sec produced a 3.3-fold increase in PC_{20} histamine ($p < 0.01$). Five slow VC breaths resulted in a higher PC_{20} histamine than that from tidal breathing for 2 min ($p < 0.05$).

DISCUSSION

The results demonstrate that nebulizer output and inspiratory time can be important determinants of the response to inhaled histamine or methacholine. Therefore they must be known and kept constant when standardizing inhalation provocation tests.

Nebulizer output is known to be determined by operating flow rate and to vary between nebulizers of different models.[15] We confirmed these earlier observations and included three models (DeVilbiss 42 and 646, and Wright) that have not been examined previously but that have been used for inhalation tests. We also found that, at a given flow rate, there was a variation in output between different nebulizers of the same model. Nebulizer characteristics are not provided by manufacturers and therefore the output of nebulizers used for inhalation tests must be measured; the outputs recorded in this study should be regarded

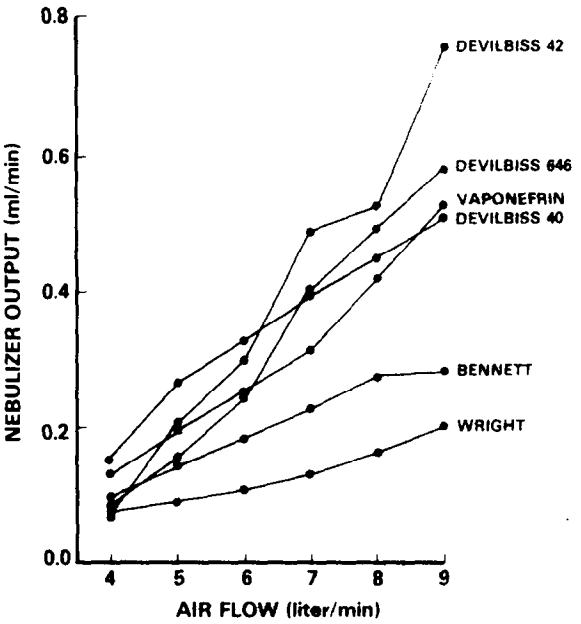

FIG. 1. Nebulizer output measured at different flow rates.

only as a guide. This is particularly so for the DeVilbiss models, which have a vent which, if open, allows auxiliary air to be drawn through the nebulizer, thus increasing the output of aerosol.[16] An accurate output of these nebulizers operated with the vent open will be obtained only if they are weighed before and after the subject has inhaled aerosol.

When aerosol was generated continuously and inhaled from RV to TLC a decrease in inspiratory time (8 to 2 sec), i.e., an increase in inspiratory flow rate, resulted in a lower total lung dose, a greater proportion of the dose deposited in the inner (large airway) zone, and a higher PC_{20} histamine. In another study[17] we delivered aerosol intermittently by using a Rosenthal-French dosimeter Model D-2A over 0.6 sec at the beginning of inspiratory capacity breaths. In this study a decrease in inspiratory time from 3 to 5 sec to 1 to 2 sec did not change lung dose or PC_{20} histamine. The difference in results obtained in the two studies suggests that the lower lung dose and higher PC_{20} histamine in the present study were the result of the decrease in dose inhaled at the mouth.

Particle size, measured only at the operating flow rate suggested by the manufacturer, varied between 0.7 and 5.2 μm AMMD. Particle size has also been documented to vary with operating flow rate, although not to a "serious" degree with the addition of auxiliary air.[16] We observed no effect of variation in particle size between 1.3 and 3.6 μm AMMD on the response to methacholine. However, the study design that we used to determine the influence of alteration in

TABLE III. Methacholine and histamine inhalation tests

	PC_{20} methacholine (mg/ml)			PC_{20} histamine (mg/ml)					
					DeVilbiss 42§			Wright‖	
								Tidal breathing	
	Wright*	Bennett†	DeVilbiss 40‡		Tidal breathing	Slow VC	Fast VC	Day 1	Day 5
Subject 1	0.41	0.39	0.19	Subject 1	0.95	2.30	7.60	3.10	3.20
2	2.90	2.60	1.90	2	0.05	0.10	0.36	0.09	0.08
3	2.90	8.40	1.30	3	0.05	0.03	0.21	0.11	0.10
4	0.10	0.08	0.02	4	2.05	3.80	7.35	5.50	7.20
5	0.07	0.09	0.02	5	1.18	4.50	12.50	2.60	3.90
6	3.43	6.00	1.65	6	0.87	1.30	3.70	2.55	3.25
7	0.16	0.29	0.04						
8	3.31	3.60	1.90						
Mean	0.68	0.88	0.26	Mean	0.42	0.73	2.40	1.02	1.15

*Wright nebulizer: output 0.26 ml, particle size AMMD 1.32 μm.
†Bennett nebulizer: output 0.26 ml, particle size AMMD 3.60 μm.
‡DeVilbiss 40 nebulizer: output 0.76 ml, particle size AMMD 3.60 μm.
§DeVilbiss 42 nebulizer: output 0.299 ml/min, particle size AMMD 4.4 μm.
‖Wright nebulizer: output 0.130 ml/min, particle size AMMD 1.32 μm.

particle size on PC_{20} involved equalizing the outputs (dose of methacholine) of two nebulizers (which produced particles of a different size) by altering the period of inhalation. We assumed that the PC_{20} would not alter when the same concentration and amount of aerosol was delivered over 70 sec as over 120 sec, which may not be the case. Therefore, the findings suggest that measurement and control of particle size produced by commercial nebulizers is not necessary for the standardization of inhalation tests, but further studies are clearly required to substantiate this.

A similar change in particle size of aerosol (1.3 μm AMMD produced by the Wright nebulizer and 4.3 μm AMMD by the Monaghan 670) did cause a different pattern of deposition in the lung, with more central deposition of the larger particles; this is consistent with previous studies.[18] The result raises the possibility that the pattern of deposition is not an important determinant of the response. This is supported by another study with inhaled histamine[17] but is contrary to the observations of Ruffin et al.[1] who observed that when histamine was deposited preferentially in central airways rather than more diffusely throughout the lung, a 15-times smaller dose was required to induce the same response. The differences between our observations and those of Ruffin et al. may be the result of differences in the ratio of central to peripheral deposition in the two studies.

Factors that influence the response to inhalation tests, whether the tests are carried out for clinical or research purposes, must be standardized so that results can be critically analyzed and compared. This study emphasizes the importance of measurement of nebulizer output and of keeping it constant. It draws attention to the control of inspiratory time, particularly when aerosol is generated continuously and inhaled by inspiratory capacity or vital capacity breaths. It also suggests that careful regulation of the particle size produced by many commercial nebulizers may not be important.

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