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Is A Longer Time Interval Between Recombinant Human Deoxyribonuclease (Dornase Alfa) and Chest Physiotherapy Better?: A Multi-Center, Randomized Crossover Trial

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Summary. Background: Although the benefits of recombinant human deoxyribonuclease (dornase alfa) in patients with cystic fibrosis (CF) are established, its optimal timing in relation to physiotherapy is unknown. As its enzymatic effect lasts for 6–11 hr, dornase alfa may be more efficacious if the time interval between inhalation and chest physiotherapy is increased. The aim of this study was to investigate if a longer time interval between dornase alfa nebulization and chest physiotherapy improves clinical outcomes of subjects with CF. Methods: A single-blind randomized cross-over trial was conducted on subjects with CF from outpatients of four hospitals. Subjects were in stable health and studied over 6 weeks (utilizing 14-day blocks of morning or evening dornase alfa administration with 14 days washout). Usual regimens for physiotherapy and exercise were unaltered. Thus changing the times altered the dwell time of dornase alfa prior to physiotherapy. Long interval was defined as dwell time of >6 hr and short as ≤ 6 hr. Outcomes were measured at pre and post each regimen. Results: Twenty subjects aged 7–40 years completed the study. At end of long interval regimen, (median interval = 11.1 hr), $FEF_{25-75\%}$ and CF-specific quality of life significantly improved compared to baseline values and to short interval regimen (median interval = 0.25 hr) outcomes. FVC, FEV_1, sputum weights, and adherence were similar in both regimens. Conclusions: A longer time interval between dornase alfa and physiotherapy is more efficacious than short interval. Administration timing of dornase alfa based on patient choice to incorporate longer interval time is likely to be the best regimen for patients previously established on dornase alfa nebulization. **Pediatr Pulmonol. 2007; 42:1110–1116.** © 2007 Wiley-Liss, Inc.

Key words: cystic fibrosis; dornase alfa; pulmonary function; quality of life; rhDNase; child; adult.

INTRODUCTION

A Cochrane review provides evidence that therapy with recombinant human deoxyribonuclease (dornase alfa) is associated with improved lung function in CF.[1] It is prescribed for many patients with CF as a nebulized enzyme that reduces viscosity of purulent respiratory secretions. Extracellular DNA released during cellular destruction is digested by dornase alfa by cleaving thereby thinning secretions. Ratjen et al.[2] concluded that the reduced

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load of DNA in broncho-alveolar lavage fluid of patients using dornase alfa may have a positive effect on clearance of lower airway secretions.

It is often assumed by clinicians that airway clearance techniques via various physiotherapy approaches will be more effective when the patient uses dornase alfa. However no studies have examined if a longer time interval between inhalation of dornase alfa and chest physiotherapy is more efficacious than a short time interval. An Australian study concluded that there was no difference in effect between taking dornase alfa before or after chest physiotherapy.[1] Product information of the only commercially available dornase alfa (Pulmozyme®, Roche) notes that "The optimal timing for the use of Pulmozyme in relation to the use of standard treatments such as physiotherapy and antibiotics has not been established", and "...no recommendation can be made as to the optimal time of day for administration of Pulmozyme."[3] In vivo studies demonstrate continued enzymatic activity at 6 hr, and animal studies note 11 hr after inhalation.[3] Thus it is biologically plausible that a longer in vivo dwell time would increase the efficacy of dornase alfa.

Furthermore the inclusion of dornase alfa to the daily regimen of patients with CF adds yet another element for required adherence. While the daily management of CF varies markedly among individuals, prescribed regimens usually include exercise, physiotherapy for clearance of airway secretions, and a variety of medications (pancreatic enzyme replacement therapy, vitamins, oral, and inhaled medications, etc.). It is known that greater complexity of treatment regimens is associated with reduced adherence, especially when no immediate benefit is perceived by the patient, such as in young people with mild spirometric impairment and minimal sputum production. In recent decades, health outcomes for persons diagnosed with cystic fibrosis (CF) have progressively improved, with at least 85% of patients transitioning from pediatric to adult care, compared to median survival of less than 1 year in 1957.[4,5] Improved longevity comes at high cost in personal and economic terms, which may be reflected in QOL measures[6,7] and poor adherence. In children, dornase alfa adherence was measured at 67–84%;[8,9] in adults varied from 24% to 82%.[10] Many attempts are made to support adherence (e.g., replacement nebulisers, educational materials). A pilot study[11] reported that some patients only respond to dornase alfa if given in a particular regimen with respect to physiotherapy, but no variables were found as predictors of response. Without clear guidelines for optimal timing, we investigated if patients' self-selected times of administration and the intervals between nebulization and physiotherapy alters the efficacy of dornase alfa.

ABBREVIATIONS

CF	cystic fibrosis
CFQ-R	cystic fibrosis questionnaire-revised
DNA	deoxyribonucleic acid
$FEF_{25-75\%}$	forced expiratory flowrate between 25% and 75% of FVC
FEV1	forced expiratory volume in 1 sec
FVC	forced vital capacity
QOL	quality of life; rhDNase, recombinant human deoxyribonuclease
VCD	verbal category descriptive score for cough.

The aim of this study was to investigate if a longer time interval (>6 hr) between dornase alfa nebulization and chest physiotherapy improves clinical outcomes of children and adults with CF. Using a randomized cross-over design with washout, we examined the clinical (spirometry, sputum weights, cough, adherence) and QOL outcomes of two time intervals ($\leq$6 hr and >6 hr) between dornase alfa nebulization and chest physiotherapy in established dornase alfa users. We tested the hypothesis that clinical and QOL measures would improve if the interval between dornase alfa administration and chest physiotherapy was longer.

METHODS

Subjects

Children and adults with CF attending outpatient clinics of four participating hospitals (Royal Children's Hospital [RCH], Prince Charles Hospital [TPCH], Gold Coast Hospital [GCH], Mater Adult Hospital [MAH]) in stable health were invited to participate. The participants of the study were enrolled between May 2003 and June 2005. Eligibility criteria were: aged over 5 years, currently using dornase alfa (rhDNase, Pulmozyme®-Roche, Dee Why, NSW, Australia, in accordance with government pharmaceutical benefit criteria), in stable health (no change in antibiotic management for 14 days prior), able to perform reliable spirometry, willing to complete daily cough checklist and collect sputum on four occasions. Patients were excluded if they were currently enrolled in another study or had significant comorbidities (e.g., unstable diabetes). Written and informed consent was obtained and the study was approved by the ethics committees of the four hospitals.

Protocol

Pediatric and adult CF outpatients were studied over 6 weeks: 14 day regimens of morning or evening dornase alfa administration, with 14 days washout between regimens. These regimens resulted in alteration of the interval time between dornase alfa nebulization and chest physiotherapy. The subject's usual routine for chest physiotherapy and exercise were unaltered. Outcomes were measured at start and end of each regimen when participants attended four study visits (each

approximately 30–60 min duration). Primary outcomes were spirometry, sputum weight (g), and CF-specific QOL(CFQ-R). Additional outcomes were subjective cough score (VCD) and medication adherence. Adherence was checked by returned empty nebule count.

Initial assessment detailed usual time of day for dornase alfa administration and for physiotherapy, and approximate durations of these activities, in addition to standard clinical and medication details. Subjects were asked to collect sputum for weighing on the day prior to testing. CF-specific QOL was measured (CFQ-R)[12] and participants scored a validated cough diary[13] and symptom checklist. Containers were supplied for return of used nebules to measure medication adherence. Compressor pumps were checked for flow between 6 and 8 L/min (Allersearch Nebuliser Pump Tester, Allersearch, Kingsgrove NSW, Australia) and a new jet nebuliser (Pari LC Plus; Pari, Midlothian, VA) supplied for dornase alfa administration. Spirometry was performed by the same two personnel (ATS methodology) using the same calibrated Microlab portable spirometer (Micro Medical Ltd, Kent, UK) and predicted values were based on Hibbert et al.[14] norms for children, and Quanjer[15] for adult participants.

Randomization tables were used to allocate groups (A or B) for timing of dornase alfa. Group A used dornase alfa in the morning for the first 14 days, their ''usual'' timing for days 15–28, and afternoon or evening use for days 29–42. To ensure investigator blinding, this information was provided in a sealed envelope to the participant, who was instructed not to inform researchers of their study phase until completion of all testing.

Statistical Analyses

For a study power of 90% at 5% significance 44 patients were required for a mean change in FEV_1% predicted of 5% with SD of 10% (paired samples). As we could not enrol sufficient subjects within the time frame of the project, the study was truncated when we achieved N of 20. This would have allowed us to show a difference in FEV_1% of 6.5 (SD 10%) for a study power of 80% (post priori calculation).

''Usual'' dornase alfa administration times and physiotherapy times were analyzed to determine ''dwell time,'' being the duration between dornase alfa inhalation and the next physiotherapy session. Based on pharmacokinetics,[3] interval times over 6 hr were considered ''long.'' Outcome measures (spirometry, CFQ-R score, cough VCD score, sputum weight, and adherence) were to be analyzed in two categories based on interval times (short defined as $\leq$6 hr and long as $>$6 hr). As the data were not normally distributed, nonparametric analyses were used throughout. All descriptive data are presented as medians and inter-quartile range (IQR). Wilcoxon test was used for paired comparisons. Statistical package SPSS Version 13.0 (SPSS, Inc., Chicago, IL) was utilized and a two-tailed P value of $\leq$0.05 was considered significant.

RESULTS

Twenty-two participants were enrolled and randomized and 20 completed the study. Both withdrawals from the study were due to exacerbations occurring after recruitment, one prior to data collection, the other at day 39 (pm arm). Health remained stable in the 20 subjects who completed the study. The median age of these 20 subjects was 13.5 years (range 7–40) and 40% (n = 8) were male. The majority of participants were enrolled from RCH (n = 13); the rest were enrolled from PCH, GCH (n = 3 each) and MAH (n = 1). Seven participants were 6–12 years of age, 8 were 12–18 years, and 5 were over 18. All participants had grown *Pseudomonas aeruginosa* in most recent sputum cultures, 60% of participants had an additional common CF pathogen including *Staphylococcus aureus*, and *Aspergillus* species in 20%. One participant was known to be chronically infected with *Burholderia cepacia* and appropriate isolation and infection control recommendations were followed with this participant. Individualized physiotherapy routines, as assessed by structured interview at entry and reassessed at the conclusion of the study, documented airway clearance technique/s and exercise used, usual time of day and duration of sessions. Analysis determined no significant differences from start to end in physical therapy techniques, time of day, or duration of sessions throughout the study.

The majority of participants used a combination of positive expiratory pressure (PEP) devices (e.g., Pari® PEP), breathing techniques (e.g., Active Cycle of Breathing Techniques), and an individualized exercise program. Less frequently used techniques included positioning with chest percussion and/or expiratory vibration; oscillating PEP (Flutter®); and Autogenic Drainage. Physiotherapy techniques not employed by any subjects in this study included High Frequency Chest Wall Compression and High Pressure PEP. The current study was not powered to undertake subgroup analysis of subjects using the various techniques.

The median interval between inhalation of dornase alfa and chest physiotherapy was 0.25 hr (IQR 0, 0.94) for the short regimen and 11.1 (10.0, 12.4) for the long regimen. There was no difference in baseline characteristics of the two regimens (short and long intervals) for all the outcomes (Table 1). At the end of the long interval regimen, FEF_{25-75} and CFQ-R of carers significantly improved compared to baseline values, also when compared to the end values of the short interval regimen (Table 1). The end of regimen CFQ-R of participants significantly improved post long interval regimen but did

TABLE 1— Baseline and End of Regimen Values for all Outcomes

Median (IQR)	Short interval	Long interval	*P*#
FEV_1			
Baseline	1.21 (1.06, 1.68)	1.69 (1.17, 2.05)	0.075
End	1.28 (1.03, 1.69)	1.57 (1.18, 2.1)	0.255
*P**	0.894	0.841	
FEV_1 % predicted			
Baseline	63.5 (37.5, 77.5)	75 (46.8, 85.0)	0.116
End	56.5 (37.5, 80.3)	76 (48.2, 87.8)	0.209
*P**	0.878	0.626	
FEF_{25-75}			
Baseline	0.87 (0.53, 1.51)	1.20 (0.65, 2.09)	0.833
End	0.89 (0.46, 1.56)	1.60 (0.89, 2.43)	0.015
*P**	0.875	0.051	
$FEF_{25-75\%}$ predicted			
Baseline	35.0 (18.3, 61.8)	47 (20.5, 78.4)	0.674
End	37.5 (12.5, 60.5)	67 (23.8, 79.0)	0.010
*P**	0.906	0.023	
FVC% (L)			
Baseline	3.12 (1.50, 2.62)	2.14 (1.5, 2.62)	0.813
End	1.88 (1.37, 2.59)	2.09 (1.54, 2.91)	0.480
*P**	0.969	0.610	
FVC% predicted			
Baseline	81.8 (54.0, 93.8)	75.5 (67.5, 94.3)	0.767
End	73.0 (61.8, 85.8)	82.0 (70.0, 90.5)	0.814
*P**	0.313	0.360	
CFQ-R-participant			
Baseline	61.2 (56.7, 71.5)	69.6 (60.1, 80.6)	0.093
End	66.3 (58.1, 73.5)	70.7 (60.4, 82.8)	0.131
*P**	0.386	0.018	
CFQ-R-carer (n = 10)			
Baseline	73.2 (66.3, 77.8)	69.5 (57.3, 73.7)	0.753
End	68.9 (64.4, 77.0)	72.3 (64.8, 76.8)	0.018
*P**	0.600	0.009	
Cough score			
Baseline	18.0 (16.3, 29.3)	17.5 (15.0, 29.5)	0.317
End	26.0 (17.8, 35.3)	21.8 (13.8, 29.5)	0.416
*P**	0.225	0.522	
Sputum weight			
Baseline	5.0 (0.3, 22.6)	2.5 (0.2, 17.4)	0.721
End	8.3 (0.2, 23.6)	3.5 (0.2, 15.1)	0.533
*P**	0.799	0.407	
Adherence			
% taken	100 (92.9, 100)	100 (96.4, 100)	1.102

*P**, comparison between data at start (baseline) and end of respective regimens.
P#, comparison between short and long interval data.

not significantly change in the short interval regimen. However, unlike the outcomes of FEF_{25-75} and CFQ-R of carers, there was no significant difference between the end CFQ-R scores of the short compared to long interval regimens (Table 1). For the outcomes of FEV_1, sputum weight and cough score, there was no significant difference between the two regimens or between end and baseline values. In the short interval regimen, no difference for any outcome was found. Adherence was very good for both regimens with no significant difference between the regimens.

DISCUSSION

This is the first study to examine whether a longer interval time between inhalation of dornase alfa and chest physiotherapy is more efficacious than a short interval. Using a randomized cross-over design, we found significant improvement in small airways function (FEF_{25-75}) and QOL measures (CFQ-R) of participant and parent when the interval time between inhalation of dornase alfa and physiotherapy was longer (over 6 hr). There was however no significant difference between the groups for

the outcomes of FEV_1, FVC, sputum weight, and adherence.

There is only one published study that has examined the efficacy of dornase alfa in relation to the timing of physiotherapy. A crossover study by Fitzgerald et al.[16] of also 2 weeks' duration for each regimen concluded that dornase alfa was equally efficacious when delivered either 30 min before or after physiotherapy and that an additional benefit was deemed possible for patients who are persistently colonized with *P. aeruginosa* if dornase alfa is administered after physiotherapy. Our study instead examined if a longer in situ dwell time of dornase alfa prior to chest physiotherapy was more efficacious than a shorter dwell time. Ashdown[17] found no consensus in a UK survey concerning dwell time or sequence of dornase alfa in relation to airway clearance. Biologically, dornase alfa continues to cleave extracellular DNA for at least 6 hr following inhalation.[3] Given the large differences in time interval between the two regimens (0.25 vs. 11 hr) it is perhaps not surprising that we found that the longer dwell time regimen was more efficacious than the short regimen. We altered the dwell time by simply altering the time of day (am or pm) of use of dornase alfa whilst maintaining their routine physiotherapy time. We did not specify the exact time dornase alfa should be given and instead allowed the subjects to choose a suitable time within the morning or evening time frames for their respective regimens. This allowed flexibility (rather than having to do physiotherapy around the same time as dornase alfa nebulization) and thus offered convenience to the patient and/or parent. Hence our finding of improved QOL with the longer dwell time (which related to an arrangement to suit individual lifestyles rather than prescribed regimens dictating specific timing of medication) is also not surprising. Greater flexibility of timing to suit individual and/or family lifestyles has been demonstrated previously to support adherence.[18] Our data supports flexibility of approach regarding the timing of these two interventions, allowing potential improvements in QOL and adherence. We used a well tested, disease specific instrument for measurement of QOL in CF covering physical, social, emotional, treatment burden, and other functional domains.[12] Janse et al.[19] highlight the importance of assessment of QOL in chronic pediatric illnesses, where discrepancies exist in understanding the impact of the disease between health care providers, parents, and patients. These authors conclude that compliance and treatment effects may be enhanced by the better understanding between parents and health care providers when QOL is assessed.

Mucus is usually cleared by airflow and ciliary movement, and sputum is cleared by cough.[20] In a study using radiolabeled aerosol particles, Laube et al.[21] allude to the possibility of dornase alfa reducing airflow obstruction in airways too small to affect changes measured by FEV_1, FVC, or indices of aerosol deposition homogeneity. Our study which showed significant differences in $FEF_{25-75\%}$ but no significant changes in FEV_1 or FVC in subjects already established on dornase alfa, is consistent with Laube's assertion.[21] Since our study group were all long term users of dornase alfa, significant changes would not be expected in FEV_1 or FVC, as these are the outcomes that improve most when dornase alfa is commenced in naive users. Whether a change in $FEF_{25-75\%}$ of 20% predicted as found in our study is clinically significant is arguable. A recent abstract notes 6% increase in MEF_{25} when dornase alfa is inhaled after ACT as significant ($P = 0.01$).[22] $FEF_{25-75\%}$ is a highly variable spirometric test.[23] However as the significant change in $FEF_{25-75\%}$ was accompanied by a significant improvement in the CFQ-R and no significant change in FVC (which influences $FEF_{25-75\%}$[23]), we are confident that the significant $FEF_{25-75\%}$ change described in our study is both real and clinically significant. Physiotherapy programs in all study centers were individualized as recommended by several authors in evidence based reviews of available literature, with recommendations such as ''Exercise should be included as part of the therapeutic regime''[24] and ''Choice of treatment should be dictated by individual needs''[24] and ''...no difference between conventional chest physiotherapy and alternative therapies in terms of respiratory function. Studies of acute exacerbations demonstrated relatively large gains in respiratory function irrespective of airway clearance technique''[25] supporting the individualized, often combined physiotherapy approach.

Median spirometry values for our patient group were quite high (Table 1). Whilst early studies focused on use of dornase alfa in patients with more significant impairment (FVC < 70% predicted),[26] an increasing body of literature has suggested the usefulness in younger and less affected patients, those receiving dornase alfa having a small improvement in FEV_1 and FEF_{25-75} over a year, versus a fall from baseline in the placebo group.[27] Our data also demonstrates a change in small airways spirometry even in relatively well patients who were established users of dornase alfa. Whether or not a longer dwell time for dornase alfa is more important in patients with more severe lung disease cannot be determined in our study because the small sample size does not allow appropriate sub analysis.

Medication adherence by self report has been shown to be unreliable,[28] and in this study was measured by counting empty medication nebules, as reported by previous authors.[8] Batch numbers were also checked. Previously, median adherence to dornase alfa over 12 weeks was shown to be 79.1% for children under12 years, and 78.4% for over 12 years.[8] Our study group showed exceptionally good adherence to dornase alfa over 6 weeks (92.9–100%). It was noted by the same authors[8] that the length of

monitoring of adherence was moderately negatively correlated with medication adherence, indicating poorer adherence with longer duration of monitoring. Flexibility of timing to maximize compliance was generally agreed in a consensus pilot study.[17] Being established users of dornase alfa would support effective habitual adherence. It is possible that patients who were not regular users of dornase alfa would not consent to enter the study.

Our study is limited by the small sample ($n = 20$) and hence our findings should be confirmed in larger groups of patients. Our group did not provide sufficient numbers for post-hoc analysis of participants with mild, moderate, or severe spirometric impairment. The small sample size was related to the recruitment difficulties despite identification of substantial numbers of dornase alfa users in the various clinics. Multiple clinical trials in progress, distance from the hospital, and in adult subjects, the requirement of ''stable health'' prior to study commencement were significant limitations on recruitment.

We conclude that a longer time interval (>6 hr) between dornase alfa and physiotherapy is more efficacious than a short interval as a modest improvement in small airways ($FEF_{25-75\%}$) and QOL are evident. Administration timing of dornase alfa based on patient choice to incorporate longer interval time is likely to be the best regimen for children and adults who have been established on dornase alfa nebulization.

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