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Bolus Inhalation of rhDNase with the AERx System in Subjects with Cystic Fibrosis

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ABSTRACT

Inhaled recombinant human deoxyribonuclease (rhDNase) delivered by nebulizer improves pulmonary function and reduces the rate of pulmonary exacerbations in cystic fibrosis subjects. Standard jet nebulizers are relatively inefficient and require a delivery time of 10–20 min. We conducted an open-label, proof-of-concept study to evaluate whether bolus inhalation of rhDNase with a more efficient delivery system was safe and effective in cystic fibrosis subjects. The AERx system used for this study aerosolized 1.35 mg of rhDNase in three inhalations at a single sitting. The predicted AERx lung dose was approximately 0.68 mg, a dose consistent with lung doses of rhDNase given by jet nebulizer. In our 16 subjects with cystic fibrosis, a mean relative increase in FEV_1 of 7.8% ($p \leq 0.001$) was observed after 15 days of bolus delivery of rhDNase with the AERx system. The safety profile of rhDNase given as a bolus was similar to that observed with traditional nebulizer delivery. This study demonstrated that bolus inhalation of rhDNase was feasible, reasonably well-tolerated, and associated with improvement in pulmonary function in this small group of cystic fibrosis subjects.

Key words: AERx, nebulizer, aerosol, rhDNase, cystic fibrosis

INTRODUCTION

Cystic fibrosis (CF) is a chronic disease characterized by persistent airway obstruction associated with accumulation of viscous purulent airway secretions, recurrent infectious exacerbations and progressive deterioration in lung function.[1] The increased viscosity of airway secretions in CF subjects is due in part to the presence of numerous polymorphonuclear neutrophils and their degradation products, including DNA, which aggregates in large fibrils that greatly increase sputum viscosity.[1] Cleaving the large DNA strands with bovine pancreatic dornase alpha was shown to reduce the viscosity of infected sputum *in vitro* over 50 years ago, and was effective when inhaled by subjects with lung infections.[2–6] However, adverse reactions to the

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bovine protein prevented the clinical development of dornase alpha until a human version of dornase alpha was developed.

Recombinant human deoxyribonuclease (rhDNase, Pulmozyme®, Genentech, South San Francisco, CA) is a 260–amino acid glycoprotein, cloned and expressed in a genetically engineered Chinese hamster ovary cell line that selectively cleaves extracellular DNA.[7] A double-blind, placebo-controlled study of 968 CF subjects[8] showed that by giving 2.5 mg of rhDNase with a Hudson-T nebulizer (Hudson Respiratory Care, Temecula, CA), the risk of respiratory exacerbations was reduced by 28% (daily dose) and 37% (twice daily dose). In addition, mean relative FEV_1 increased by 7.9% and 9.0% after 7 days of treatment with rhDNase 2.5 mg once or twice daily, respectively. Improvement in FEV_1 was maintained at 5.8% and 5.6%, respectively, over the ensuing 23 weeks of treatment. Since rhDNase was approved for use in 1993, it is widely used in CF subjects of all severities.[9]

RhDNase is approved for use with specific nebulizer/compressor combinations shown in clinical trials to effectively deliver the drug. These systems include the Acorn II (Marquest Products, Eaglewood, CO) with the Pulmo-Aide compressor (DeVilbiss Health Care, Inc., Somerset, PA), the PARI LC Jet™ nebulizer with ProNeb compressor (Pari, Richmond, VA)[10] and recently the SideStream nebulizer with MobilAire compressor (Invacare Corporation, Elyria, OH).[11]

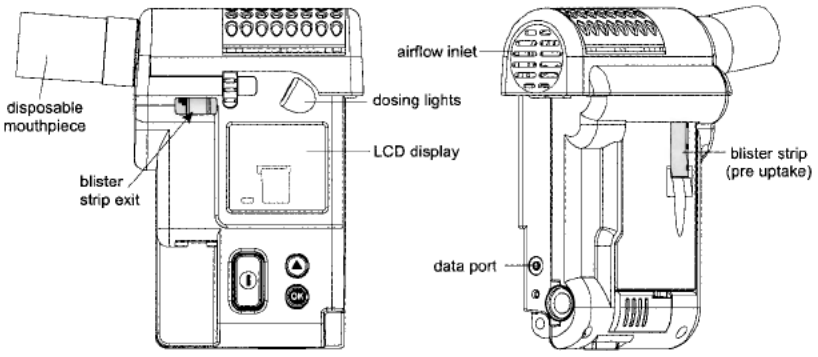
Although the safety and efficacy of rhDNase delivered with nebulizers systems have been clinically demonstrated, these systems present certain disadvantages for subjects such as lack of portability, low proportion of respirable particles, low delivery efficiency, and protracted delivery times. Including set up and cleaning, the time cost of delivering rhDNase via nebulizer may be up to 45 min per day for patients taking two doses per day. CF patients may take multiple inhaled drugs on a daily basis, including rhDNase, bronchodilators, and antibiotics, with total treatment time exceeding 2 h per day for some. Clearly more efficient delivery methods are required to reduce the time burden of taking multiple inhaled drugs in CF patients.

There are nebulizer/compressor systems available that reduce the administration time of rhDNase for the convenience of CF subjects. For example, two studies showed the SideStream nebulizer coupled with a powerful compressor reduced the delivery time and maintained efficacy.[11,12] Whether taking a further step and delivering rhDNase by bolus inhalation could be safe and effective was unknown.

The AERx® System is under development for the pulmonary delivery, via oral inhalation, of aerosolized liquid formulations of various small and large molecules. The AERx System is a drug/device combination (a "drug product") consisting of a hand-held, microprocessor-controlled device capable of aerosolizing a liquid formulation of drug filled into disposable unit-dose blisters incorporating single-use nozzles (dosage forms) through which the drug solution is extruded to form a fine aerosol. The AERx System uses no propellants (Fig. 1).

The AERx device contains aerosol generation hardware and electronics associated with breath actuation and automatic compliance monitoring. The AERx System has been designed to assist subjects in remembering and complying with instructions for use. Features include the following:

- Breath actuated; delivery of an aerosol "bolus" when the optimal inspiratory flow rate is



achieved within a preprogrammed inspired volume range
- Visual cues for the subject to inhale deeply within the optimum range of inspiratory flow rates
- A timer for the breath-holding maneuver

The AERx System has been used for the oral pulmonary delivery of insulin,[13] morphine,[14] fentanyl,[15] and numerous other compounds.

The AERx system generates an aerosol with more than 90% of the particles in the 2–3-μm range. Conventional nebulizers typically have a delivery efficiency of only 10–20%.[16] In a previous scintigraphic study[17] of inhaled rhDNase, the nebulizers delivered 0.16–0.78 mg of the 2.5-mg loaded dose into the lung. This represents a delivery efficiency of only 6–31%. The AERx system typically delivers 50–70% of the loaded drug dose to the lung. The AERx system used in this study when tested *in vitro* was predicted to deliver a lung dose of rhDNase equivalent to that of a jet nebulizer in only three inhalations at a single session, compared to the 10 min or more required by most nebulizers. The dose for this study (1.35 mg loaded dose; 0.68 mg estimated lung dose) was selected to target the high end of the nebulizer lung-delivery range.

The purpose of this study was to evaluate the feasibility of bolus inhalation of rhDNase via the AERx system in cystic fibrosis subjects.

MATERIALS AND METHODS

This was a multicenter, open-label study consisting of three phases: screening and washout, treatment, and post-treatment follow-up. Subjects already using Pulmozyme were screened, then withdrawn from Pulmozyme treatment for 14 days prior to study treatment. Subjects not previously using Pulmozyme were screened three days prior to study treatment. Subjects received rhDNase with the AERx System for 15 days. Subjects were seen in the clinic for safety and efficacy evaluation on treatment days 1, 2, 8, 11, and 15. On these study-visit days, the subjects took their dose of rhDNase in the clinic. They also performed pulmonary function tests before and 30 min after the dose to detect any bronchospasm that might be caused by bolus drug administration. Subjects returned to clinic 2 weeks after the last dose of the safety evaluation, all subjects had a blood sample for anti-rhDNase antibodies collected prior to the first dose and 4 weeks after the last dose.

Subjects with a confirmed diagnosis of cystic fibrosis were recruited from five sites across the United States. The study protocol was approved at each institution's human subjects Institutional Review Board. Informed consent was obtained from all subjects and/or from their guardians if the subject was <18 years of age. Subjects were screened within 14 days prior to any other study procedure. Screening procedures included a complete physical exam, medical history, and a review of inclusion and exclusion criteria.

Eligible subjects met the following inclusion criteria: (1) ages 8–19 years; (2) either using rhDNase or not using rhDNase for a minimum of 3 months prior to the study; (3) FEV_1 of 25–75% of predicted values, and an FVC of ≥1.5 L; and (4) able to perform reproducible spirometry maneuvers in accordance with American Thoracic Society Guidelines.[18] An FVC of 1.5 L was necessary for the subject to inhale the complete drug dose from the AERx device.

Subjects were excluded from the study if they: (1) used an investigational product within the 28 days of enrollment; (2) used inhaled tobramycin within 3 months of enrollment[19]; (3) had a qualitative change in inhaled antibiotic, bronchodilator, or steroid regimen within 14 days of enrollment; (4) were hospitalized within 14 days of enrollment; or (5) were pregnant. Females of child bearing potential were required to have a pregnancy test at screening and to use an effective method of birth control for the expected duration of the study. Subjects were required to demonstrate proficient use of the AERx device prior to taking it home.

Each AERx dosage blister contained 0.45 mg of rhDNase in 45 μL solution. The daily dose of AERx rhDNase in this study consisted of three inhalations (1.35 mg total loaded dose, or 0.45 mg loaded dose per inhalation) taken over 1–2 min at a single session. Assuming a 50% delivery efficiency, the AERx System was expected to deliver approximately 0.68 mg of rhDNase to the lung.

All efficacy assessments were conducted in the clinic. Prior to performing pulmonary function tests (FEV_1, FVC, $FEF_{25–75}$) on study-visit days, subjects were not to use long-acting bron-

chodilators within 8 h of their clinic visit. Pulmonary function tests were performed according to ATS standards for spirometry.[18] Pulmonary function results were converted to the percent predicted for age, gender, and height using the equations of Knudsen.[20] The baseline pulmonary function was calculated as the mean of measurements taken 3 days before and immediately prior to the first AERx treatment. Efficacy was assessed by comparing the pulmonary function on treatment days 8, 11, and 15 with the baseline pulmonary function values.

Safety was assessed by measuring bronchoconstrictor response, calculated from pulmonary functions prior to and 30 min after treatment on treatment days 1, 2, 8, 11, and 15, and testing for antibodies to rhDNase. Serum specimens were tested with a radioimmunoassay[21] for total antibodies to rhDNase, including IgE, IgG, and IgM antibodies. Monitoring for adverse events was performed at each visit.

Subjects were to be withdrawn from treatment if they experienced an acute respiratory exacerbation resulting in a >25% drop in FEV_1, required intravenous antibiotics, were hospitalized, experienced an anaphylactic reaction after treatment or if they experienced severe or life threatening (grade 3 or 4) toxicity according to the World Health Organization Common Toxicity Criteria.[22]

The primary efficacy analysis was based on the subset of evaluable subjects. A subject was considered as evaluable if he or she completed all treatments and assessments according to protocol. Subjects who reported missing two consecutive or a total of three nonconsecutive doses were not considered to be evaluable. The intent-to-treat and on-treatment population for safety analyses consisted of all subjects who completed one or more doses of study drug. Compliance with treatment was assessed by history and by reviewing the AERx device electronic dosing record. The primary efficacy outcome variable for the study was the overall mean improvement in FEV_1 (mean of treatment days 8, 11, and 15) relative to baseline. The mean relative improvement in all pulmonary function parameters (FEV_1, FVC, FEF_{25-75}) was also calculated separately for treatment day 8, 11, and 15 relative to baseline. A 95% confidence interval was calculated for each parameter. The Wilcoxon Signed Rank test was used to calculate the statistical significance of at 30 min post-treatment was calculated on treatment days 1, 2, 8, 11, and 15. The mean change on each of these days was considered as significant if the 95% confidence interval excluded zero.

The patient population selected for this study had the same characteristics as the subset of subjects with a moderately low baseline FEV_1 and the most robust response to rhDNase in the pivotal phase 3 study.[8] In that study, CF subjects aged 8–19 years with a baseline FEV_1 of 25–75% of predicted, demonstrated an 11.1% ([±13.9% [SD]) improvement in FEV_1 after 14 days of treatment with rhDNase. If the true effect of the AERx rhDNase systems was an 11% change from baseline, then with 16 efficacy evaluable subjects, the probability was 0.88 that the observed mean improvement should be greater than or equal to 7%. A sample size of 16 evaluable subjects was therefore selected for this study.

RESULTS

A total of 20 subjects were enrolled from five clinical trial sites in the United States. The mean age of study participants was 13.8 ± 2.7 years (range, 9–18 years). Fifteen males and five females were enrolled. Sixteen subjects were using Pulmozyme® prior to entering the study.

One subject withdrew prior to dosing due to a pulmonary exacerbation during the 14-day washout period and is not included in the efficacy or on-treatment safety analysis. Two subjects were not considered evaluable for efficacy due to mild pulmonary exacerbations that resulted in a drop in FEV_1 of >25% during the dosing period, and one noncompliant subject missed dosing on 2 consecutive days. These three subjects were included in the safety analyses only. The efficacy analysis is based on the results of 16 subjects: 13 subjects using Pulmozyme® regularly prior to the study and three subjects not using Pulmozyme prior to the study. All 19 subjects who took one or more doses of study medication completed the final study visit and provided safety data. Compliance as recorded by the electronic log was consistent with the stated history with one notable exception. One subject reported compliance with therapy but according to the electronics log took the majority of his doses on only 3 days.

The sixteen subjects who were using Pulmozyme prior to the study underwent a 14-day

TABLE 1. PULMONARY FUNCTION IMPROVEMENT WITH AERx rhDNase SYSTEM

Statistic	*Overall (days 8, 11, 15)*	*Day 8*	*Day 11*	*Day 15*
N	16	16	16	16
Mean relative change in FEV_1 (% predicted)	7.8	6.6	6.9	9.9
95% CI	4.0, 11.7	2.0, 11.3	3.1, 10.7	5.0, 15.0
p-value[a]	0.001	0.008	0.003	0.002

[a]*p*-values are from Wilcoxon signed rank test.

subjects had a decrease in FEV_1 during that time and the remaining 11 subjects had an increase in FEV_1 or remained unchanged by the end of the washout period. There was no significant mean change in FEV_1 observed for the group during the 14-day washout (1.68 L [96% CI 1.45, 1.90]) vs. 1.76 L [96% CI 1.50, 2.02] for days −14 and 0, respectively).

A significantly mean increase in FEV_1 compared to baseline was seen on treatment days 8, 11, 15, and overall (mean of treatment days 8, 11, and 15) for the primary efficacy population (Table 1).

Individual subject changes in FEV_1 on treatment days 8 and 15 (Primary Analysis group) are presented in Figure 2. With the exception of two subjects, most subjects remained stable or had an increase in FEV_1 over the 15-day treatment period, with 12 subjects improving in the first week.

A significant mean relative increase was seen on day 15 compared to baseline for FVC (7.48%, $p < 0.001$) and $FEF_{25–75}$ (16.45%, $p < 0.018$). There were too few subjects who were Pulmozyme naive ($n = 3$) versus chronic users ($n = 13$) to draw any conclusions as to the effect of prior treatment on differential response to rhDNase treatment. After day 15, the thirteen Pulmozyme non-naive subjects were returned to their regular rhDNase delivered by nebulizer. Pulmonary function measurements did not differ when assessed 2 weeks after the final AERx bolus dose for these subjects.

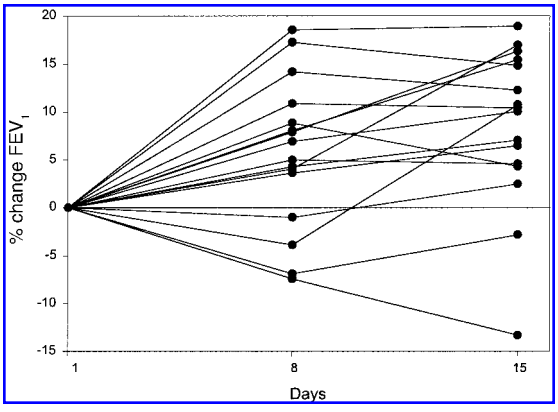


FIG. 2. Percent change in FEV$_1$ from baseline. Primary

Adverse events reported by at least 10% of all subjects included cough (26%), upper abdominal pain (16%), sore throat (16%), and rhinorrhea (11%). These symptoms are seen frequently in CF subjects, and are similar to those reported in previous rhDNase trials. No hospitalizations or other serious adverse events were reported during the study.

The study was conducted between the months of December and June, the peak season for respiratory viruses. Two subjects experienced acute respiratory exacerbations during treatment, resulting in a drop in FEV_1 of >25% from baseline measurements on treatment day 11. Both subjects recovered from their chest infections uneventfully with oral antibiotics.

Nineteen subjects provided both pre-treatment and 4-week post-treatment blood specimens. No serum antibodies to rhDNase were detected in any subject prior to or after treatment in the study.

Overall there were no significant changes in the mean difference in FEV_1 (% predicted) measured prior to and 30 minutes post-treatment on any study-visit dosing day (Table 2). Airway reactivity to rhDNase administration defined as ≥10% drop in FEV_1 30 minutes after treatment was infrequent, occurring on only two individual occasions in different subjects. Both subjects continued on therapy without a recurrence in FEV_1 drop or other significant sequelae. Neither subject required rescue medications as a result of the

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