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Effect of mannitol and repetitive coughing on the sputum properties in bronchiectasis

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Summary
Mucociliary clearance increases with increasing doses of mannitol and clearance is enhanced when mannitol inhalation is followed by repetitive voluntary coughing.

The aim of the study was to investigate: 1) the effect of increasing doses of mannitol and repetitive coughing on the sputum physical properties; 2) if the changes in sputum properties can predict the efficacy of mucus clearance measured by radioaerosol technique in bronchiectasis patients.

Sputum was collected from 14 patients, age: 63 ± 6 yr, who participated on the mucociliary and cough clearance studies at baseline, with mannitol (160, 320 and 480 mg) and control (Daviskas et al. ERJ 2008; 31:765-772). Sputum was collected: 1) on the screening visit before and after mannitol challenge (635 mg); 2) at the start and end of each clearance study after 100 repetitive voluntary coughs except on the control study (no mannitol or repetitive coughing). The sputum solids content, surface tension, contact angle and rheology were measured.

Mannitol in association with coughing and coughing alone reduced the solids content, surface tension, contact angle and viscoelastic sputum properties ($p < 0.0001$) and this effect, unlike mucociliary clearance, was not dose dependent. The control produced no effect. Total mucus clearance correlated only with the percentage reduction in surface tension on 480 mg mannitol and with the reduction in solids content at baseline.

In conclusion: Inhaled mannitol and voluntary repetitive coughing improved the sputum physical properties in bronchiectasis patients and this effect was not dose dependent. Changes in sputum properties do not predict efficacy of mucociliary and cough clearance.

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Introduction

Several studies have confirmed that patients with bronchiectasis have abnormal clearance of mucus that accumulates in the airways causing chronic cough and recurrent infections.[1–6] Osmotic agents, such as mannitol, improve clearance of mucus primarily by increasing the hydration at the airway luminal surface. The increase in mucociliary clearance acutely after a single dose of mannitol (400 mg) has been well documented, using a radioaerosol technique, in patients with bronchiectasis.[3,4] In a pilot study, it has also been shown that 400 mg mannitol reduce the solids content and surface properties of sputum in patients with bronchiectasis.[7]

In a recently published study in patients with bronchiectasis, using a radiaoaerosol technique, we investigated the effect of increasing doses of mannitol on the mucociliary and cough clearance.[5] We reported an increase in mucociliary clearance that was dose dependent and that the overall clearance was enhanced when mannitol was associated with repetitive voluntary coughing.[5] The effect of increasing doses of mannitol and of repetitive coughing on the sputum physical properties in patients with bronchiectasis remained unknown. In addition, the correlation of the physical properties of sputum with the mucociliary and cough clearance following treatment has not been investigated in vivo. If efficacy of the clearance of mucus following treatment can be predicted from measurements of the physical properties of sputum, this may minimise exposure of subjects to radioactivity.

In the present study, in the same subjects with bronchiectasis who had mucociliary and cough clearance measured,[5] we investigate: 1) if the effect of mannitol on the sputum properties is dose dependent; 2) if repetitive voluntary coughing alone is effective in changing the sputum properties; and 3) if the efficacy of clearance of mucus can be predicted by the sputum properties following treatment in patients with bronchiectasis. The hypothesis is that the changes in the sputum properties would correlate with clearance of mucus following treatment.

This study reports the effect of mannitol alone, of increasing doses of mannitol in association with cough and of cough alone on the sputum properties in patients with bronchiectasis. The study also reports the association of the sputum properties with the clearance of mucus following treatment that we reported previously in the same patients with bronchiectasis.[5]

Materials and methods (details on Supplementary data)

Subjects

Fourteen subjects, who participated in the mucociliary and cough clearance studies,[5] with stable bronchiectasis, diagnosed by HRCT, lifelong non-smokers, participated in the sputum studies (Table 1). All subjects withheld their regular medication approximately for 20 h on each visit. No subject was being treated with an antibiotic for an exacerbation for at least 4 weeks prior to study or at the time of the study.

The study was approved by the Ethics Review Committee of Sydney South West Area Health Service (part two, 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 sputum studies

The study consisted of 6 visits. On visit 1 (screening), sputum was collected before and after a standard airway challenge with mannitol. The standard challenge with 635 mg mannitol[8] involved 18 spirometric manoeuvres in total (2 after each dose) and any spontaneous cough induced by the mannitol.

On visits 2–6, involving mucociliary and cough clearance studies, using a radioaerosol technique,[5] sputum was collected at the start and end of each clearance study (Table 2). The intervention involved: resting breathing (baseline study) or inhalation of mannitol (160 or 320 or 480 mg) in a randomised order. Cough clearance during the last 30 min of the total study time (90 min) involved 100 repetitive voluntary deep coughs (about 4 coughs per minute). Subjects had 4 successive coughs followed by rest each minute while in the supine position. The control day involved no mannitol or repetitive voluntary coughing.

Measurements of the physical properties of sputum

Solids content

The percentage of solids content in the sputum was calculated by measuring the weight of a 50 μL aliquot of sputum before and after lyophilization to dryness for 24 h using a freeze dryer (Kinetics, Stone Ridge, NY, USA).

Interfacial tension (surface tension)

Surface tension was measured with a semiautomatic tensiometer (Fisher Tensiomat Model no. 21, Fisher Scientific, Pittsburgh, PA) using the du Nouy ring method described previously.[9,10]

Wettability (contact angle-glass)

Wettability of mucus, its ability to spread over a solid planar surface, is characterized by the contact angle (θ).[11] The contact angle of a 20 μL aliquot of sputum drop on glass was measured as previously described.[7,10]

Work of adhesion

The work of adhesion was calculated from the surface tension and contact angle as follows:

$$\text{Work of adhesion}(W_{ad}) = \text{surface tension} \times (1 + \cos(\theta))\text{mN/m}.$$

Rheology of mucus

The viscosity and elasticity of sputum was measured using a 20-μL aliquot and a controlled stress rheometer with

Table 1 Mean characteristics and lung function in 14 subjects with bronchiectasis.

Subject	Age (yr)	Height (cm)	FEV_1 % Pred	FEV_1 /FVC %	FEF_{25-75} % Pred	%fall FEV_1 post 635 mg mannitol
Mean	63	163	77	66	64	5.3
SD	6	7	14	6	22	5.3

geometry 20 mm, 0.5° aluminium cone and plate (AR 2000, TA Instruments, New Castle, DE) as previously described.[7,10]

Statistical analysis

Comparison of sputum properties collected at start and end of each visit was performed using a 2-factor analysis of variance (ANOVA with repeated measures). Post-hoc analysis was performed using Fisher PLSD (protected least significant difference). Probability values less than 0.05 were considered statistically significant. Pearson's correlation was performed between the clearance of mucus and the sputum properties. Statistical analyses were performed using a statistical package (Statview, Abacus Concepts Inc; Berkeley, CA, USA).

Results

All sputum properties (%solids, surface tension, contact angle and viscoelasticity) were significantly reduced after all doses of mannitol in association with voluntary repetitive coughing and this effect was not dose dependent. Voluntary repetitive coughing alone (baseline) reduced the sputum properties to a similar magnitude.

Sputum solids content and surface properties

The solids content, surface tension and contact angle were significantly reduced after all doses of mannitol in association with coughing and after coughing alone (baseline) (Fig. 1) ($p < 0.0001$). Initial values on each study day were similar (mean range: solids: 7.1–7.6%, $p > 0.9$; surface tension: 83–86 mN/m, $p > 0.7$; contact angle: 51–56°, $p > 0.05$). While the changes were significant with coughing alone and with each mannitol dose, the magnitude of the reduction in solids content, surface tension and contact angle after all doses of mannitol and coughing and after coughing alone, was similar ($p > 0.2$) (Table 3). A significant reduction, of similar magnitude, in solids, surface tension and contact angle was also measured after 635 mg mannitol following the airway challenge on the screening day, when there were spirometric manoeuvres but there was no voluntary repetitive coughing ($p < 0.0001$) (Fig. 1) (Table 3). The solids content, surface tension and contact angle remained unchanged in the absence of mannitol and when there was no repetitive voluntary coughing as on the control study (Fig. 1) ($p > 0.2$) (Table 3).

The work of adhesion was similarly reduced after all doses of mannitol in association with coughing and with cough alone (baseline) ($p < 0.002$) (Fig. 2) (Table 3).

Sputum rheology

The elasticity and viscosity were reduced significantly after all doses of mannitol in association with coughing and also after coughing alone (baseline) ($p < 0.0005$). There was no significant change for the control study ($p > 0.5$). The magnitude of the reduction in elasticity and viscosity after mannitol and coughing was not dose dependent and it was not different to coughing alone ($p > 0.15$) (Table 4). A significant reduction in elasticity and viscosity was also measured after 635 mg mannitol alone on the screening day ($p < 0.0001$) (Table 4).

There was no significant change in $\tan\delta$ (ratio of viscosity to elasticity) at 1 rad/s ($p > 0.4$) but there was at 100 rad/s ($p < 0.001$).

Assay of mannitol

The amount of mannitol in the sputum collected at the end of the study was estimated following a validated assay for measuring mannitol in the sputum supernatant (ARL Pathology, Melbourne, Australia). Supernatant of sputum was obtained by ultracentrifuge method at 45,000 revs/min. The estimate of mannitol in sputum was necessary in order to ascertain that mannitol was still present in the airways after all the coughing and that the changes in all sputum properties were not just due to repetitive coughing alone. The mean ± SD amount of mannitol (mg/mL) in the sputum supernatant was dependent on the inhaled mannitol dose: 160 mg: 0.079 ± 0.142 mg/mL; 320 mg: 0.290 ±0.582 mg/mL; 480 mg: 1.408 ± 2.059 mg/mL. A greater amount of mannitol was measured in the sputum when 480 mg was inhaled compared to when160 and 320 mg was inhaled ($p < 0.03$). An even greater amount of mannitol was measured in the sputum after the 635 mg was inhaled, 10.712 ± 13.842 mg/mL. This value was significantly greater than that measured in the sputum after the mannitol (160, 320 and 480 mg) and voluntary coughing ($p < 0.0005$).

Table 2 Study design of sputum collection on each visit involving mucociliary (MCC) and cough clearance.

Spirometry and sputum collection	Initial MCC 15 min	Intervention 15 min	Post intervention MCC 30 min	Cough clearance 30 min	Spirometry and sputum collection

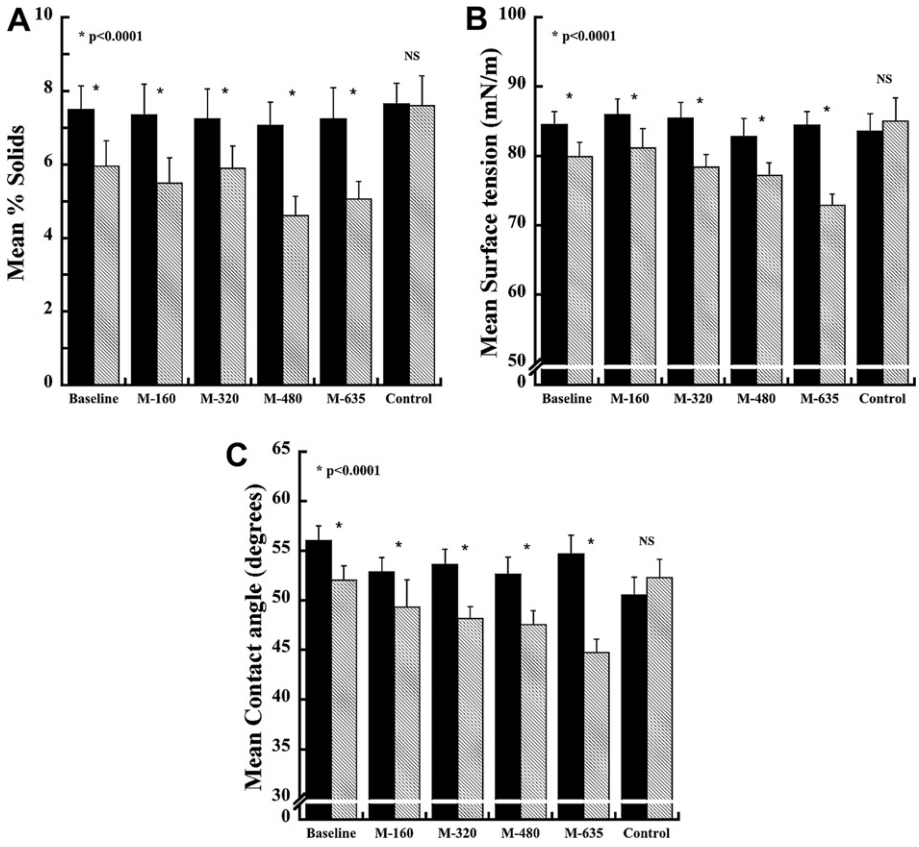

Figure 1 Solids content (A), surface tension (B) and Contact angle (C) in 14 subjects with bronchiectasis at start (black bars) and at the end (striped bars) of each visit involving: baseline clearance (resting breathing followed by repetitive voluntary coughing alone), mannitol 160, 320, and 480 mg followed by repetitive voluntary coughing and mannitol 635 mg after challenge on the screening visit. The control involved no mannitol or voluntary coughing. The solids content, surface tension and contact angle were significantly reduced after all doses of mannitol in association with voluntary coughing, after mannitol alone (635 mg) and after coughing alone (baseline visit) ($p < 0.0001$). There was no significant difference in the magnitude of the reduction in solids content and surface tension. The reduction in the contact angle after 635 and 480 mg was significantly greater than the reduction after coughing alone (baseline) ($p < 0.05$). There was no change in the sputum properties on the control study.

Correlations of sputum properties with clearance of mucus

The change in the solids content after the repetitive voluntary coughing alone (Baseline visit) correlated inversely with the total whole right lung clearance on the day[5] ($r = -0.609$, $p < 0.02$) (Fig. 3). No significant correlation was found between the changes in the surface and rheological properties and the clearance of mucus after coughing alone.

The % change in surface tension from initial value, when 480 mg mannitol was inhaled in association with coughing, correlated inversely with the total whole right lung clearance on the day[5] ($r = -0.569$, $p = 0.032$) (Fig. 4). The % clearance by cough over 30 min when 480 mg mannitol was

Table 3 Mean reduction (95% CI) from initial values on each study day in solids content, surface tension contact angle and work of adhesion at the end of each study. All reductions are significant but similar in magnitude, except on the control day. In addition, the magnitude of the reduction in Contact angle with mannitol 480 and 635 mg is significantly different compared to Baseline ($p < 0.05$).

	Solids%	Surface tension mN/m	Contact angle degrees	Work of adhesion mN/m
Control	0(−1.0 to 1.0)	1.5(−5.8 to 8.8)	1.8(−1.5 to 5.1)	−0.033(−8.6 to 8.5)
Baseline	1.5(0.2−2.8)	4.6(−1.6−10.8)	4.0(1.5−6.5)	2.7(−5.0−10.4)
Mannitol160 mg	1.9(1.0−2.8)	4.8(1.1−8.5)	4.4(0.5−8.1)	3.0(−4.1−10.0)
Mannitol320 mg	1.3(0.2−2.4)	7.1(1.6−12.6)	5.4(2.8−8.0)	5.2(−2.2 to 12.6)
Mannitol480 mg	2.4(1.1−3.7)	5.6(1.6−9.6)	5.1(1.9−8.3)	3.4(−2.4 to 9.1)
Mannitol635 mg	2.2(0.7−3.7)	11.6(6.9−16.3)	9.9(5.2−14.6)	8.2(1.7−14.7)

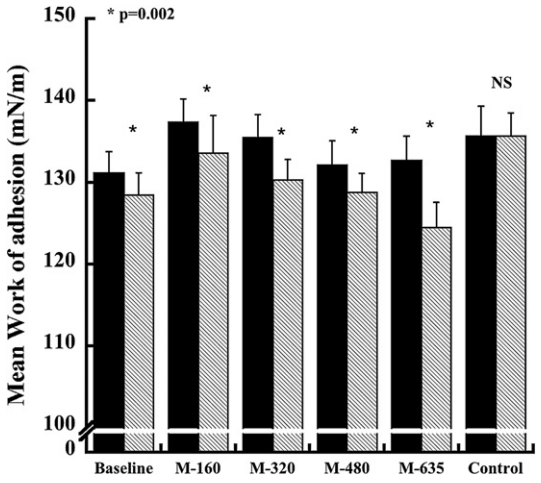

Figure 2 Work of adhesion was significantly reduced after all doses of mannitol in association with voluntary coughing, after mannitol alone (635 mg) and after coughing alone (baseline) ($p < 0.002$).

inhaled correlated with the amount of mannitol measured in the sputum[5] ($r = 0.680$, $p = 0.009$).

There were no other significant correlations found between any of the sputum properties or their changes and the total clearance of mucus or cough clearance in the presence of mannitol associated with coughing.

Discussion

This study investigated the changes in the physical properties of sputum in patients whose mucociliary and cough clearance was assessed with resting breathing followed by repetitive voluntary coughing (baseline) and after three doses of mannitol followed by repetitive voluntary coughing. All sputum properties (solids content, surface tension, contact angle and viscoelasticity) were significantly reduced to a similar magnitude after all doses of mannitol and coughing and after coughing alone. There was no significant change in any of the properties of sputum on the control study when there was no mannitol or voluntary coughing involved.

In the presence of mannitol, the only sputum property that was related to the total clearance of mucus was the % reduction in surface tension after 480 mg and cough. In addition, the percentage of clearance by cough, 30 min after this dose, correlated with the amount of mannitol in the sputum. This dose of mannitol was, however, the highest dose administered in conjunction with voluntary cough and significantly more mannitol was still in the airways after the 100 coughs compared to the lower doses. It is also a dose close to what is administered clinically (400 mg) in patients with bronchiectasis.

We expected to find dose dependent changes in the sputum properties as we did with mucociliary clearance in the same subjects with bronchiectasis. However, the changes in the sputum properties, while significant, were similar in magnitude after all doses of mannitol followed by voluntary coughing and after coughing alone (baseline study) and the reason is unclear. It is possible that the physical properties of sputum collected after 100 voluntary coughs were mostly influenced by the effect of cough, as after 100 coughs most mannitol had been cleared from the airways especially with the lower doses. However, similar changes in the properties were found following the dose of 635 mg that did not involve voluntary coughing and significantly more mannitol was still present in the sputum compared to the lower doses of mannitol in association with coughing. It is of interest that similar changes in the sputum properties were found after increasing doses of mannitol alone, in asthmatics with excessive secretions, when there was no voluntary coughing involved.[10]

The effectiveness of repetitive coughing on the clearance of mucus and its relationship with the changes on the sputum properties has not been investigated before in patients with bronchiectasis. It was found that the

Table 4 Mean (±SD) sputum dynamic elasticity (storage modulus G'), dynamic viscosity (loss modulus G'') at 1 and 100 rad/s at the start (initial) and end of baseline, mannitol 160, 320, 480 and 635 mg and control visit.

	G'1 Pa		G''1 Pa	
	Initial	End	Initial	End
Control	23.3 ± 18.1	25.2 ± 15.1	11.9 ± 9.1	12.8 ± 8.6
Baseline	31.7 ± 22.7	21.2 ± 19.1*	17.7 ± 19.0	12.8 ± 13.7*
M160	29.9 ± 23.9	25.2 ± 22.7*	18.4 ± 23.4	14.2 ± 14.7*
M320	30.7 ± 28.7	22.8 ± 18.9*	16.1 ± 18.2	12.6 ± 12.3*
M480	24.2 ± 18.1	16.8 ± 11.3*	13.3 ± 8.4	8.8 ± 6.0*
M635	33.6 ± 26.9	16.3 ± 9.8*	20.1 ± 15.9	8.6 ± 7.0*
	G'100 Pa		G''100 Pa	
	Initial	End	Initial	End
Control	38.1 ± 25.0	38.0 ± 23.4	176.9 ± 87.9	166.7 ± 47.5
Baseline	52.4 ± 53.7	33.2 ± 32.0*	238.0 ± 184.9	197.9 ± 148.6#
M160	55.4 ± 54.5	40.1 ± 43.5*	206.6 ± 91.3	202.7 ± 113.6#
M320	56.3 ± 66.1	45.5 ± 34.0*	165.0 ± 60.2	173.7 ± 96.3#
M480	44.6 ± 30.6	31.2 ± 23.8*	166.5 ± 43.8	146.6 ± 63.4#
M635	57.4 ± 39.0	28.2 ± 26.4*	253.1 ± 160.9	170.4 ± 96.3#

* # significantly different from initial value (2-factor AVOVA with repeated measures), *$p < 0.0005$, #$p < 0.03$.

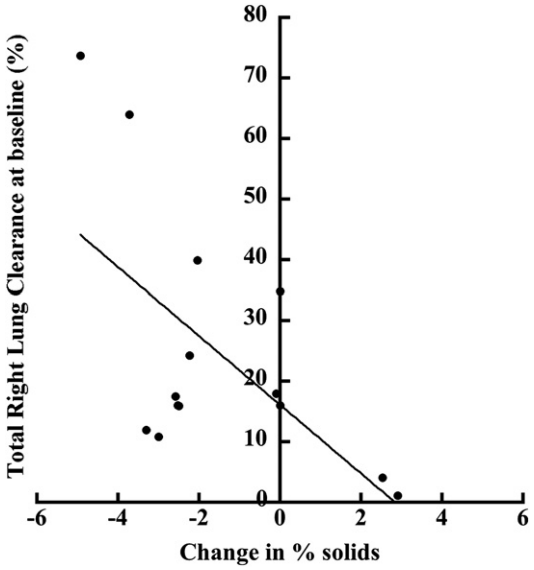

Figure 3 Correlation between the total whole right lung clearance and the change from initial value in % solids content on the baseline study (cough alone, no mannitol) ($r = -0.609$, $p < 0.02$).

reduction in solids content after coughing alone correlated with the total clearance at baseline. Coughing is regarded as a mechanical stimulus that can stimulate release of ATP that can potentially increase the hydration at the airway lumen.[12,13] The reduction in solids content after repetitive coughing is consistent with an increase in hydration at the airway lumen. The reduction in surface tension, contact angle and viscoelasticity is also consistent with an increase in hydration at the airway lumen. However, there was no correlation found between the surface and rheological properties of sputum or their changes and the clearance of mucus following repetitive coughing alone. In contrast, simulated repetitive coughing *in vitro* has been shown to

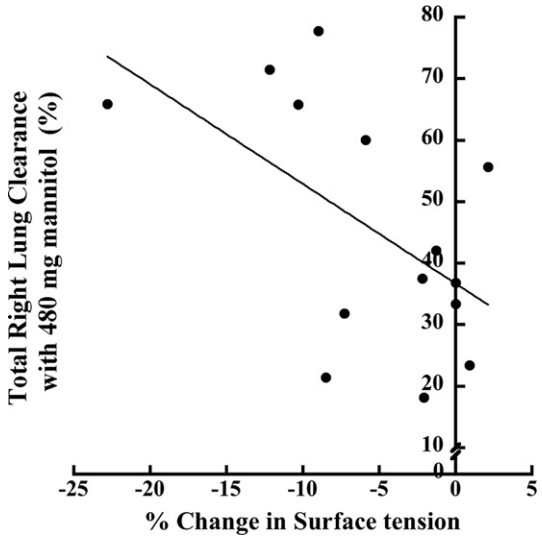

Figure 4 Correlation between the total whole right lung clearance in response to 480 mg mannitol in association with voluntary coughing and the % change from initial value in surface tension ($r = -0.569$, $p = 0.032$).

increase mucus transportability that correlated with shear thinning and a change in viscosity.[14]

The baseline solids content was around 7%, more than double the value of solids that is regarded as normal (2−3%),[15] reflecting significant dehydration in this group of bronchiectasis patients. Dehydration of mucus can occur as a result of imbalance between the load of mucus secreted and the water available at the airway surface.[15] Dehydrated mucus is viscous and sticky and difficult to clear and mucus transport is significantly decreased as solids increase from 2.5 to 6%,[15,16] consistent with these subjects having poor mucociliary clearance.[5] Mannitol, being an osmotic agent, creates the driving force for water efflux into the airway lumen. We proposed that 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 forces created. However, we were unable to find significant differences in the concentration of solids with the different doses of mannitol and especially after 635 mg and the reason for this surprising finding is unclear.

A significant change in surface tension, work of adhesion and viscoelastic properties was measured after mannitol and cough and after cough alone. In our pilot study, involving a smaller cohort of subjects with bronchiectasis, we found no change in the viscoelastic properties after inhalation of 400 mg mannitol.[7] However, baseline solids and viscoelastic properties in the present study were higher compared to the previous study[7] suggesting that these subjects may have had a more severe disease.

Based on the current findings, changes in the physical properties of sputum *in vivo* may guide us to assess the effect of a treatment qualitatitively, but they do not guide us to assess efficacy of clearance of mucus with different treatments quantitatively as measured by the radioaerosol technique. We were unable to find consistent correlations between clearance of mucus and any of the sputum properties or their changes after coughing alone or after treatment with mannitol and coughing. The only correlation found was between the solids content and clearance after cough at baseline and between the reduction in surface tension and clearance after 480 mg of mannitol in association with coughing. Hasani et al.[17] also found no correlation between clearance during cough and the viscoelastic properties of sputum. The lack of other significant correlations in the changes of sputum properties and clearance of mucus may not be surprising given that there was no difference in the changes of sputum properties with increasing doses of mannitol and cough. While the physical properties of mucus can affect its clearance, the measurement of the clearance of mucus depends on many other factors including deposition of the radioactive aerosol. In our study the deposition was well controlled within the same individual between studies and it was predominantly in the central region. Expectorated sputum originates from the large airways, however, under stimulated conditions and after 60 min of treatment, sputum may originate from smaller airways that has been transported to the large airways. It is possible that there are limitations to our technique of sputum collection. Perhaps, different results may have been obtained if samples of mucus were collected directly from the lower airways bypassing the upper airways and avoiding any salivary contamination.

In conclusion, inhaled mannitol in association with repetitive voluntary coughing and coughing alone improved the solids content, surface tension, contact angle, work of adhesion and viscoelasticity in patients with bronchiectasis. There was no mannitol dose effect in association with coughing in the improvement of the physical properties of sputum in patients with bronchiectasis, in contrast to the effect on the clearance of mucus. This is the first study that shows a change in the sputum properties after coughing alone, although it has been suggested that coughing is a mechanical stimulus and it can potentially increase the hydration in the airways.[12] Repetitive voluntary coughing can be beneficial in patients with bronchiectasis as it enhances the clearance of mucus when used shortly after mannitol inhalation. The findings of this study suggest that the changes in the sputum properties after treatment may indicate an improvement in the clearance of mucus but they may not predict the magnitude of the improvement.

Conflict of interest

Evangelia Daviskas MBiomedE PhD, Dr Daviskas is an employee of the South West Sydney Area Health Service that owns the patent relating to the use of mannitol for enhancing clearance of secretions described in the paper and may benefit from royalties in the future. Dr Daviskas owns self funded shares in Pharmaxis Ltd. Dr Daviskas, in her capacity as an employee of the SSWAHS, consults for Pharmaxis.

Sandra D Anderson PhD DSc, Dr Anderson is the inventor of the mannitol test. The patent is owned by her previous employer Sydney South West Area Health Service (SSWAHS). SSWAHS invoiced Pharmaxis for my time, until March 2009 she had not received personally any monies from Pharmaxis other than to cover travelling expenses. Since March 2009 she has received US$ 2000 for consulting fees. She has not received a speaker's fee from Pharmaxis to date. She owns shares in Pharmaxis that she purchased herself. She does not own any options. She receives a share of 10% of the royalties paid to SSWAHS. She has acted in an honorary capacity on the Scientific Board of Pharmaxis. She has spoken for Merck and Nycomed in the last 3 years for which she has received an honorarium.

Iven H Young MBBS PhD, Professor Young is employed by the Sydney South West Area Health Service (SSWAHS) who is the owner of the intellectual property of the use of mannitol for clearance of mucus, that has been licensed to Pharmaxis Ltd.

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The application for the use of mannitol described in this paper is covered by U.S. patent No. 5817028, Australian patent no. 682756, and International patent PCT/AU 95/00086. The patent is owned by the Sydney South West Area Health Service and has been licensed to Pharmaxis Ltd, Frenchs Forest, Australia, for development.

Appendix. Supplementary data

Supplementary data associated with this article can be found in online version at doi:10.1016/j.rmed.2009.10.021.

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