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RhDNase nebulisation in children with Cystic Fibrosis before bedtime or after waking up?

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Abbreviations

ACT	Airway clearance therapy
CF	Cystic fibrosis
CSS	Cough symptom score
FEV_1	Forced expiratory volume in one second
FVC	Forced vital capacity
PFT	Pulmonary function test
MEF_{25}	Expiratory flow at 25% of the actual forced vital capacity
rhDNase	Recombinant human deoxyribonuclease
TSI	Tobramycin solution for inhalation
VAS	Visual analogue scale

Keywords

Airway Clearance Therapy, Children, Cystic Fibrosis, Pulmozyme, rhDNase, Timing

Abstract

Objective

To compare in patients with CF who are on maintenance therapy with rhDNase the efficacy and possible side effects of nebulisation of rhDNase before bedtime or after waking up.

Methods

Design: randomized, double blind, double dummy, cross over study. Inclusion criteria: CF, stable clinical condition, rhDNase maintenance therapy. Children in Group I inhaled rhDNase before bedtime, and a placebo after waking up in week 1-2. The protocol was reversed during week 3-4. Group II performed the reversed sequence. Patients continued their daily routine sputum expectoration. Primary endpoint: MEF_{25}. Pulmonary functions tests were performed on day 0, 7, 14, 21 and 28. At 1, 2, 3 and 4 weeks oxygen saturation and cough frequency were measured during the night.

Results

24 patients completed the study. Mean age: 13 years (range 6-19). MEF_{25} (%pred) as primary endpoint did not show a significant difference between nebulisation of rhDNase before bedtime or after waking up. Nocturnal cough, oxygen saturation, or the other secondary end points were not significantly different between the two study periods.

Conclusion

In children with CF who are on maintenance treatment with rhDNase it is equally effective and safe to nebulise rhDNase before bedtime compared to after waking up.

Introduction

Cystic Fibrosis (CF) lung disease is characterized by excess mucus production and impaired mucociliary clearance(1). This causes the airways to become chronically infected with micro organisms, leading to chronic airway inflammation and progressive structural lung damage (2). CF sputum contains large amounts of extracellular DNA released by disintegrated inflammatory cells, particularly neutrophils (3, 4). Patients need daily treatment to reduce the amount of mucus in the airways, but tend to feel this as a great burden (5). Airway clearance therapy (ACT) and nebulisation of recombinant human deoxyribonuclease (rhDNase) are the most frequently used methods to mobilize sputum. RhDNase cleaves extra cellular DNA and reduces sputum viscosity, transforming it from a non-flowing viscous gel into a flowing liquid (6). Daily treatment with rhDNase reduces the number of pulmonary exacerbations, improves pulmonary function, and is well-tolerated and safe in mild, moderate and severe CF (7-11). Though the effectiveness of rhDNase nebulisation is well established, little is known about optimal timing. The Epidemiologic Registry of Cystic Fibrosis reported that 51% of the patients used rhDNase in the morning, 37 % in the evening, and 8% at variable times; timing was not specified for 3% (12).

Theoretically, it could be effective and time efficient for patients to nebulise rhDNase before bedtime. Firstly, it would allow sufficient time during sleep for rhDNase to act upon the free DNA present in the airway lumen. Its half-life in rodents was found to be at least 11 hours after inhalation (13). Secondly, postural shifts during sleep might act like postural drainage therapy; allowing gravity dependent mobilization of the sputum. Adults were found to have an average of 16 position shifts per night (14). Postural drainage is reported to be effective when relatively large quantities of mucus of low adhesion are present in the airways (15). Following nebulisation of rhDNase the mucus will become less viscous. Postural drainage during sleep is therefore likely to improve mucociliary clearance. On the other hand, there are

several theoretical arguments against the nebulisation of RhDNase before bedtime. Firstly, there are indications that mucociliairy clearance is depressed during sleep in normal subjects (16) and in subjects with asthma (17). Still, in CF the dehydrated secretions are capable of triggering coughing spells during sleep (18). Secondly, nightly spontaneous cough might be less effective to expectorate the sputum relative to daytime cough or active ACT. Hence, before bedtime nebulisation of rhDNase might even be detrimental to lung function. Thirdly, nebulisation-induced increased cough during sleep might affect sleep quality.(18) Finally, nebulisation of rhDNase before bedtime might induce ventilation perfusion mismatch due to additional occlusion of peripheral airways by the more liquid mucus. However, this was never systematically studied.

We conducted a study in children with CF on maintenance therapy with rhDNase, comparing the efficacy and possible side effects of nebulisation before bedtime to those of nebulisation after waking up. Efficacy was evaluated with pulmonary function tests. Side effects were monitored using nocturnal oxygen saturation and cough frequency. We hypothesized that before bedtime nebulisation of rhDNase would be safe and more effective to improve peripheral airway flow than nebulisation after waking up.

Material and methods

Study subjects

Children followed by the CF centre in the Erasmus MC – Sophia Children's Hospital were eligible when they fulfilled the following criteria: proven CF, at least five years of age, ability to perform reproducible spirometry, FVC >40%, daily ACT, maintenance treatment with rhDNase, and clinical stability. The latter was defined as no need for intravenous antibiotics and no hospitalizations for at least one month prior to the study. CF was defined as clinical symptoms characteristic for CF plus an abnormal sweat test and/or the presence of two CF mutations. We excluded children who used rhDNase more than once a day, those who were considered to have poor therapy compliance (<50% of treatments) as judged by the CF team, or those who were mentally retarded.

A total of 152 CF patients were identified. Forty-nine patients either did not meet the study inclusion criteria or met the exclusion criteria. Most patients were excluded since they were too young to do spirometry. Of the remaining 103, 43 patients were randomly selected and invited to participate.

Throughout the study, subjects continued to receive their standard treatment.

Study design

The study had a randomized, double blind, double dummy, cross over design. All subjects nebulised both rhDNase (2.5 mg of rhDNase in 2.5 ml buffered solution: 8.77 mg/ml sodium chloride and 0.15 mg/ml calcium chloride) (19) and a placebo (2.5 ml of a buffered solution: 8.77 mg/ml sodium chloride and 0.15 mg/ml calcium chloride) once daily for a period of four weeks. Placebo was similar to rhDNase in both colour and taste. Subjects were randomized into one of two groups. One group (I) used rhDNase before bedtime and placebo in the

morning directly after waking up in the first two weeks. This order was reversed in the following two weeks. (Figure 1) The other group (II) used placebo before bedtime and rhDNase after waking up in the first two weeks, and in reverse sequence thereafter.

Based on previous studies week 1 and week 3 were considered wash-in and wash-out periods (10, 20, 21). Because patients were on maintenance treatment with rhDNase a true wash-out period without rhDNase was considered unethical and unpractical. Patients continued to perform routine airway clearance therapy (ACT) during the study period. ACT in the morning was done 30 minutes after nebulisation.

Placebo and rhDNase were administered using a Sidestream nebuliser (Respironics, Murrysville, Pennsylvania, USA) and one of the following compressors: Portaneb, Freeway Lite, - Freedom or CR60 (Respironics).

We selected the MEF_{25} %pred as primary endpoint, because it is more sensitive to changes in the peripheral compartment in early lung disease than are FVC and FEV_1.(22)

The study was conducted between September 2005 and June 2006. The Erasmus MC Medical Ethical Review Board approved the protocol. The study was performed according to ICH-GCP guidelines.

Equipment specifications

Pulmonary function tests (PFTs) were carried out using a handheld spirometer (MicroLoop, Micro Medical Ltd, Rochester, UK). The following PFT results were obtained: forced expiratory volume in one second (FEV_1); forced vital capacity (FVC); and MEF_{25}. The PFT results were expressed as a percentage of predicted values (23). Interrupter resistance (Rint) (kPa/L/s) was measured using the MicroRint (Micro Medical Ltd, Rochester, UK).

Calibrations of flow were carried out using a 1liter precision pump.

Procedures

PFTs were carried out at the patient's home. Weight and height were measured on day 0. Rint measurements and spirometry were performed on days 0, 7, 14, 21 and 28. Rint was always measured first. Throughout the study, lung function measurements were done with the same spirometer, in the afternoon on the same day of the week for each patient. Before PFTs the children were asked to clear their throat by coughing. For Rint measurements, children were instructed to sit upright while breathing quietly. The head was positioned in slight extension. The hands of the investigator supported the patient's cheeks and chin to reduce upper airway compliance (24). A test measurement was done before the actual measurements to familiarize children with the sound of the shutter. Minimally five correct tracings (maximal 10) were obtained. The expiratory Rint ($Rint_e$) was measured, because expiratory interruptions are more sensitive in detecting airway obstruction relative to inspiratory interruptions (25). Spirometry was performed in triplicate according to ERS guidelines (26).

For those patients who used Tobramycin solution for inhalation (TSI) the study was timed in the four-week TSI-free interval. To estimate adherence to study medication patients were asked to keep empty drug vials for vial count.

Oxygen saturation was determined by pulse oximetry (Mars Pulse oximeter, Respironics, Murrysville, Pennsylvania, USA) on days 7, 14, 21, and 28. To measure the nocturnal oxygen saturation profile a pulse oximeter sensor was attached to the subject's finger or a toe during sleep. Data were stored on the pulse oxymeter. From this recording we computed mean oxygen saturation.

Cough recordings were made on a digital audio player/recorder (Archos^{TM} Gmini 120, Shenzhen, China) during nights 7, 14, 21, and 28, next transferred to a personal computer and stored on compact disc. The recordings were analysed using free open

source audio record & edit software, which provided a graphical display for audio analysis applications (Audacity, Boston, USA).

Cough episodes were identified during which at least one explosive cough was present. Next we counted duration of each cough episode in seconds (cough seconds (cs)). If several cough sounds occurred successively, we counted the duration of the total episode. Finally, total duration of cough seconds was summed and divided by total recording time. Cough was thus expressed as seconds of cough per hour (cs/h). This measure therefore encompasses an estimate of the length of peals of coughs (27). Counting of cough sounds from digital audio recordings has excellent agreement with simultaneous video recordings (28).

During the second and fourth weeks the children recorded in a diary their day and nighttime cough frequencies with a validated cough symptom score (CSS) (Table 1) (29). Furthermore they rated sputum viscosity, sputum production, sleep quality, appetite in the morning and cough frequency on a visual analogue scale (VAS) (29). A VAS is a horizontal line, 10 cm in length, anchored by word descriptors at each end. Rating is by placing a mark on this line in the position that best represents the child's perception. The VAS score is the distance in cm between 'no symptom' (left = 0 cm) and the mark placed by the patient.

Data analysis

The pre-planned primary outcome variable was MEF_{25} %pred. With 24 evaluated patients, the power for this parameter in the comparison of the two treatment schedules would be greater than 80 percent for an effect-size (difference of means / standard deviation) of 0.8 at an alpha (two-sided) of 5 percent.

Secondary outcome variables were FVC %pred, FEV_1 %pred, $Rint_e$, oxygen saturation, cough frequency and diary scores.

Outcome variables were compared using the paired t-test after ensuring that there were no significant carry-over or period effects. The mean values of diary scores for the second and fourth weeks were analysed. Statistical analysis was performed with SPSS version 11.0, and P=0.05 (two-sided) was considered the limit of significance in all analyses.

Results

As from the 43 randomly selected children 13 children declined to participate, and 5 did not respond, the study group included 25 children; randomly divided over group I (12) and group II (13). Final analysis was for 24 children, however, as one child, randomized to group I, withdrew in the third week due to a common flu. Baseline characteristics are summarized in Table 2, type of ACT and type of nebulisers are listed in Table 3. The two randomized groups were comparable at baseline with respect to age, sex and lung function (Table 2). No carry-over or period effect was observed for any of the endpoints.

Mean adherence to study treatment, as calculated from the vial count, was 97% (range 82%-100%).

The primary endpoint, MEF_{25} %pred, did not significantly differ (P=0.25) between after waking up (A) or before bedtime nebulisation (B): the difference (A minus B) after 2 weeks is 3.38 (95% CI: -2.6% to 9.3%) (Figure 2).

None of the secondary endpoints or safety parameters did significantly differ between the two schedules (Tables 4 and 5).

Discussion

In this study we tested the hypothesis that before bedtime nebulisation of rhDNase would be safe and prove more effective than nebulisation after waking up. Our findings, however, do not support the last part of the hypothesis. Both the primary endpoint MEF_{25} %pred and secondary endpoints such as cough frequency and nocturnal oxygen saturation did not show significant improvement after two weeks of nebulising rhDNase before bedtime, compared to nebulisation after waking up. In addition cough frequency and nocturnal oxygen saturation were not significant different between the study periods, which support the first part of the hypothesis, i.e. nebulisation of rhDNase before bedtime would be safe.

Several possible explanations present themselves why we did not find improved efficacy after nebulisation before the night. First, sputum after nebulisation with rhDNase might still be too viscous to allow any extra positive effect by gravity.

Second, during sleep mucociliary clearance is depressed (16, 17), and breathing patterns change, resulting in reduced minute ventilation and tidal breathing pattern (30, 31) as well as and increased airway resistance (32). If gravity and mucociliary clearance fail to mobilize the viscous sputum spontaneously it can only be cleared by high expiratory flows (33). Sleep, however, is mostly characterized by quiet tidal volume breathing, which is likely to contribute little or nothing to sputum transport.

As a third explanation, the two-week treatment period in each arm may have been too short. In a recent study (34) we compared the effect of nebulisation of rhDNase before and after ACT. The primary end point MEF_{25} showed no significant difference after two weeks, but after three weeks was significantly increased by 6% when rhDNase was used 30 minutes before ACT. A longer study period might well have resulted in a difference between the two treatment arms. The result of the previous

study was not known when the current study started. Extension of the treatment period implied exclusion of those patients treated with TSI, since TSI greatly influences spirometry results (35). Alternatively, each treatment arm could have been scheduled into the TSI negative or positive treatment period. This approach, however, would have made the total study duration for each patient longer with risk of higher noise and drop out rates.

The absence of differences in nocturnal cough frequency or oxygen saturation between treatment arms suggests no major differences in sputum mobilisation.

A positive finding was that before bedtime rhDNase administration did not result in increased cough or reduced oxygen saturation during the night in this group of patients with mild to moderate lung function abnormalities. As suggested by some physicians, before bedtime rhDNase could have increased nightly cough periods due to its effect on the sputum. Yet we did not find any increase in cough seconds per hour when rhDNase was nebulised before bedtime. This might be a result of the depressed cough reflex during sleep (36). This condition could prevent the liquefied sputum to be evacuated by cough clearance. However, we think this is unlikely since oxygen saturation did not differ between the two study periods. Substantial increase of peripheral airway obstruction due to liquefied sputum would have resulted in more severe ventilation-perfusion mismatch and thus in reduced oxygen saturation. The oxygen saturation finding is, therefore, in line with our PFT and cough results.

In contrast to our expectations the children showed a trend towards better sleep quality and less daytime and nighttime coughing (Table 5) when rhDNase was nebulised before the bedtime. Nebulisation of rhDNase before bedtime in patients with mild to moderate lung function abnormalities seems thus to be safe**.**

How do our results translate to daily treatment for CF patients? Based on our results, there are no arguments against the pre-sleep rhDNase treatment. However, long-

term studies in larger cohorts, and with additional secondary end points such as exacerbation rate, are needed to confirm our results.

We cannot exclude that our observations would have been different for children with severe or end-stage lung disease. This must be investigated in a separate controlled trial, using similar monitoring of safety end points as in our current study.

In conclusion, this study showed that for children with CF lung disease on maintenance treatment with rhDNase it is equally effective and safe to nebulise rhDNase before bedtime and doing ACT in the morning or to nebulise rhDNase after waking up by ACT. Hence, it is up to the children themselves to choose the most convenient time to nebulise rhDNase.

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Table 1

Cough Symptom Score

Day time

0 = no cough

1 = cough for one or two short periods

2 = cough for more than two short periods

3 = frequent cough not interfering with normal activities

4 = frequent cough interfering with school or other activities

5 = distressing cough most of the day

Night time

0 = no cough

1 = cough on waking only/ cough on going to sleep only

2 = awoken once or woken early due to coughing

3 = frequent waking due to coughing

4 = frequent coughs most of the night

5 = distressing cough

Table 2

Characteristics of the study population (numbers of patients or mean with SD) at baseline for group I and II.

	Group I	Group II
N	11	13
Sex(male/female)	3 / 8	5 / 8
Age (year)	12.5 (4.5)	13.5 (3.4)
FVC (% predicted)	81 (9)	82 (22)
FEV_1 (% predicted)	74 (12)	76 (27)
MEF_{25} (% predicted)	49 (32)	49 (34)
$Rint_e$ (kPa/L/s)	0.5 (0.21)	0.5 (0.23)
Use of TSI (n)	3	5

Note: In the first two weeks group I nebulised rhDNase before bedtime and placebo in the morning directly after waking up. In the following two weeks the order in which rhDNase and placebo were taken was reversed. Group II nebulised placebo before bedtime and rhDNase after waking up in the first two weeks, and the reverse sequence thereafter.

Table 3

Maintenance treatment

ACT (n / %)	
PEP mask	15 / 62%
Flutter	3 / 13%
Autogenic drainage	4 / 17%
Combination	2 / 8%
Nebuliser (n / %)	
Portaneb	12 / 50%
Freeway Lite	1 / 4 %
Freeway Freedom	9 / 38%
Freeway Elite	1 / 4%
CR 60	1 / 4%

Table 4. Mean values with standard deviation after one and two weeks rhDNase before bedtime or after waking up.

	RhDNase before bedtime	RhDNase morning	p- value
After 1 week			
MEF_{25}%pred	47.2 (30.0)	45.5 (29.9)	0.59
FVC %pred	83.3(18.8)	83.0 (16.8)	0.78
FEV_1%pred	75.5 (21.4)	75.1 (21.6)	0.82
$RINT_e$	0.51 (0.22)	0.55 (0.27)	0.18
Saturation%	95.5 (1.3)	95.8 (1.9)	0.61
Cough (cs/h)	2.7 (4)	3.7 (4)	0.24
After 2 weeks			
MEF_{25}%pred	43.0 (28.4)	46.4 (28.5)	0.25
FVC %pred	83.0 (18.8)	83.1(17.7)	0.97
FEV_1%pred	74.2 (21.8)	75.5 (20.6)	0.38
$RINT_e$	0.54 (0.27)	0.54 (0.23)	0.97
Saturation%	96.1 (1.6)	96.2 (1.4)	0.78
Cough (cs/h)	3.3 (5)	3.7 (8)	0.85

Table 5. Mean diary scores with standard deviation after two weeks rhDNase before bedtime or after waking up.

	rhDNase before bedtime	**rhDNase morning**	**p-value**
VAS viscosity	2.1 (1.6)	2.5 (1.6)	0.22
VAS sputum amount	1.9 (1.8)	2.1 (1.6)	0.26
VAS daytime coughing	2.2 (1.6)	2.5 (1.7)	0.27
VAS nighttime coughing	1.0 (1.0)	1.3 (1.4)	0.08
CSS daytime	1.3(0.9)	1.6 (0.9)	0.07
CSS nighttime	0.8 (0.8)	1.0 (0.9)	0.27
VAS appetite	3.0 (2.7)	3.3 (2.6)	0.35
VAS sleep quality	1.1 (1.0)	1.5 (1.30)	0.08

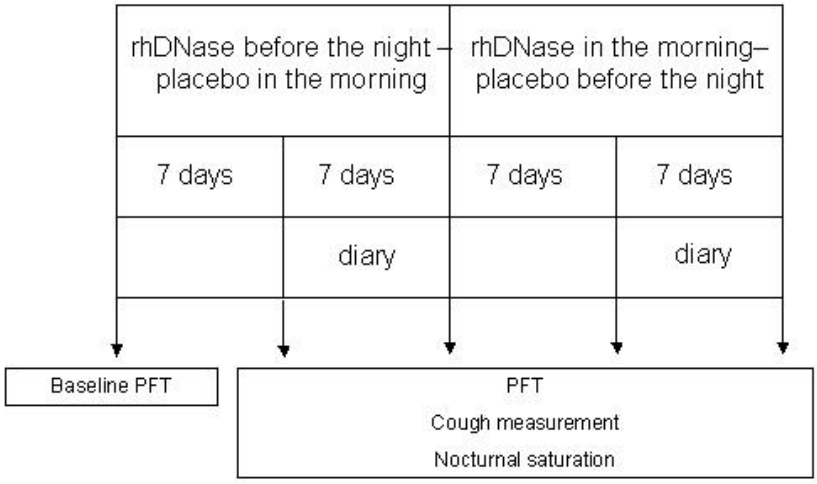

Figure 1. Study design and time points for the primary and secondary endpoint measurements for children in group I. In group II the order of the rhDNase and the placebo were reversed.

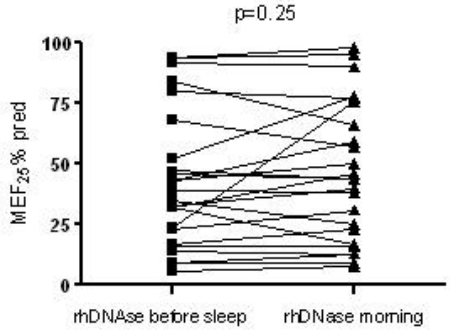

Fig 2. MEF_{25}% pred according to nebulisation of rhDNase before going to sleep and in the morning after 2 weeks. Lines connect individual data points.