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Effect of increasing doses of mannitol on mucus clearance in patients with bronchiectasis

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ABSTRACT: Bronchiectasis is characterised by hypersecretion and impaired clearance of mucus. A 400-mg dose of inhaled mannitol improves mucus clearance however, the effect of other doses is unknown.

A total of 14 patients, aged 63.3±5.7 yrs, were studied on five visits. Mucus clearance at baseline and with mannitol (160, 320 and 480 mg) was measured using technetium-99m-sulphur colloid and imaging with a gamma camera over 45 min, followed by a further 30 min involving 100 voluntary coughs. A control study assessed the effect of cough provoked by mannitol during the intervention.

Whole right lung clearance over 45 min was 4.7±1.2 and 10.6±2.6% on baseline and control days, respectively, and increased to 16.7±4.2, 22.8±4.2 and 31±4.7% with 160, 320 and 480 mg mannitol, respectively. Clearance over 45 min with 480 mg mannitol was greater than clearance with 320 and 160 mg. Total clearance over 75 min, after mannitol administration and voluntary coughs, was 36.1±5.5, 40.9±5.6 and 46.0±5.2% with 160, 320 and 480 mg mannitol, respectively, all significantly different from baseline (24.1±6.0%) and control (13.1±3.0%). Total clearance over 75 min with 480 mg mannitol was greater compared with 160 mg.

In conclusion, mucus clearance increases with increasing doses of mannitol and can be further increased by cough in patients with bronchiectasis.

KEYWORDS: Bronchiectasis, hyperosmolarity, mannitol, mucus clearance

Bronchiectasis patients usually have increased and persistent mucus secretion along with impaired mucociliary transport, which results in mucus accumulation, cough and recurrent infections [1–5]. Most patients with bronchiectasis are troubled by the disease and have a poor quality of life [6, 7]. Treatment of mucociliary dysfunction consists mainly of pharmacological and physical therapy, which aim to reduce secretion or to increase clearance of mucus [8–12]. An improvement in the clearance of mucus by ciliary and cough action can be achieved by increasing the airway hydration and by optimising the viscoelastic and surface properties of mucus. This has been the role of mucoactive agents, such as osmotic agents, including mannitol.

Previous studies [4, 5] have confirmed that inhalation of 400 mg of mannitol increased clearance of mucus acutely and over 24 h in bronchiectasis patients. In addition, mannitol improved the health-related quality of life in patients with bronchiectasis when administered over 2 weeks [13, 14].

Although it is clear that 400 mg of mannitol is effective in improving clearance of mucus, it is not known whether this effect is dose dependent. In order to test that clearance of mucus increases with increasing doses of mannitol and that significant clearance of mucus can be achieved with doses <400 mg of mannitol, the present study investigated the effect of increasing doses of mannitol alone, and in association with cough, on the clearance of mucus in bronchiectasis patients.

MATERIALS AND METHODS

Subjects

A total of 14 subjects with stable bronchiectasis took part in the study (table 1). Bronchiectasis was diagnosed with a high-resolution computed tomography (HRCT) scan. Based on the HRCT scan, all subjects except one had extensive bronchiectasis involving at least the lower lobes, bilaterally. In one subject only, the bronchiectatic changes were localised on the lower left lobe and were reported as mild. None of the subjects had a diagnosis of cystic fibrosis and all had long-standing symptoms of bronchiectasis. All subjects withheld their

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regular medication (table 1) for ~20 h on each study day. All subjects were never-smokers and none were being treated with antibiotics for exacerbations at the time of the study.

The present study was approved by the Ethics Review Committee of Sydney South West Area Health Service (Australia; Protocol No. X05 0259) and was performed under the Clinical Trial Notification Scheme of the Therapeutic Goods Administration of Australia (CTN No. 2005/602). Written informed consent was obtained from all subjects.

Design of the study

Screening: visit 1

An airway challenge with mannitol was performed in potential subjects following an approved protocol [15] in order to identify airway hyperresponsiveness to dry powder mannitol. Subjects were eligible to proceed to subsequent visits if they had a decrease in FEV_1 to <15% of baseline FEV_1 after the airway challenge with mannitol. Subjects' medical histories and medications were recorded.

Mucus clearance studies: visits 2–6

There were five study days: 1) day 1, baseline; 2) day 2, administration of 160 mg of mannitol; 3) day 3, administration of 320 mg of mannitol; 4) day 4, administration of 480 mg of mannitol; and 5) day 5, control. Study days 1–4 were randomised.

The procedures on each study day were: 1) spirometry; 2) radioaerosol inhalation; 3) dynamic imaging over 15 min in order to assess initial deposition and clearance of the radioaerosol; 4) intervention, consisting of either breathing while at rest, mannitol doses of 160, 320 or 480 mg or cough control over 15 min; 5) dynamic imaging over 30 min in order to assess post-intervention clearance with and without mannitol; 6) dynamic imaging over a further 30 min in order to assess the effect of cough on days 1–4 (during this interval, subjects were asked to voluntarily cough deeply 100 times, corresponding to about 4 coughs·min^{-1}); and 7) spirometry.

On the baseline study day, subjects rested in the sitting position during the intervention period. On the control day, subjects were asked during the intervention period to inhale through the device loaded with an empty capsule and to cough the maximum number of spontaneous coughs counted on the mannitol test days. For this reason, the control study was performed last. In addition, the control study acted as a no-cough control for the last 30 min; therefore, no voluntary coughing was requested during this interval.

Dry powder mannitol

Dry powder mannitol (Pharmaxis Ltd, Frenchs Forest, Australia) was inhaled from capsules using the low-resistance dry powder inhaler (RS 01; Plastiape, Osnago, Italy). Mannitol was delivered in doses of 160 mg (four capsules of 40 mg), 320 mg (eight capsules of 40 mg) and 480 mg (12 capsules of 40 mg).

Measurement of lung function

Spirometry was measured using a SpiroScore® card (Bird Healthcare, Melbourne, Australia). All subjects were clinically stable and had reproducible spirometry values (table 1). Predicted values for adults were taken from QUANJER *et al.* [16].

Measurement of mucus clearance

Clearance of mucus was measured using a radioaerosol technique and dynamic imaging with a double-head gamma camera (Biad; Trionix, Twinsburg, OH, USA). The radioaerosol, ^{99m}Tc-sulphur colloid (CIS-US Inc.; Bedford, MA, USA) was generated by a jet nebuliser (mass median aerodynamic diameter of the particles of 6.3 μm, span 2; Medic-Aid, Peckham, UK) at 8 L·min^{-1}. Subjects inhaled the radioaerosol with a controlled breathing pattern following a

TABLE 1 Subject details and baseline lung function values

Subject	Sex	Age yrs	Height cm	FEV_1 % pred	FEV_1/FVC %	$FEF_{25–75\%}$ pred	FEV_1% decrease post-635 mg mannitol	Regular medication
1	F	69	160	87	61	52	13.3	Ciclesonide, salbutamol
2	F	66	159	89	70	80	-3.9	Flixotide, salbutamol
3	M	56	179	44	60	34	0.7	Salbutamol
4	F	60	159	96	73	94	6.0	Flixotide
5	F	61	163	79	70	45	-4.0	Doxycycline
6	F	56	166	52	61	33	6.2	Flixotide, salbutamol
7	F	53	159	75	70	69	13.0	Nil
8	F	66	163	82	75	94	8.9	Nil
9	F	65	163	81	74	95	5.0	Terbutaline,doxycycline
10	F	68	163	86	59	73	8.3	Terbutaline
11	F	68	157	73	65	49	4.0	Flixotide, salbutamol
12	F	66	153	78	64	43	8.0	Ciclesonide, salmeterol
13	M	72	168	81	64	61	7.0	Nil
14	M	60	172	70	64	70	2.0	Symbicort, nuelin, doxycycline
Mean±SD		63±6	163±7	77±14	66±6	64±22	5.3±5.3	

FEV_1: forced expiratory volume in one second; % pred: % predicted; FVC: forced vital capacity; $FEF_{25–75\%}$: mean forced expiratory flow between 25 and 75% of FVC. F: female; M: male.

target volume of 450 mL and a target inspiratory time on a computer screen aimed at maximising deposition in the conducting airways [4, 5, 17]. The breathing pattern aimed at a peak inspiratory flow rate of ~40 $L \cdot min^{-1}$. Approximately 5 min after the radioaerosol inhalation, simultaneous anterior and posterior dynamic imaging were commenced with a 1-min frame rate and the patient in the supine position. The lung fields of the subjects were delineated from a transmission scan with a cobalt-57 sheet source.

All images were decay corrected to the imaging start time. The anterior and posterior transmission and emission images were combined into geometric mean (GM) images [18]. The right lung was divided into central, intermediate and peripheral regions [19]. The initial lung radioaerosol distribution (*i.e.* penetration index) was characterised by the ratio of the counts per pixel in the peripheral to the counts per pixel in the central region of the GM emission image obtained 5 min after the radioaerosol inhalation.

A mono- or bi-exponential function was fitted to each curve obtained from the dynamic GM images, using a nonlinear least squares method (IDL 5.0, Research Systems Inc, Boulder, Co, USA). The total counts of the whole right lung and defined regions in the first emission GM image were taken 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. The percentage retention curves gave an overall estimate of the total clearance from start (100% retained). Activity that had not been retained had cleared. Counts from the best fit of the percentage retention curves were used to calculate total percentage clearance over the specified intervals as follows:

$$(\text{counts at start of interval} - \text{counts at end of interval}) \times 100 / \text{counts at start of interval} \quad (1)$$

Statistical analysis

A one-factor ANOVA with repeated measures was performed on the calculated total percentage clearance at the same specified intervals on the five study days. *Post-hoc* analysis was performed using Fisher-protected least significant difference. A p-value <0.05 was considered to be statistically significant. Data are presented as mean±SEM and 95% confidence intervals (CIs) are reported where applicable.

RESULTS

Whole right lung

Baseline day mucus clearance was very poor, 5.2±1.4% over the first 60 min without any intervention, as shown in the percentage retention curves (fig. 1). Clearance of mucus increased with all doses of mannitol compared with clearance measured on the baseline and control days (fig. 1, table 2).

Clearance over the 45-min period from the start of intervention increased with increasing doses of mannitol in the majority of subjects (table 2, fig. 2). Clearance with 480 mg was significantly greater than clearance with 160 and 320 mg mannitol (table 2, fig. 2). When the mannitol dose was increased from 160 mg to 320 mg, the absolute increase (95% CI) in clearance over 45 min was 6.1% (1.1–11.1%; p=0.07) and when increased to 480 mg it was 14.3% (7.6–21.1%; p<0.0001). When the mannitol dose was increased from 320 mg to 480 mg, the increase in clearance was 8.2% (3.2–13.2%; p<0.02). More importantly, the increase in clearance over 45 min with each mannitol dose compared with control was 6.1% (0.5–11.7%; p=0.07), 12.2% (5.7–18.7%; p<0.001) and 20.4% (12.4–28.4%; p<0.0001) for 160, 320 and 480 mg, respectively.

Clearance of mucus was further enhanced in response to 100 requested voluntary coughs (mean±SE 108±2%) over a period of 30 min commenced 30 min after intervention. The cough clearance, calculated over this 30-min period, was not dose dependent and not different to the clearance on the baseline study day (21.3±5.9, 24.3±4.3, 24.4±5.2 and 23.0±3.8% on the baseline day and for 160, 320 and 480 mg mannitol, respectively; p>0.5). However, total clearance over 75 min, taken from the start of intervention and including the 30 min cough period, with 160, 320 and 480 mg mannitol was greater compared with clearance on baseline and control days (table 2). Total clearance with 480 mg mannitol was 10% greater than that with 160 mg (p<0.03).

Regional mucus clearance

Clearance increased with increasing doses of mannitol in the central and intermediate regions, but not in the peripheral region (fig. 1, table 3). In the central and intermediate regions, clearance with 480 mg mannitol was greater than clearance with 160 mg and 320 mg mannitol. In contrast, the increase in clearance in the peripheral region was similar with all doses.

The baseline day clearance over 45 min from start of intervention was very poor (table 3). All doses of mannitol significantly increased clearance over this period compared with baseline in all regions (table 3). Clearance on the control day, involving coughing without mannitol during the intervention period, was not significantly increased during this 45-min period in any region over the baseline day clearance. Clearance with all doses of mannitol was greater than clearance on the control day in all regions, with the exception of the lowest dose of mannitol used (160 mg), which was similar to control in the central region over 45 min (table 4). Clearance started to increase during the intervention period while mannitol was inhaled, except in the peripheral region where it started to increase in the post-intervention period.

Clearance with all doses of mannitol was further increased in all regions after the 100 voluntary coughs over 30 min. However, the cough clearance over the 30-min period was not dose dependent and not different, in any region, to the clearance on the baseline study day. In the central region, cough clearance was 28.2±6.8, 31.1±5.9, 32.7±6.4 and 31.4±3.9% on the baseline day and with 160, 320 and 480 mg mannitol, respectively (p>0.4). Total clearance over the 75 min, taken from the start of intervention, was significantly increased with all doses compared with baseline in all regions (table 3). Total clearance with 480 mg was almost double compared with baseline and triple compared with control in all regions (fig. 1 and table 3).

The number of coughs provoked by mannitol inhalation and all spontaneous coughs are shown in figure 3. There was no significant difference in the number of spontaneous coughs provoked by mannitol (p>0.06). In addition, there was no

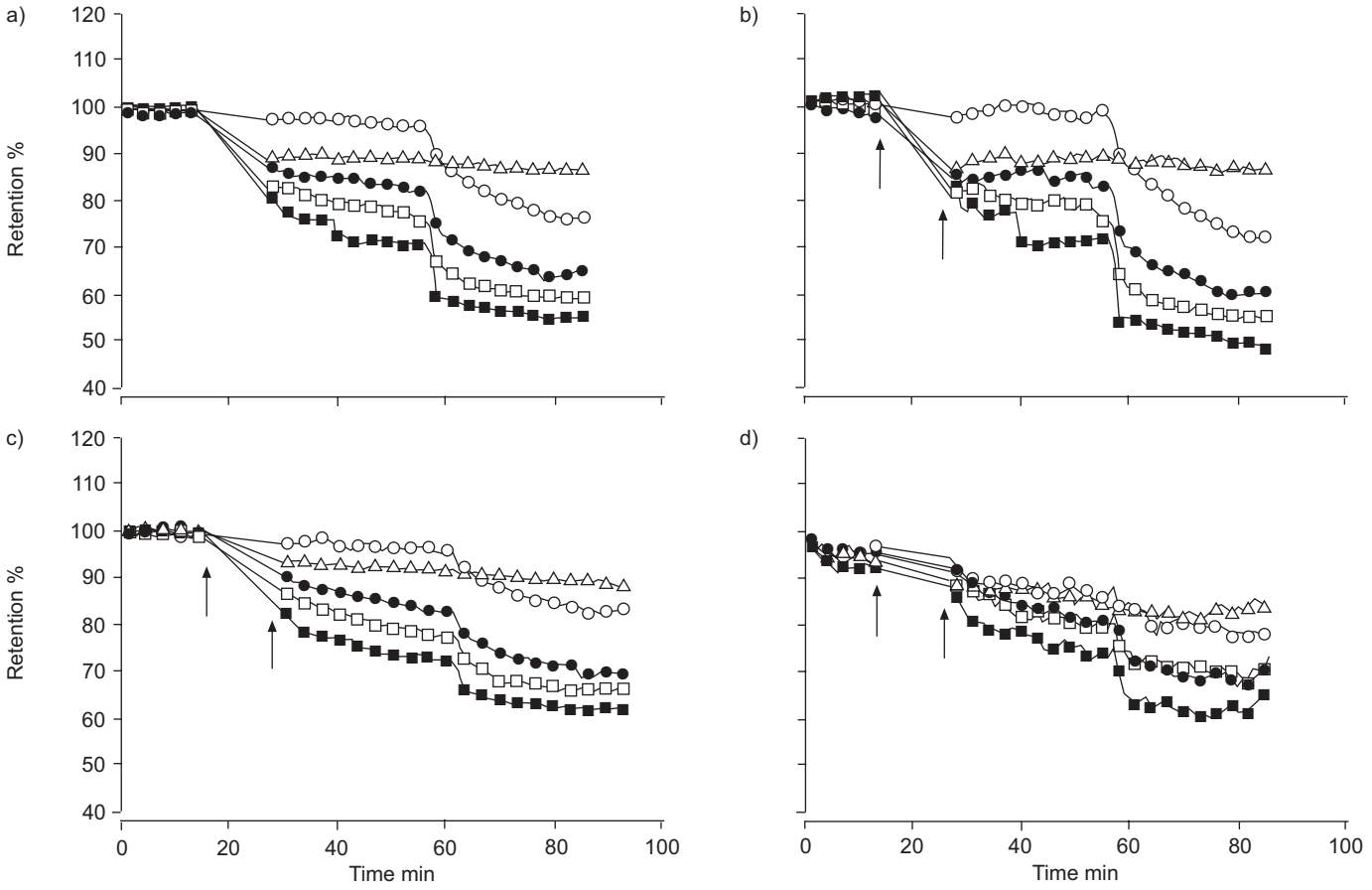

FIGURE 1. Mean percentage retention curves over 90 min of total imaging time in a) the whole right lung, b) right central, c) right intermediate and d) right peripheral regions in 14 subjects with bronchiectasis studied on five visits involving: baseline (○); control (△); 160 mg mannitol (●); 320 mg mannitol (□); and 480 mg mannitol (■). Arrows represent intervention period. During the last 30 min, subjects were asked to cough 100 times voluntarily. The control visit involved the maximum number of coughs provoked by the mannitol doses during the intervention but did not include coughing during the last 30 min. The results demonstrate an increase in clearance in response to all doses of mannitol compared with baseline and control. Clearance increased in the central and intermediate regions with increasing dose of mannitol but not in the peripheral region.

significant correlation between clearance in the whole right lung and central region and the number of coughs over the intervention period or over the 45-min period from the start of mannitol inhalation ($p>0.6$).

Radioaerosol deposition

The initial radioaerosol distribution in the whole right lung was similar on all study days (22.2±2.4, 26.1±2.9, 26.3±3.6, 25.4±3.0 and 25.4±3.4% for baseline, control, 160, 320 and

TABLE 2 Clearance at specified intervals in the whole right lung in 14 subjects with bronchiectasis

Study	Clearance			Total clearance from start intervention	
	Over initial 15 min	During intervention, over 15 min	Post-intervention, over 30 min	Over 45 min	Over 75 min
Baseline	2.0±0.8	2.4±0.7	2.8±1.3	4.7±1.2	24.1±6.0
Control	2.8±2.1	9.6±2.4*	3.0±1.4	10.6±2.6	13.1±3.0*
Mannitol dose mg					
160	3.0±1.3	11.9±3.8*	6.0±2.0	16.7±4.2***	36.1±5.5**, ###
320	2.0±1.0	15.3±4.5***	8.5±2.0*, #	22.8±4.2***, ###	40.9±5.6***, ###
480	1.7±0.8	20.8±5.4***, ##, ¶	11.9±3.0**, ##, ¶	31.0±4.7***, ###, ¶¶¶, +	46.0±5.2***, ###, ¶

Data are presented as mean percentage clearance±SEM. *, ** and ***: $p<0.05$, $p<0.01$ and $p<0.001$, respectively, *versus* baseline; #, ## and ###: $p<0.05$, $p<0.01$ and $p<0.001$, respectively, *versus* control; ¶ and ¶¶¶: $p<0.05$ and $p<0.001$, respectively, *versus* 160 mg mannitol; +: $p<0.05$ *versus* 320 mg mannitol.

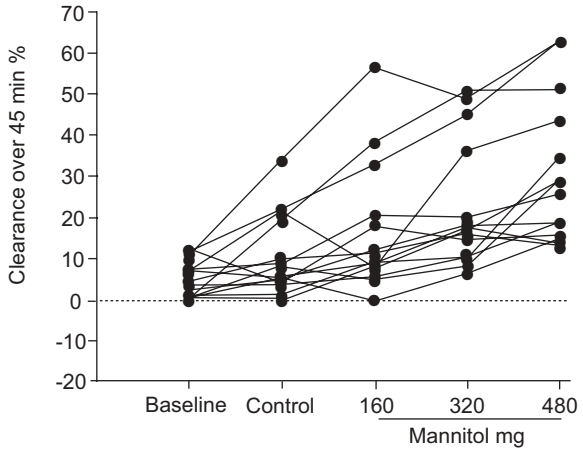

FIGURE 2. Clearance over 45 min, measured since the start of the intervention, in the whole right lung of 14 subjects with bronchiectasis. Clearance increased with increasing doses of mannitol in the majority of subjects. Mean±SE percentage clearance over 45 min for baseline, control, 160, 320 and 480 mg mannitol were 4.7±1.2, 10.6±2.6, 16.7±4.2, 22.8±4.2 and 31.0±4.7%, respectively.

480 mg mannitol, respectively; p>0.2). The radioaerosol deposition was consistent with the FEV1 % predicted being similar on all study days (76.7±3.6, 76.5±3.7, 77.0±3.7, 76.0±3.4 and 76.6±3.7% for baseline, control, 160, 320 and 480 mg mannitol, respectively; p>0.4).

DISCUSSION

The present study investigated the acute effect of clearance of mucus in response to three doses of mannitol. The key finding herein is that clearance of mucus increases with increasing doses of mannitol and that clearance can be further enhanced by voluntary cough in patients with bronchiectasis.

The majority of subjects had extensive bronchiectasis and poor baseline clearance (<10% over 60 min), consistent with previous findings [1–5]. However, the majority of subjects had improved clearance, which was dose dependent after mannitol. Clearance over 45 min from the start of intervention increased to 16.7, 22.8 and 31% with mannitol doses 160, 320 and 480 mg, respectively. These findings are in agreement with previous findings of increasing concentrations of hypertonic saline (3, 7 and 12%) in cystic fibrosis, although the increases in clearance achieved with 7 and 12% hypertonic saline compared with control were very similar [20].

Mannitol increased clearance in all regions of the lung, consistent with previous clearance studies [5] and with deposition studies [21, 22], showing that mannitol is deposited in the lung in a diffuse distribution pattern. The magnitude of

TABLE 3 Clearance at specified intervals in the right central, intermediate and peripheral regions of the lung in 14 subjects with bronchiectasis

Region	Clearance		Total clearance from start intervention	
	During intervention, over 15 min	Post-intervention, over 30 min	Over 45 min	Over 75 min
Central				
Baseline	3.6±1.3	3.3±1.7	4.8±1.5	30.2±7.2
Control	13.0±3.3	3.2±1.8	13.1±3.6	15.7±4.0**
Mannitol dose mg				
160	14.1±5.3*	6.1±2.8	16.2±5.5*	40.4±6.7$^{\#\#\#}$
320	19.0±6.4**	6.6±3.0	23.3±6.2***, $^{\#}$	46.3±7.2**, $^{\#\#}$
480	24.0±7.2***, $^{\#}$, ¶	12.6±4.5*, $^{\#}$	33.7±5.8 ***, $^{\#\#\#}$, ¶¶¶, +	53.7±5.2***, $^{\#\#\#}$, ¶
Intermediate				
Baseline	2.0±0.6	2.8±0.9	4.4±1.0	17.4±4.1
Control	6.4±1.3*	3.1±1.1	8.4±1.7	11.6±2.0
Mannitol dose mg				
160	10.1±2.1***	9.5±1.9**	18.3±3.2***, $^{\#\#\#}$	31.9±4.4***, $^{\#\#\#}$
320	11.9±1.8***, $^{\#\#}$	11.5±2.1***, $^{\#\#\#}$	21.9±2.6***, $^{\#\#\#}$	33.5±4.3***, $^{\#\#\#}$
480	19.3±2.9***, $^{\#\#\#}$, ¶¶¶, +++	12.2±2.1***, $^{\#\#\#}$	28.8±3.6***, $^{\#\#\#}$, ¶¶¶, +	38.9±4.8***, $^{\#\#\#}$
Peripheral				
Baseline	3.3±1.7	6.2±2.0	8.4±2.1	18.2±4.2
Control	5.5±1.6	7.0±2.1	10.3±2.5	12.6±3.5
Mannitol dose mg				
160	4.8±1.8	15.5±3.3**, $^{\#\#}$	19.0±4.0*, $^{\#}$	31.6±6.3*, $^{\#\#\#}$
320	7.0±2.3	15.1±2.4**, $^{\#\#}$	18.2±3.2*, $^{\#}$	32.1±4.7*, $^{\#\#\#}$
480	7.9±2.4	15.3±2.6**, $^{\#\#}$	20.5±3.8**, $^{\#\#}$	34.1±6.2**, $^{\#\#\#}$

Data are presented as mean percentage clearance±SEM. *, ** and ***: p<0.05, p<0.01 and p<0.001, respectively, *versus* baseline; $^{\#}$, $^{\#\#}$ and $^{\#\#\#}$: p<0.05, p<0.01 and p<0.001, respectively, *versus* control; ¶ and ¶¶¶: p<0.05 and p<0.001, respectively, *versus* 160 mg mannitol; + and +++: p<0.05 and p<0.001, respectively, *versus* 320 mg mannitol.

TABLE 4 Total increase in clearance[#] over 45 min with each dose of mannitol in the whole right lung and defined regions in 14 subjects with bronchiectasis

Lung region	Mannitol		
	160 mg	320 mg	480 mg
Whole right lung	6.1 (0.5–11.7)[+]	12.2 (5.7–18.7)***	20.4 (12.4–28.4)[¶]
Central	2.9 (-6.2–11.9)	10.2 (-0.3–20.7)***	20.5 (9.7–31.5)***
Intermediate	9.9 (4.6–15.1)***	13.5 (8.0–19.0)[¶]	20.3 (13.3–27.4)[¶]
Peripheral	8.7 (1.1–16.3)*	8.0 (2.3–13.7)*	10.3 (1.1–19.4)**

Data are presented as total mean absolute clearance (95% confidence intervals). [#]: compared with clearance on the control study. *, ** and ***: $p<0.05$, $p<0.01$ and $p<0.001$, respectively, *versus* control; [¶]: $p<0.0001$ *versus* control; [+]: $p=0.07$ *versus* control.

the increase in clearance in these bronchiectasis subjects was dose dependent in the central and intermediate regions, but not in the peripheral region. Clearance over 45 min with 480 mg of mannitol was 34, 29 and 20% in the central, intermediate and peripheral regions, respectively, compared with ~5–8% at baseline.

It is evident that the highest dose of mannitol used in the present study (480 mg) increased clearance of mucus the most in the majority of subjects compared with other doses of mannitol (160 and 320 mg). The 95% CIs showed an overlap in the increase in clearance between the doses used. However, the increase in clearance with each dose above the control, suggests that the effect was quite distinct between doses of 160 and 480 mg mannitol. Apparently, a dose difference of 160 mg was too small to achieve a sufficiently distinct increase in clearance to avoid overlap in the 95% CIs.

The clearance over 45 min with 480 mg is very similar to the clearance found with 400 mg in different groups of bronchiectasis patients [4, 5]. More importantly, clearance with 480 mg was ~30% over 45 min, a baseline clearance rate found in young, healthy subjects [23, 24]. Short-term clinical trials [13, 14] in patients with bronchiectasis using 400 mg of mannitol administered twice daily provide evidence that this dose is sufficient to improve the health-related quality of life. Patients with a range in severity of bronchiectasis were included in those studies [13, 14], but were not classified accordingly. As the present study finds that mucus clearance is dose dependent, the clinical effect of mannitol with doses <400 mg needs to be investigated in patients with differences in the severity of bronchiectasis.

The increase in clearance of mucus in response to mannitol is probably due to many factors. Mucus in patients with bronchiectasis has a higher than usual percentage of solid content, reflecting dehydration [13]. Dehydration of mucus can occur as a result of imbalance between the load of mucus secreted and the water available at the airway surface [25]. Dehydrated mucus is viscous, sticky and difficult to clear. As mannitol is an osmotic agent, it creates the driving force for water efflux into the airway lumen. There is evidence that mannitol reduces the proportion of solid content and improves the surface properties of sputum [13, 26].

The increase in clearance with increasing doses of mannitol may relate to the greater efflux of water into the airway lumen in response to the higher osmotic force. While the increase in

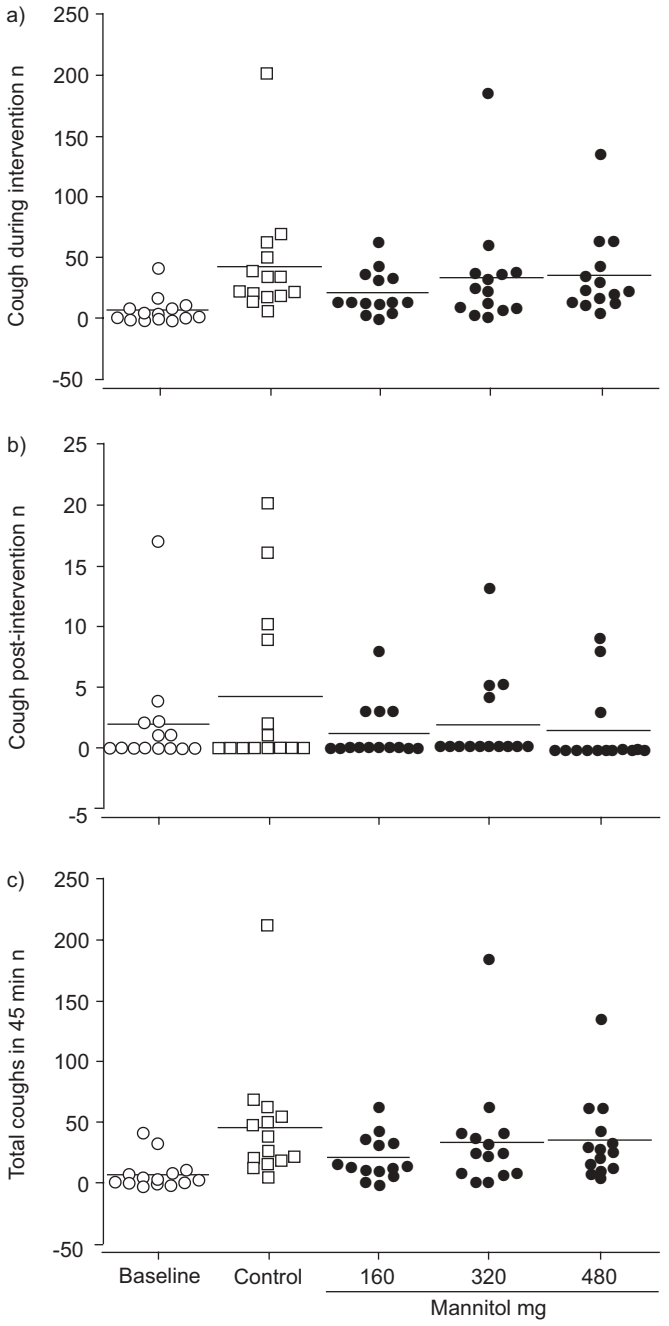

FIGURE 3. Number of coughs a) during intervention, b) post-intervention and c) in total over 45 min from the start of the intervention on the five visits. The control involved the maximum number of coughs provoked by the mannitol doses during the intervention. The horizontal bars are mean values. The median values are: a) 3, 27, 14, 23 and 22 for baseline, control, 160, 320 and 480 mg mannitol, respectively; b) 0 for all conditions; and c) 4, 33, 15, 24 and 28 for baseline, control, 160, 320 and 480 mg mannitol, respectively.

the osmolarity of the airway fluid after mannitol deposition is likely to be transient due to re-establishment of the osmotic equilibrium, the increase in volume of water in the airway lumen should be sustained for as long as the mannitol is present. Mannitol is not absorbed and it has low permeability; therefore, unless it is expectorated with the sputum, it should remain in the lumen, sustaining its effect, for some hours [27].

Inhalation of mannitol provoked cough both during and after the administration; however, the increase in clearance that occurred during the intervention with mannitol is unlikely to be accounted for by the cough it provoked. This is because the cough number was greater during the intervention on the control day and yet the clearance was significantly less compared with any of the mannitol doses, assuming that the intensity and effectiveness of voluntary cough is the same as that of spontaneous cough. Also, evidence that the dose effect is not cough related is provided by the fact that the clearance was dose related but the cough frequency during intervention was not (*i.e.* a similar cough frequency was recorded, irrespective of the mannitol dose). In addition, there was no correlation between the number of coughs provoked by mannitol and clearance of mucus, an observation consistent with previous findings [4, 5].

Mannitol has a pleasant taste and the cough provoked by mannitol is well tolerated. Most patients with bronchiectasis undergo physiotherapy treatment. Physiotherapy also provokes cough, aiming to help expectoration of excessive secretions. Therefore, mannitol could also be used as a help to physiotherapy treatment in patients with bronchiectasis, especially since voluntary coughing, 30 min after inhalation of mannitol, further enhanced clearance in all regions. Most of these subjects had a well-preserved expiratory airflow, which is required for the cough to be effective in clearing mucus [28]. However, it is obvious from the present study that clearance in the presence of mannitol combined with cough manoeuvres can be twice that of the clearance with cough in the absence of mannitol, an enhancement that is seen even in the peripheral region. In the presence of mannitol, it is likely that both an increase in water in the airway lumen and changes in the sputum properties contribute to a greater increase in overall clearance.

Some patients with bronchiectasis have airway hyperresponsiveness to mannitol. The prevalence of hyperresponsiveness in patients with bronchiectasis taking inhaled corticosteroids is 18.9%, and 27.6% in those not taking them [29]. In the present study, three subjects who had a decrease in FEV_1 >15% after inhaling 635 mg of mannitol, following an approved protocol [15], were excluded from the study. It is highly recommended that patients are assessed prior to receiving treatment with inhaled mannitol in order to avoid bronchoconstriction.

In conclusion, the present study confirms the acute increase in the clearance of mucus in response to inhaling mannitol in patients with bronchiectasis. These findings have been extended to show that the acute beneficial effect of mannitol on the clearance of mucus is increased when the dose is increased. The results of the present and previous studies support doses >400 mg of mannitol for achieving clearance of mucus three-fold from baseline clearance and for achieving long-term clinical outcomes in patients with bronchiectasis. It is arguable whether the results of the present study support clinical use of lower doses of mannitol in mild to moderate disease. However, a lower dose, such as 320 mg, may prove to be clinically effective in patients with mild to moderate disease. It is likely that the dose of mannitol needed for effective clearance of mucus may differ among patients according to the severity of the disease.

In addition, clearance can be further improved if administration of mannitol is followed, shortly after, by some cough manoeuvres. The present data provide guidance concerning the dose of mannitol for treatment in patients with bronchiectasis and for clinical trials with a larger number of subjects to show the long-term clinical effect with each dose of mannitol.

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