Timing of dornase alpha inhalation does not affect the efficacy of an airway clearance regimen in adults with cystic fibrosis: a randomised crossover trial

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**Introduction**

Treatment of sputum retention and the associated chronic infection in the airways of people with cystic fibrosis involves several therapeutic approaches. Antibiotics are administered to suppress infection (Southern et al 2004, Ryan et al 2003, Smyth and Walters 2003), manual physiotherapy techniques and other physical interventions are used to clear infected mucus from the airways (van der Schans et al 2000), and various mucoactive medications are used to improve the properties of the mucus to facilitate its clearance (Jones and Wallis 2010, Wark and McDonald 2009). One of these mucoactive medications is recombinant human deoxyribonuclease, or dornase alpha (Pulmozyme®). It reduces the viscosity of sputum in people with cystic fibrosis by cleaving strands of the deoxyribonucleic acid (DNA) released by neutrophils (Lieberman 1968). This makes the sputum flow more easily (Shak et al 1990). Regular use of dornase alpha improves lung function and quality of life, and reduces the number and severity of respiratory exacerbations (Hubbard et al 1992, Ramsey et al 1993, Fuchs et al 1994).

Although dornase alpha has been used widely in the management of cystic fibrosis for more than 15 years, the optimal timing of administration with respect to physical airway clearance techniques is still unclear. During its clinical development, trials allowed dornase alpha to be administered either before or after physical airway clearance techniques. Only recently have trials started to address this potentially important aspect of its administration.

**Conclusion**: Timing of dornase alpha can be selected according to convenience, patient preference, or to accommodate the timing of other medications in the treatment regimen. **What this study adds**: The timing of dornase alpha inhalation does not affect the efficacy of an airway clearance regimen in adults with cystic fibrosis: a randomised crossover trial. **Journal of Physiotherapy 57: 223–229**

**Key words**: Cystic fibrosis, Dornase alpha, Airway clearance techniques, Lung function, Quality of life

**What is already known on this topic**: The timing of dornase alpha in relation to physiotherapy techniques may alter the effect of these two interventions on airway clearance. However, this has not been examined in adults with cystic fibrosis.

**What this study adds**: The timing of dornase alpha does not strongly influence the efficacy of the airway clearance regimen in adults with cystic fibrosis. Therefore dornase alpha can be timed according to convenience, patient preference or to accommodate other medications in the treatment regimen.
and 75% of the FVC and one measure of quality of life), administration of dornase alpha before physical airway clearance techniques was less favourable. These somewhat conflicting results make it difficult for clinicians to advise people – especially adults – with cystic fibrosis about how to structure their treatment regimen for airway clearance.

This study was designed to compare the effectiveness of dornase alpha administered before versus after airway clearance techniques, in adults with cystic fibrosis. We were also interested in whether the response of some subgroups of participants might differ from others, defined by their baseline lung function, or by their baseline sputum production. Therefore, the research questions for this study were:

1. Does the inhalation of dornase alpha before or after airway clearance techniques influence the effect on lung function?
2. Does the inhalation of dornase alpha before or after airway clearance techniques influence 24-hour sputum production, the percentage of daily sputum produced during the airway clearance regimen, oxygen saturation, peak oxygen consumption during an incremental exercise test, oxygen desaturation during exercise, and quality of life?
3. Do particular subgroups of participants differ in their response to the two regimens?

**Method**

**Design**

A randomised trial with concealed allocation and intention-to-treat analysis and blinding of participants, therapists, and assessors was undertaken at the Cystic Fibrosis Unit at Westmead Hospital, Sydney. Participants were recruited from the outpatient clinic of the Cystic Fibrosis Unit. Before entry into the study, each participant had their airway clearance techniques reviewed and optimised by one investigator (JRB). The range of techniques used included conventional postural drainage and percussion, positive expiratory pressure via a mask interface, and active cycle of breathing techniques (Pryor and Prasad 2008). All participants were then encouraged to perform at least 15 min of the techniques each day for the 28 days before randomisation was scheduled, to ensure familiarity with the techniques. Participants were assessed in the Cystic Fibrosis Unit 14 days prior to randomisation and on the day of randomisation (Day 0) to confirm clinical stability at the time of enrolment. Randomisation occurred within the hospital pharmacy to maintain concealment of the random allocation list, which used a block size of four participants. Dornase alpha and placebo in blinded packaging were dispensed through the hospital pharmacy to maintain blinding. Participants inhaled dornase alpha before and placebo after performing their airway clearance techniques for 14 days, and placebo before and dornase alpha after the techniques for the other 14 days. The order of the two 14-day periods was randomised. Participants were assessed at the beginning and end of each 14-day period, as presented in Figure 1.

**Participants**

Outpatients attending the Cystic Fibrosis Unit were eligible to participate if they were aged 18 years or more and had a diagnosis of cystic fibrosis confirmed by a clinical history, a positive sweat test and/or nasal potential difference measurement. Exclusion criteria were: FVC less than 40% of the predicted value, clinical instability (> 10% change in forced expiratory volume in 1 sec (FEV₁) over 14 days) or hospitalisation within the 14 days prior to randomisation, current use of dornase alpha, inability to perform reproducible lung function tests, and poor adherence with therapy (defined as < 85% adherence based on self report and vial counts).

**Intervention**

The experimental intervention was to take dornase alpha after and the placebo before performing the airway clearance techniques once daily for 14 days. The control intervention was to take dornase alpha before and the placebo after the airway clearance techniques for 14 days.

The active ampoules contained 2.5 mg of dornase alpha in 2.5 mL. The placebo ampoules contained 2.0 mL of 0.9% saline. To preserve blinding, all ampoules were stored under refrigeration – a requirement of dornase alpha. Each participant was supplied with two jet nebulisers to be used for inhaling the trial solutions. The nebulisers were colour-coded to match the trial solution packaging, but were otherwise identical. Separate nebulisers were necessary because dornase alpha can be denatured by traces of other compounds in the nebuliser chamber. At the start of the trial, all nebuliser pumps were tested to ensure that they produced adequate flow rates (6–8 L/min) with sufficient driving pressures (10–12 pounds per square inch, 69–83 kPa).

All participants received usual medical and allied health management by the Cystic Fibrosis Unit if required during the trial period, and were encouraged to continue with their other usual therapies. Participants who were already taking bronchodilators were advised to inhale them before the inhalation of the first trial solution at each daily treatment session. Participants who were already taking inhaled antibiotics were advised to inhale them after the inhalation of the second trial solution at each daily treatment session.

Demographic and clinical data including age, gender, body mass index, bacterial colonisation of sputum, usual medication use, lung function, oxhaemoglobin saturation, and quality of life were recorded at baseline (Day 0).

On Day 1, participants received the blinded therapy under clinical supervision. Lung function was measured before and after each nebulisation and both before and after the physical airway clearance techniques to assess any acute changes during the intervention. Cumulative sputum weight was measured after each spirometry measurement. Subsequent doses were inhaled independently at home. On the first day of the second treatment arm (Day 15) the same measurements were performed.

**Outcome measures**

All outcome measures were recorded at the start and end of the first 14-day period (Days 1 and 14) and at the start and end of the second 14-day period (Days 15 and 28), as presented in Figure 1. All measurements were performed by an investigator who was blinded to whether the participant was in the experimental or control arm of the study. Participants were also blinded throughout the study, including when they completed the quality of life questionnaires.
Patients screened for participation (n = 30)

Excluded (n = 10)
• Already taking dornase alpha (n = 7)
• Declined to participate (n = 3)

Review of physical airway clearance techniques and assessment of clinical status (n = 20)

Withdraw (n = 3)
• Time constraints (n = 3)

Randomised (n = 17)
(n = 9) (n = 8)

Experimental arm
• usual care
• dornase alpha after airway clearance techniques

Control arm
• usual care
• dornase alpha before airway clearance techniques

Lost to follow-up (n = 0)

Experimental arm (n = 9) Control arm (n = 8)

Day 1
Measured lung function, sputum weight, oxygenation, exercise capacity and quality of life

Lost to follow-up (n = 0)

Day 14
Measured lung function, sputum weight, oxygenation, exercise capacity, and quality of life
(n = 9) (n = 8)

Day 15
Measured lung function, sputum weight, oxygenation, exercise capacity, and quality of life
(n = 9) (n = 8)

Lost to follow-up (n = 0)

Day 28
Measured lung function, sputum weight, oxygenation, exercise capacity, and quality of life
(n = 9) (n = 8)

Figure 1. Design and flow of participants through the trial.
Lung function was measured using a standard spirometer\(^b\) according to American Thoracic Society guidelines (American Thoracic Society 1995). The spirometric measures recorded were FEV\(_1\) in litres and as a percentage of the predicted value, and FVC as a percentage of the predicted value. Predicted values were calculated using the equations of Knudson and colleagues (1976).

The sputum expectorated within a 24-hr period was collected in a plastic flask by the participants and weighed on an electronic scale. The amount of sputum expectorated during a session of airway clearance techniques was collected independently in a separate flask, so that it could be calculated as a proportion of the 24-hour sputum weight.

Oxygenation was measured using a standard pulse oximeter with a finger probe. Stable readings were required for 10 sec before recording the data. Oxygenation was also continuously monitored during the exercise test (described below) to determine the greatest reduction during the exercise test.

Exercise capacity was measured using the original 10-m shuttle test (Singh et al 1994) or the Multi Stage Fitness Test (Léger and Lambert 1989). Oxygen uptake at peak exercise was estimated from the exercise testing using standard equations (Singh et al 1994, Léger and Lambert 1989).

Participants completed the adult Australian Cystic Fibrosis Quality of Life (CFQOL) questionnaire\(^c\) independently. This questionnaire results in an overall score between 0 (worst) and 100 (best).

**Data analysis**

A change in FEV\(_1\) of 10% is used as a threshold for Australian government reimbursement of the cost of dornase alpha. We therefore nominated 10% as the between-group difference we sought to identify. Assuming a within-patient SD of 10%, 18 participants would provide 80% power, at the 2-sided 5% significance level, to detect a 10% difference in FEV\(_1\) between the experimental and control arms as statistically significant. We recruited 20 participants to allow for loss to follow-up.

Continuous data were summarised as means and standard deviations and categorical data were summarised as frequencies and percentages. The normality of the distribution of the data was examined with the Kolmogorov-Smirnov test. Although some of the raw data were not normally distributed, the within-subject differences were normally distributed. Therefore the data were analysed using parametric statistics. Between-group differences in change from baseline were analysed using paired t-tests. Mean differences (95% CI) between groups are presented. Data were analysed by intention-to-treat. The effect of the timing regimen on FEV\(_1\) was correlated against baseline FEV\(_1\) and against baseline sputum production, and the strength of the relationship was reported using the coefficient of determination (r\(^2\)).

**Results**

**Flow of participants, therapists and centres through the trial**

Thirty adults from the Cystic Fibrosis Unit were screened for eligibility. Twenty met the initial eligibility criteria, but three withdrew during the 14-day period of regular use of airway clearance techniques, citing time constraints. The remaining 17 participants were randomised: 9 to the experimental arm (dornase alfa after physical airway clearance techniques) first (post-pre group), and 8 to the control arm (dornase alfa before physical airway clearance techniques) first (pre-post group). The flow of participants through the trial is illustrated in Figure 1. The characteristics of the participants were similar at the start of each arm of the study (Table 1 and the first two columns of Table 2). Twelve participants were using positive expiratory pressure as their physical airway clearance technique. Seven participants were using active cycle of breathing techniques, of whom 4 were using percussion as well. One participant used positive expiratory pressure once daily and percussion once daily.

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**Table 1. Characteristics of participants.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n = 17)</th>
<th>Exp first (n = 9)</th>
<th>Con first (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr), mean (SD)</td>
<td>25 (11)</td>
<td>27 (15)</td>
<td>23 (4)</td>
</tr>
<tr>
<td>Gender, n males (%)</td>
<td>9 (53)</td>
<td>5 (56)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>BMI (kg/m(^2)), mean (SD)</td>
<td>20.6 (3.3)</td>
<td>20.9 (4.1)</td>
<td>20.3 (2.2)</td>
</tr>
<tr>
<td>Bacterial colonisation of sputum, n (%)</td>
<td>16 (94)</td>
<td>8 (89)</td>
<td>8 (100)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>5 (29)</td>
<td>1 (11)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>1 (6)</td>
<td>0 (0)</td>
<td>1 (13)</td>
</tr>
<tr>
<td>MRSA</td>
<td>1 (6)</td>
<td>1 (11)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Burkholderia cepacia</td>
<td>11 (65)</td>
<td>7 (78)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Exercise</td>
<td>8 (47)</td>
<td>5 (56)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Bronchodilator</td>
<td>10 (59)</td>
<td>5 (56)</td>
<td>5 (63)</td>
</tr>
<tr>
<td>Antiinflammatory</td>
<td>7 (41)</td>
<td>3 (33)</td>
<td>4 (50)</td>
</tr>
<tr>
<td>Oral steroids</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>10 (59)</td>
<td>4 (44)</td>
<td>5 (63)</td>
</tr>
<tr>
<td>Dornase alpha naïve, n (%)</td>
<td>11 (65)</td>
<td>7 (78)</td>
<td>4 (50)</td>
</tr>
</tbody>
</table>

Exp = experimental condition (dornase alpha after physical airway clearance techniques), Con = control condition (dornase alpha before physical airway clearance techniques), BMI = body mass index, MRSA = Methicillin-resistant Staphylococcus aureus.

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\(^b\)Cystic Fibrosis Unit, \(^c\)Australia's Cystic Fibrosis Association.
The airway clearance regimen, including tailoring of the physical techniques and confirming the appropriate nebulisation procedures, was determined by the Cystic Fibrosis Unit physiotherapist, who had 6 years of clinical experience, including 4 years in the cystic fibrosis area. The Cystic Fibrosis Unit of Westmead Hospital in Sydney was the only centre to recruit and test patients in the trial. The Cystic Fibrosis Unit managed approximately 60 adult patients during the time of the study.

Compliance with trial method

All randomised participants completed both arms of the trial. According to diary card entries and vial counts, compliance with the allocated therapies was > 85%. No participants in either arm had adverse clinical changes during the intervention that required cessation of the intervention. One participant with a history of recurrent haemoptysis had a single episode after the first 14-day intervention period (during which he was taking dornase alpha before airway clearance techniques). This was considered unlikely to be related to treatment and resolved spontaneously despite continuation of the allocated treatment regimen.

Effect of intervention

Group data for all outcomes for the experimental and control interventions are presented in Tables 2 and 3, while individual data are presented in Table 4 (see eAddenda for Table 4).

The timing of the inhalation of dornase alpha did not have statistically significant effects on lung function. The best estimate of the average effect of changing from inhaling dornase alpha before to after the physical techniques was to increase FEV$_1$ by only 40 mL (95% CI –140 to 230 mL). When the FEV$_1$ data were considered in terms of a percentage of the predicted value, the best estimate of the effect and the limits of the confidence interval all indicated that any effect was too small to be clinically worthwhile.

Table 2. Mean (SD) for outcomes for each group, mean (SD) difference within groups, and mean (95% CI) differences between groups.

<table>
<thead>
<tr>
<th></th>
<th>Exp (n = 17)</th>
<th>Con (n = 17)</th>
<th>Exp minus Con</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV$_1$ (L)</td>
<td>2.29 (0.81)</td>
<td>2.35 (0.95)</td>
<td>0.01 (0.21)</td>
<td>–0.14 to 0.23</td>
</tr>
<tr>
<td>FEV$_1$ (% predicted)</td>
<td>67 (22)</td>
<td>68 (25)</td>
<td>1 (6)</td>
<td>–4 to 6</td>
</tr>
<tr>
<td>FVC (% predicted)</td>
<td>83 (22)</td>
<td>81 (23)</td>
<td>–2 (6)</td>
<td>–11 to 0</td>
</tr>
<tr>
<td>SpO$_2$ (%)</td>
<td>97 (2)</td>
<td>96 (5)</td>
<td>0 (7)</td>
<td>–4 to 6</td>
</tr>
<tr>
<td>Estimated VO$_2$ peak (mL/kg/min)</td>
<td>27 (8)</td>
<td>27 (8)</td>
<td>0 (3)</td>
<td>–3 to 2</td>
</tr>
<tr>
<td>SpO$_2$ change with exercise (absolute %)</td>
<td>–4.8 (4.0)</td>
<td>–4.7 (3.3)</td>
<td>–0.4 (1.2)</td>
<td>–1.8 to 0.3</td>
</tr>
<tr>
<td>Quality of life (0 to 100)</td>
<td>73 (9)</td>
<td>71 (13)</td>
<td>0 (10)</td>
<td>–9 to 3</td>
</tr>
</tbody>
</table>

Table 3. Mean (SD) for outcomes for each group at the end of the intervention period, and mean (95% CI) difference between groups.

<table>
<thead>
<tr>
<th></th>
<th>Exp (n = 17)</th>
<th>Con (n = 17)</th>
<th>Exp minus Con</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>24-hour sputum weight (g)</td>
<td>21 (23)</td>
<td>24 (36)</td>
<td>–3 (–12 to 7)</td>
<td></td>
</tr>
<tr>
<td>Proportion of 24-hr sputum obtained during airway clearance regimen (%)</td>
<td>17 (15)</td>
<td>23 (25)</td>
<td>–5 (–15 to 4)</td>
<td></td>
</tr>
</tbody>
</table>

Exp = experimental condition (dornase alpha after physical airway clearance techniques), Con = control condition (dornase alpha before physical airway clearance techniques).
FVC tended to favour the inhalation of dornase alpha before airway clearance techniques, but the result was only of borderline statistical significance.

Daily sputum production did not appear to be influenced by the timing regimen, and nor did the amount of sputum obtained during the airway clearance regimen as a proportion of the daily amount.

There was little change in resting oxygen saturation levels in all participants throughout both arms of the study. The timing of inhalation of dornase alpha did not have a significant effect on this outcome. Exercise capacity was also quite stable among all participants, with no significant effect due to the timing of dornase alpha. On average, participants showed a fall in oxygenation of about 5% (absolute) during the exercise test at the start and end of both arms of the study.

The quality of life data showed that most patients’ quality of life scores improved during the study regardless of the timing of dornase alpha. Change in quality of life score showed a good correlation with change in FEV1 (r² = 0.4, p < 0.001). The effect of the timing regimen on FEV1 was not significantly correlated with baseline FEV1 (r² = 0.11). It was also not significantly correlated with baseline sputum production (r² = 0.02).

Discussion

This is the first study to consider the effect of the timing of dornase alpha in relation to airway clearance techniques in adults with cystic fibrosis. The main finding is that the timing of dornase alpha does not have a substantial impact on clinical outcomes over a 14-day period. This finding is likely to be accurate because many aspects of the study design eliminated sources of potential bias. For example, the groups were similar on their baseline measures and are likely to have been similar on unmeasured characteristics as well, due to the use of randomisation and concealment of allocation, which circumvents some potential confounders of the randomisation process. Potential sources of bias were also eliminated from the outcome data through blinding of participants, the assessors, and the physiotherapist who explained the intervention to the participants and who taught them how to administer the trial solutions. The study was adequately powered, with no loss to follow-up after randomisation, resulting in a confidence interval around the primary outcome that excluded the possibility that the timing of dornase alpha has clinically important effects. Previous large multi-centre studies have shown that the maximum effect of dornase alpha on FEV1 is achieved within the first 7 to 14 days (Fuchs et al 1994), so presumably the duration of the study arms was sufficient to identify the effect on lung function.

In addition to the strengths of the study design, we acknowledge that there were some limitations in the methods. Peak oxygen consumption was not measured directly and one of two exercise tests was used to estimate it. Also, there was a minimal washout period between the two study arms. However, there was minimal difference between the groups at the end of the first treatment period, suggesting that the lack of a long washout period was not a substantial confounder.

The results of the study were also consistent with similar studies in children with cystic fibrosis. Fitzgerald and colleagues (2005) examined the effect of timing of dornase alpha in children with less severe cystic fibrosis lung disease than our cohort. This trial also did not identify an effect of timing on any outcome. Interestingly, post-hoc analysis of their cohort identified that inhalation of dornase alpha after physical airway clearance techniques was more beneficial for FEV1 in those participants who were colonised with Pseudomonas aeruginosa. Although virtually all the participants in our study were colonised with Pseudomonas aeruginosa, it did not demonstrate a clear advantage of inhaling dornase alpha after physical airway clearance techniques. In a different study, dornase alpha inhaled 30 min before physical airway clearance techniques improved expiratory flow at 25% of the forced vital capacity (van der Giessen et al 2007). However, FEV1, FVC, and visual analogue scores of sputum and cough were not affected differently by the two timing regimens in that study.

Although the other studies in this area reported the amount of sputum expectorated, ours was the only study to report the amount of sputum obtained during the airway clearance regimen as a proportion of daily sputum production. We believe this is an important measure because it reflects the immediate efficacy of airway clearance interventions and the extent to which the person with cystic fibrosis will be productive of sputum throughout the remainder of the day when they may be undertaking work, study or social activities. On average, about one-fifth of daily sputum production occurred during the airway clearance regimen.

The correlational analyses we conducted confirmed that our overall result – the timing of dornase alpha inhalation had little effect on lung function – can be considered applicable to all people with cystic fibrosis who meet the eligibility criteria for this study. That is, the lack of an effect on lung function in this study was not due to a real effect in some participants being diluted or masked by a weak or adverse effect in participants with different characteristics such as baseline lung function or baseline sputum production.

The knowledge that the timing of dornase alpha inhalation in relation to physical airway clearance techniques does not affect clinical outcomes is useful for patients and clinicians, because the regimen of dornase alpha can be prescribed according to other priorities. For most patients, the timing of dornase alpha in relation to airway clearance can be tailored to patient preferences or timing in relation to other inhaled therapies. The correlation between change of quality of life scores and change in FEV1 suggests that the majority of patients can assess a true improvement subjectively. N-of-1 trials may therefore be useful in determining a suitable timing regimen for an individual patient.

In summary, the timing of dornase alpha inhalation does not appear to have a strong influence on the efficacy of the overall airway clearance regimen in adults with cystic fibrosis. The inhalation of dornase alpha can be prescribed according to convenience, patient preference, or to accommodate the timing of other medications in the treatment regimen.

Footnotes: aSidestream, Medic-Aid, Pagham, UK, bVitalograph, Vitalograph Ltd, Buckinghamshire, UK, cRoche Products Pty Limited, Dee Why, Australia
eAddenda: Table 4 available at jop.physiotherapy.asn.au

Ethics: The Western Sydney Area Health Service Human Research Ethics Committee approved this study, HREC 98/9/4.8 (695). All participants gave written informed consent before data collection began.

Competing interests: Nil.

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