

RAPID PUBLICATION

Inhalation of dry-powder mannitol increases mucociliary clearance

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Inhalation of dry-powder mannitol increases mucociliary clearance. E. Daviskas, S.D. Anderson, J.D. Brannan, H-K. Chan, S. Eberl, G. Bautovich. ©ERS Journals Ltd 1997.
ABSTRACT: Inhalation of hypertonic saline stimulates mucociliary clearance (MCC) in healthy subjects and those with obstructive lung disease. We investigated the effect of inhaling the osmotic agent mannitol on MCC.

We used a dry-powder preparation of mannitol British Pharmacopoeia (BP) which was encapsulated and delivered using a Dinkihaler™. MCC was measured for 75 min in six asthmatic and six healthy subjects on two occasions before and after the mannitol inhalation or its control, using ^{99m}Tc-sulphur colloid and a gamma camera. The inhaled dose of mannitol was 267±171 mg (mean±SD) and 400 mg and the percentage fall in forced expiratory volume in one second (FEV₁) was 22±3 and 4±2% in the asthmatic and healthy subjects, respectively.

The total clearance in the whole right lung for the 60 min from the start of inhalation of mannitol was greater by 26.3±11.9% in the asthmatic and 18.1±4.9% in the healthy subjects compared to the control. The total clearance over 75 min was 54.7±9.6% and 33.6±9.4% on the mannitol and control day (p<0.002), respectively, in the asthmatic subjects and 40.5±7.1% and 24.8±7.8% (p<0.002) in the healthy subjects.

In conclusion, inhalation of dry-powder mannitol increases mucociliary clearance in asthmatic and healthy subjects and may benefit patients with abnormal mucociliary clearance.

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Mucociliary clearance (MCC) is a physiological function of the respiratory tract to clear locally produced debris, excessive secretions or unwanted inhaled particles. For normal MCC to occur it is necessary that the epithelial cells are intact, the ciliary activity and the rheology of mucus is normal and that the depth and chemical composition of the periciliary fluid layer is optimal. An increase in the osmolarity of the airway surface fluid by inhalation of hypertonic saline aerosol has been shown to increase MCC in asthmatic and healthy subjects [1] and in patients with bronchitis [2] and cystic fibrosis [3]. It is possible that inhalation of other osmotic agents will have the same effect on MCC.

We have recently developed a preparation of the osmotic agent mannitol suitable for inhalation as a dry powder [4]. Mannitol is a naturally occurring sugar alcohol (C₆H₁₄O₆, molecular weight 182) which is not absorbed by the gastro-intestinal tract, does not cross the blood-brain barrier and is not metabolized to any substantial extent when injected. Mannitol is stable as a powder and resists moisture resorption at relatively high humidities. These characteristics make it an ideal substance to encapsulate for inhalation, for diagnostic and therapeutic purposes [4].

Like hypertonic saline, mannitol exerts osmotic effects in the lower airways and as a dry powder is easy to deliver. We therefore decided to investigate its potential to stimulate MCC. For this initial study we inves-

tigated the effect of inhaling a dry-powder mannitol on MCC in healthy and stable asthmatic subjects with normal baseline lung function. These subjects are known to have a normal baseline clearance and an increased MCC rate in response to inhaling hypertonic saline, and for this reason they were considered the best subjects to investigate the effect of the osmotic agent mannitol on MCC. The long-term aim is to develop a practical and effective treatment for patients with abnormal mucociliary clearance, such as patients with cystic fibrosis and bronchiectasis, to clear excessive secretions.

Materials and methods

The study was approved by the Ethics Review Committee of Central Sydney Area Health Service and informed consent was obtained in writing from all subjects before they participated in the study.

Subjects

Six asthmatic (mean age 20±3 yrs) and six healthy (mean age 21±3 yrs) subjects took part in the study. The asthmatic subjects had stable asthma and normal baseline lung function. The mean (±SD) baseline forced expiratory volume in one second (FEV₁) was 95 (±9) %

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predicted (ranging 82–108%). Similarly, the healthy subjects had a mean (\pm SD) baseline FEV₁ 105 (\pm 11) % pred (ranging 90–122%). Most of the asthmatics were taking β -agonists as needed and taking inhaled corticosteroid medication (beclomethasone) daily. None of the asthmatic subjects was taking theophylline. All asthmatics withheld their medications for at least 8 h before beginning the protocol each day. All subjects were asked not to take any nonsteroidal anti-inflammatory drugs or antihistamines for at least 48 h prior to each study day. None of the subjects had a history of cigarette smoking and none had a lower respiratory tract infection in the 6 weeks prior to each study.

Dry-powder mannitol

The mannitol powder (Mannitol British Pharmacopoeia (BP), Rhône Poulenc Chemicals Pty Ltd, Brookvale, NSW, Australia) was prepared, at the Department of Pharmacy at the University of Sydney, by spray drying a solution containing 15 mg·mL⁻¹ (Buchii 190 Mini Spray Drier, Buchii, Flawil, Switzerland). It was irradiated with approximately 4.7 KGys at Steritech (Wetherill Park, NSW, Australia) and a bioburden analysis was carried out at Stanford Laboratories (Rydalmere, NSW, Australia). The results for both yeast and mould showed a value less than 10 colony-forming units (cfu)·g⁻¹ and no coliforms or other pathogens were detected.

The particle size of dry-powder mannitol was measured using a multi-stage liquid impinger (Astra Pharmaceuticals, Lund, Sweden) and assayed by vapour pressure osmometry (Knauer, Berlin, Germany). Gelatine capsules (Parke-Davis, Sydney, Australia) were hand-filled with 5, 10, 20 and 40 (\pm 0.2) mg on an analytical balance (Sartorius BA110S, Gottingen, Germany) as required under controlled conditions (relative humidity 40%, temperature 20 \pm 1°C). The capsules were stored in a container with silica gel and kept in a cool environment. The mannitol was inhaled at flow rates between 60–120 L·min⁻¹ using a Dinkihaler™ (Rhône Poulenc Rorer, Collegeville, Pennsylvania, USA). Over these inhaled flow rates the percentage of particles by mass in the aerosol cloud was between 47% (less than 5.6 μ m), at 95 L·min⁻¹ and 67% (less than 7 μ m) at 60 L·min⁻¹. The capsules were emptied well and there was no obvious residual amount of mannitol left in the device. Therefore, the dose inhaled was very similar to the loaded dose.

Study design

The study involved three visits, which were at least 48 h apart. The procedure on each visit was as follows: Visit 1: assessment of airway responsiveness to dry-powder mannitol; Visits 2 and 3: 1) spirometry; 2) radio-aerosol inhalation; 3) transmission/emission anterior/posterior images (1 min each), 10 min after the mid-inhalation time of the radioaerosol; 4) dynamic emission anterior/posterior images (20 s each) for 10 min; 5) intervention: inhalation of dry-powder mannitol or control; 6) dynamic emission anterior/posterior images (20 s each) for 45 min.

The number of coughs induced spontaneously by the inhalation of mannitol were counted. The control study

day involved the same inhalation manoeuvres and number of inhalations through the Dinkihaler™ loaded with an empty capsule. Also, on the control day all subjects were asked to cough as many times as they coughed spontaneously on the mannitol day. Therefore the number of coughs was identical on both test days.

Measurement of lung function

Spirometry was measured using a hot wire anemometer (Minato, AS-500, Osaka, Japan), before and after inhalation of dry-powder mannitol, on the first visit. All subjects had reproducible spirometry which was within the normal range at rest. Predicted values were taken from QUANJER *et al.* [5] for adults.

Measurement of MCC

Mucociliary clearance was measured using a radio-aerosol technique and a gamma camera. The method for delivering the radioaerosol, the image collection and analysis has been published in detail previously [1, 6, 7].

In brief, approximately 1 giga becquerel (GBq) ^{99m}Tc-sulphur colloid (Chedoke-McMaster Hospitals, Hamilton, Ontario) was diluted in 5 mL of isotonic saline. The radioaerosol (mass mean aerodynamic diameter 7 μ m, span 1.6 determined by laser diffraction technique on a Malvern Mastersizer X (Malvern Instruments Ltd, Malvern, Worcestershire, UK)) was generated by an Acorn jet nebulizer (Medic-Aid, Peckham, Sussex, UK). Lung images were obtained using a single-headed rotating gamma camera (Philips Diagnost Tomo; Hamburg, Germany), in a 64 \times 64 matrix, linked to an on-line computer (DEC PDP11, Maynard, MA, USA). The lung fields of the subjects were delineated with anterior and posterior transmission images [8] taken with a moving line source [9]. Geometric mean (GM) images were obtained from the anterior and posterior images for the transmission and emission images [10]. The right lung was divided into three regions of interest: central, intermediate and peripheral [11]. The initial lung radio-aerosol distribution (penetration index) was characterized by the ratio of the activity in the peripheral region to the activity in the central region.

A bi-exponential function was fitted to the curve obtained from the dynamic GM images, using a nonlinear least squares method (PCNONLIN, SCI, Software, Lexington, KY, USA). The total counts of the whole right lung and defined regions in the first emission GM image were taken as the initial counts expressed as 100% retention. The counts of the whole right lung and defined regions in the dynamic emission GM images, measured before and after the intervention, were expressed as a percentage of the initial counts. Data from the best fit were used to calculate the percentage clearance during, post-intervention, in 1 h since the start of intervention and over the 75 min of measurement.

Statistical analysis

A two-factor analysis of variance (ANOVA), with repeated measures, was performed on the calculated clearance with mannitol and its control in asthmatic and

healthy subjects. Probability values less than 0.05 were considered statistically significant. Ninety five per cent confidence intervals (95% CIs) were calculated from the mean clearance and differences of clearance between study days. Spearman's test was used for correlation analysis.

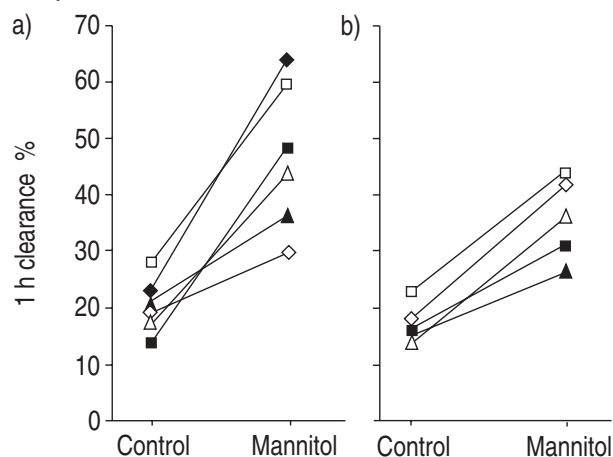


Fig. 1. – Total mean percentage clearance in the 60 min from the start of the inhalation of mannitol and its control in the whole right lung in all: a) asthmatic and b) healthy subjects. The inhaled dose of mannitol ranged 115–485 mg in the asthmatic subjects while it was a fixed dose of 400 mg in the healthy subjects. This figure clearly shows that mannitol compared to its control markedly increased clearance in all subjects. The mean (95% confidence interval (95% CI)) clearance was 20.4 (14.8, 26.0) % and 46.7 (31.7, 61.7) % on the control and mannitol day ($p=0.0001$) in the asthmatic subjects, resulting in a mean (95% CI) increase in clearance of 26.3 (16.0, 36.6) %. Similarly, it was 16.4 (12.7, 20.1) % and 34.5 (26.6, 42.3) % ($p=0.0001$) in the healthy subjects, resulting in a mean (95% CI) increase in clearance of 18.1 (13.9, 22.3) %.

Results

The key finding of this study was that the inhalation of a dry-powder mannitol markedly increased mucociliary clearance in the whole right lung and all lung regions both in asthmatic and healthy subjects (fig. 1, table 1). This increase in MCC rate was immediate and the total clearance in response to mannitol was more than double when compared to the control (fig. 2).

Mannitol increased MCC in a similar manner in both the asthmatic and healthy subjects. However, the magnitude of the increase tended to be greater in the asthmatic subjects, although the mean inhaled dose of mannitol was smaller, compared to the healthy subjects (fig. 1). For the asthmatic subjects, the dose of mannitol was determined by their airway responsiveness measured on the initial visit. The mean±SD (range) inhaled dose of mannitol, in the asthmatic subjects, was 267±171 (115–485) mg which induced a mean ±SD (range) fall in FEV1 of 22±3 (19–27) %. By contrast, the healthy subjects had a fixed dose of dry-powder mannitol (400 mg) and had no significant reduction in FEV1 with a mean±SD (range) 4±2 (2–9) %. Mannitol was well tolerated by all subjects. During and after inhalation of the mannitol the asthmatic subjects coughed mean (±SD) 13 (±10) times and the healthy subjects coughed eight (±11) times. Care was taken to reproduce this same number of coughs on the control day so that the differences in clearance between the two test days could not be accounted for by differences in cough number.

Regional analysis showed that mannitol increased MCC in all regions (table 1) and in all asthmatic and

Table 1. – Total percentage clearance over the total time of measurement (75 min) in the whole right lung and its defined regions in asthmatic and healthy subjects on the control and mannitol study day ($p<0.002$)

Lung regions	Asthmatic subjects		Healthy subjects	
	Control	Mannitol	Control	Mannitol
Whole right lung	33.6 (22.7, 44.5)	54.7 (44.0, 65.4)	24.8 (16.0, 33.8)	40.5 (32.5, 48.5)
Central	37.6 (24.1, 51.1)	64.5 (51.1, 77.9)	28.0 (10.5, 45.5)	52.3 (40.2, 64.4)
Intermediate	30.5 (15.9, 45.1)	53.3 (44.9, 61.7)	15.1 (0.5, 29.7)	31.3 (19.4, 43.2)
Peripheral	27.1 (20.9, 33.3)	38.2 (27.2, 49.2)	21.5 (6.5, 36.5)	27.3 (10.3, 44.3)

Values are means (95% confidence intervals). These results include the baseline clearance (15 min) before intervention.

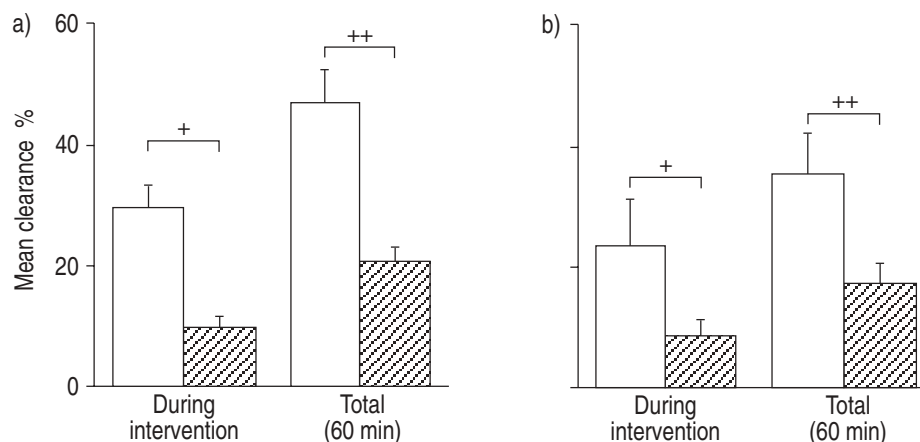


Fig. 2. – Mean percentage clearance during intervention and total in 60 min from the start of the intervention with mannitol and its control in the whole right lung in: a) asthmatic (n=6) and b) healthy (n=6) subjects. This figure demonstrates that the total clearance (60 min) in response to mannitol was more than double compared to the control in both groups. It also demonstrates that most of the increase in clearance in response to mannitol occurred during the time of the inhalation of dry-powder mannitol in both groups. The inhaled dose of mannitol was 267±171 mg (mean±SD) and 400 mg in the asthmatic and healthy subjects, respectively. □ : mannitol; ▨ : control. +: $p<0.02$; ++: $p<0.003$.

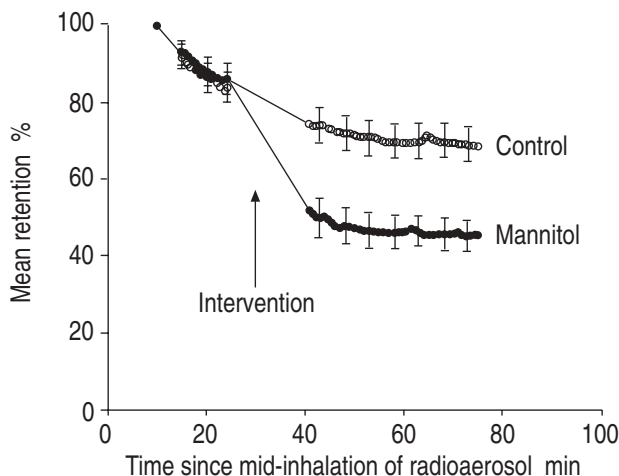


Fig. 3. — Mean percentage retention curves over the total time of measurement (75 min) with control and mannitol in the whole right lung in asthmatic subjects ($n=6$). This figure demonstrates the reproducibility of the clearance in the first 15 min before intervention in both study days. It also demonstrates the immediate and marked increase in clearance in response to mannitol compared to control. Similar curves were obtained in healthy subjects.

healthy subjects (fig. 1) except in the peripheral region in which only four of the six asthmatic and three of the six healthy subjects had an increase in MCC. Comparison of the clearance in each region showed that the magnitude of the increase in clearance in response to mannitol was larger in the central and smaller in the peripheral region ($p<0.03$), both in asthmatic and healthy subjects.

The mean percentage retention curves in the whole right lung in asthmatic subjects (fig. 3) over the 75 min of measurement demonstrate the reproducibility of the baseline clearance during the interval before the intervention and the increased clearance in response to inhaled mannitol compared to the control study. Similar curves were obtained in the healthy subjects.

The initial radioaerosol distribution was well matched in the two study days, and was similar in both groups. The mean (\pm SD) penetration index was 37.2 (± 6.7) and 40.0 (± 8.4) % for mannitol and control day, respectively, in the asthmatic subjects and similarly 33.4 (± 7.8) and 30.3 (± 9.4) % in the healthy subjects. There was no correlation between the small differences (maximum approximately 6%) in the penetration index and the differences in percentage clearance in the whole right lung and defined regions on the two study days in both groups ($p>0.2$).

Discussion

The inhalation of dry-powder mannitol caused a marked increase in MCC in the whole right lung and all lung regions both in asthmatic and healthy subjects. This increase in MCC was immediate and although it occurred mostly during the time of inhalation of the dry-powder mannitol, the total clearance in response to mannitol was more than double when compared to the control.

The increase in MCC was of a similar magnitude in both asthmatic and healthy subjects although it tended to be greater in the asthmatic subjects. Direct comparison of the effect of the dose of mannitol on MCC was

not possible in the present study. Taking into consideration that the asthmatic subjects inhaled a smaller dose of mannitol compared to healthy subjects, it may be suggested that the effect of mannitol on MCC is greater in the asthmatic subjects. The airways of asthmatics are sensitive to the effects of increased osmolarity and respond by narrowing [4]. For this reason, the dose of mannitol chosen for the measurement of MCC was that which had provoked only a 20% fall in FEV₁. This dose varied between subjects (range 115–485 mg). It is probably for this reason that some differences were observed in the percentage clearance at 1 h in the asthmatic subjects. While we could have premedicated the asthmatics so that their airways would not have been responsive, we considered that such an intervention would have confused the outcome. By contrast to the asthmatics, the healthy subjects inhaled a fixed dose of dry mannitol and the variation of the response was smaller. Inhalation of dry-powder mannitol was well tolerated by all subjects and induced only a mild cough which was reproduced on the control day.

The short duration of the effect of dry-powder mannitol on MCC rate is similar to the duration of the effect of hypertonic saline [1]. There are two possible reasons which could explain the relatively short duration of the increase in MCC rate in response to inhalation of dry-powder mannitol: 1) the hyperosmolar stimulus may be present only for a short time. This could be a result of an increase in water flux in response to the osmotic gradient that is created at the airway lumen and subsequently in the epithelial cells and submucosa; 2) the amount of radioactive mucus that had to be cleared may have reached its maximum in a relatively short period of time. This last possibility is quite likely as the clearance was more than double compared to the control.

The regional differences in the magnitude of the increase in MCC may relate to the differences of the deposited dose of mannitol powder in each region. The pattern of deposition of mannitol is unlikely to be diffuse because it was inhaled at relatively high inspiratory flow rates. This concept will need to be confirmed with a deposition study of radiolabelled mannitol. However, as the clearance in the peripheral region in the healthy subjects was small and only occurred in half the subjects, it may not have any biological significance.

The mechanism whereby inhalation of dry-powder mannitol increases MCC, most likely relates to the indirect effects of increased osmolarity on the ciliary activity and on the rheology of the mucus. An increase in the osmolarity of the airway lumen causes mechanical and electrical changes on the epithelial cells which trigger a series of biochemical changes intracellularly. Water moves out of the cells due to the osmotic stimulus causing the cells to shrink. Shrinking of the epithelial cells is followed by a regulatory volume increase (RVI) which initiates biochemical events leading to an increase in intracellular calcium [Ca^{2+}]_i [12]. An increase in [Ca^{2+}]_i has been shown to increase ciliary activity [13–15]. Hyperosmolarity also causes depolarization of the epithelial cell membrane [16] which could cause an increase in ciliary beat frequency, as the work by MAO and WONG [17] suggests, dependent on the influx of sodium or Ca^{2+} .

Hyperosmolarity also triggers release of mediators

from human lung mast cells (*e.g.* histamine) [18] and possibly neuropeptides (*e.g.* substance P) [19] from sensory nerves, which have been shown to increase ciliary beat frequency [20–22]. Histamine has been demonstrated to increase MCC both in asthmatic and healthy subjects [23, 24]. Histamine was also found to increase $[Ca^{2+}]_i$ [25] and it is possible that other mediators may do so as well. The increase in MCC in response to hyperosmolarity induced by inhalation of dry-powder mannitol in asthmatic and healthy subjects is most likely due to an increase in ciliary activity modulated by intracellular biochemical mechanisms with changes in intracellular calcium playing a key role.

The transportability of mucus from patients with cystic fibrosis and bronchiectasis has recently been shown to increase markedly when incubated with dry powders of hyperosmotic agents such as sodium chloride, urea, mannitol and glucose [26]. The reason for this is not known but an alteration in the rheological properties of the mucus was suggested.

The magnitude and duration of the effect of inhaling dry-powder mannitol on mucociliary clearance is similar to the effect of inhaling wet aerosols of hypertonic saline [1]. However, the advantage of using a dry powder of mannitol over the wet aerosol of hypertonic saline is that it does not require an ultrasonic nebulizer for delivery. The superiority of the use of dry-powder mannitol over hypertonic saline as a therapeutic tool needs to be investigated in patients who require a therapeutic intervention to improve MCC, *e.g.* patients with cystic fibrosis and bronchiectasis. Asthmatic subjects in remission with normal baseline lung function have normal clearance [1, 6, 27]. Therefore, the present study was not aiming to improve MCC in these asthmatic subjects *per se* but simply to investigate if inhaling an osmotic agent like mannitol could increase MCC in a manner similar to hypertonic saline [6].

In conclusion, increasing the osmolarity of the airway fluid by inhaling a dry-powder preparation of mannitol increases mucociliary clearance in asthmatic and healthy subjects. The mechanism of this increase in clearance remains unclear but it is likely that hyperosmolarity stimulates ciliary beat frequency indirectly through intracellular biochemical changes. Mannitol may also alter the rheological properties of the mucus as it is reported to increase transportability of mucus. We conclude that inhaling a dry powder of suitably prepared mannitol has the potential to benefit patients with abnormal mucociliary clearance, such as those with cystic fibrosis and bronchiectasis.

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