

Safety and Efficacy of Recombinant Alpha₁-Antitrypsin Therapy in Cystic Fibrosis

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Summary. Neutrophil elastase (NE) is thought to be the most important protease which damages the cystic fibrosis (CF) lung. Attempts have been made to suppress this activity using the plasma-derived inhibitor, alpha₁-antitrypsin (AAT). In this pilot study, the safety and efficacy of inhaled recombinant human AAT (rAAT) as a treatment for CF were investigated. Thirty-nine patients participated in a prospective, double-blinded, randomized, placebo-controlled phase II trial to examine the effect of rAAT (500, 250, and 125 mg) on sputum NE activity. Sputum myeloperoxidase (MPO), interleukin-8, tumor necrosis factor receptors, sputum and plasma NE/AAT complexes, and safety parameters were also measured. Subjects were randomized to receive nebulized treatment once a day for 4 weeks, followed by 2–4 weeks with no study treatment, and then a 2-week rechallenge phase. Trends toward a reduction in NE activity were observed in patients treated with 500 mg and 250 mg of rAAT compared to placebo. Sputum NE/AAT complex and MPO levels were lower on rAAT compared to placebo. No major adverse events and, in particular, no allergic reactions to rAAT were observed. Although significant differences between rAAT and placebo for sputum NE activity were not observed, some improvements were found for secondary efficacy variables. This study demonstrated that nebulized rAAT is safe and well-tolerated, but has a limited effect on NE activity and other markers of inflammation. **Pediatr Pulmonol.** 2006; 41:177–183. © 2005 Wiley-Liss, Inc.

Key words: cystic fibrosis; neutrophil elastase; alpha₁-antitrypsin; inflammation.

INTRODUCTION

In cystic fibrosis (CF), chronic infection in the airways leads to the recruitment of large numbers of neutrophils into the lungs.¹ These neutrophils release high levels of a serine protease, elastase (NE), that is neutralized in normal individuals by the endogenous inhibitor alpha₁-antitrypsin (AAT). In CF, however, the amount of free NE present overwhelms AAT, leading to aberrant destruction of lung epithelium,^{2,3} as well as a reduction in the ability of the host immune system to combat infection^{4,5} and the stimulation of further mucus production.⁶

With active antiproteases, such as AAT, there is therefore an opportunity for a therapeutic augmentation approach to suppress the burden of NE in the airways of patients with CF.⁷ Previous studies in CF patients showed that delivery of plasma-derived AAT to the airways resulted in inhibition of NE activity in bronchoalveolar lavage fluid.⁸ This treatment may also correct some of the other effects of excess elastase, including excessive lung-tissue inflammation and interference in the killing of bacteria. The commercial development of recombinant alpha₁-antitrypsin (rAAT) was recently described.⁹

This pilot study was designed to evaluate both the acute and long-term effects of rAAT on sputum free NE activity

and to assess the risk, if any, of stopping and restarting rAAT before the initiation of larger-scale studies. The secondary objectives were to determine the effect of rAAT therapy on inflammatory markers such as sputum

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interleukin-8 (IL-8), myeloperoxidase (MPO), and tumor necrosis factor (TNF) receptors, as well as sputum and plasma NE/AAT complexes.

MATERIALS AND METHODS

Study Subjects

Thirty-nine patients (32 males) aged 16 years and older, who attended the clinics of the investigators and who had a vital capacity $\geq 40\%$ of predicted, participated in the study. All patients had the diagnosis of CF confirmed by sweat test ($\text{Cl}^- > 60$ mmol/l) or genotype, and were free from pulmonary exacerbation in the 14 days prior to initial dosing. Patients taking oral prednisolone at a daily dose > 10 mg and those who smoked or were ex-smokers of less than 6 months' duration were excluded from the study. This study was conducted in compliance with ethical principles originating from the Declaration of Helsinki of 1996 and Good Clinical Practice. The study protocol and amendments were reviewed and approved by the independent Ethics Committees of all participating hospitals, and all patients gave informed consent.

Study Design

This pilot study was performed at five centers throughout the UK (Belfast, Cambridge, Cardiff, Edinburgh, and Sheffield) as a double-blinded, randomized, placebo-controlled, parallel-group trial. Patients made eight study visits over a period of 16 weeks during which a physical examination, pulmonary function tests, sputum, blood, and urine collection, and the measurement of safety parameters were carried out. It was planned that a total of 48 evaluable patients would be recruited, with non-evaluable patients replaced up to a maximum of 60 patients recruited. Previous work by McElvaney et al.⁸ involving 12 patients with CF showed that treatment with aerosolized AAT at approximately 3 mg/kg resulted in a reduction of measured sputum neutrophil elastase to almost zero in most patients. It was therefore determined that 12 patients per group, to include three treatment groups and one placebo group, would give a two-sample *t*-test a 5% significance level and approximately 80% power. This would be sufficient to detect any reduction in free sputum elastase activity over 28 days of treatment. However, due to pulmonary exacerbation and the availability of eligible and willing patients, the study was closed after 39 evaluable patients had been recruited. Patients were randomized to one of four treatment groups, according to a predetermined randomization list. The randomization was stratified by center and for colonization with *Burkholderia cepacia* complex (BCC), which is known to be predictive of a poor prognosis in patients with CF. The active study medication comprised rAAT (PPL Therapeutics, Roslin, Scotland, UK) at either 500-mg,

250-mg, or 125-mg doses, in a histidine and sodium chloride buffer (reconstituted volumes were 10, 5, and 2.5 ml, respectively). The placebo medication comprised histidine and sodium chloride buffer alone. After an initial screening visit (visit 1), eligible patients returned to the clinic for a supervised first dosing and then subsequently administered once daily the study medication as a nebulized solution, using the Pari LC-Star nebulizer with the Turbo Boy[®] compressor (Pari GmbH, Starnberg, Germany) for a period of 4 weeks (visits 2–5). The inhalation times were usually 20–30 min in duration. This was followed by a period of 2–4 weeks with no treatment, and then a 2-week rechallenge phase for safety and immunogenic analysis. For the duration of the study, patients were required to attend the clinic once a week to allow assessment. Patients then had a safety follow-up visit approximately 2 weeks after the last dosing.

Methods

A Vitalograph[®] was used to measure vital capacity (VC), forced vital capacity (FVC), and forced expiratory volume in one second (FEV_{1}). At each visit, each of these variables was measured in triplicate, and the best value chosen by the Vitalograph[®] was recorded as the result for that time point. Pulmonary function tests were performed according to the current American Thoracic Society guidelines. A sample of blood was taken for routine clinical chemistry and hematology. Samples of sputum and plasma were obtained for the measurement of inflammatory markers. Sputum was collected into sterile, disposable plastic containers during a 30-min physiotherapy session. Sputum was weighed and processed by centrifugation at 30,000g for 1 hr at 4°C within 15 min of the end of the collection period. The resultant supernatant (sol) was recovered and specifically inhibited, using the combinations of inhibitors described by Martin et al.¹⁰ All samples were stored in aliquots at -70°C , pending measurement of inflammatory mediators.

Inflammatory markers were analyzed as a single batch at one site by a blinded investigator. Free NE activity was detected by spectrophotometric assay, utilizing the substrate Suc-Ala-Ala-Pro-Val-*p*-nitroanilide (Bachem, Walden, Essex, UK).¹¹ All other parameters were measured by commercially available enzyme-linked immunosorbent assays (ELISA) supplied by R&D Systems (Abingham, Oxon, UK), with the exception of NE/AAT complexes, which were detected by an ELISA developed within the authors' laboratory, where relevant performance and quality-control checks were conducted.

Safety was assessed at each visit by the reporting of adverse events, laboratory safety data, pulmonary function tests, pulmonary exacerbation frequency, chest X-ray, skin-prick testing, plasma rAAT concentrations, and the presence or absence of antibodies to rAAT. The latter two

parameters were detected by ELISAs developed within the authors' laboratory, where relevant performance and quality-control checks were conducted. The rAAT ELISA was dependent on the development of an antibody able to distinguish between the recombinant and plasma forms of AAT. This was achieved by exploiting differences in glycosylation on the surface of AAT, as sheep produce N-acetyl neuraminic acid, whereas humans produce N-glycolyl neuraminic acid. The assay to detect antibodies to rAAT was a simple direct assay.

Statistical Analysis

As baseline values varied considerably from patient to patient for most variables, it was not possible to analyze changes from baseline for actual values. For all laboratory measurements except TNF receptors, the following data transformations were applied. Prior to analysis, a log transformation was applied to each value. Analysis was then performed on the difference between these log-transformed values between visit 5 (at the end of the 4-week dosing period) and baseline (screening visit 1) (i.e., $\log_e(\text{visit 5}) - \log_e(\text{baseline})$). The analysis for TNF receptor data was performed on the difference between actual values between visit 5 and baseline (i.e., visit 5 – baseline). For patients who withdrew from the study prior to visit 5, the last available measurement after starting the study medication was used. Sputum NE activity even after this transformation did not appear normally distributed. Therefore, the nonparametric Kruskal-Wallis test was used for an overall comparison between treatment groups,

and the Wilcoxon two-sample test was used for comparisons between each active group and placebo. Due to the small numbers of patients in each treatment group, there were no adjustments for any covariates. All other variables were subjected to analysis of variance (F-test) for an overall test of differences between treatments in terms of the transformed variables. In addition, pairwise comparisons of each of the active treatments with placebo were performed using two-sample *t*-tests.

RESULTS

Analysis Population

Ten patients received 500 mg rAAT, of whom all reached the end of the first treatment phase (visit 5). Ten patients were randomized to receive 250 mg rAAT, of whom 9 reached visit 5. Nine patients received 125 mg rAAT, of whom 8 reached visit 5. Ten patients received placebo, of whom 9 reached visit 5 (36/39 patients in total). In total, 38 patients were included in the safety population, as one patient from the 250-mg group withdrew from the study due to exacerbation, prior to receiving any study medication.

Baseline Characteristics

Demographic data and baseline characteristics are summarized by treatment group in Table 1. All patients were Caucasian, with a mean age of 27.5 years (range, 16.6–69.2 years). There was a higher mean age in the 125-mg rAAT and placebo groups (31.8 ± 15 years and

TABLE 1—Demographic and Baseline Characteristics of Patient Population¹

Parameter	500 mg rAAT (n = 10)	250 mg rAAT (n = 10)	125 mg rAAT (n = 9)	Placebo (n = 10)
Sex (male/female)	9/1	9/1	7/2	7/3
Age (years)	24.9 ± 5.2 (18.8–35.6)	27.4 ± 8.4 (17.5–39.7)	31.8 ± 15.0 (19.2–69.2)	31.2 ± 10.4 (16.8–51.3)
Body mass index (kg/m ²)	22.2 ± 2.3 (18.0–26.1)	20.1 ± 2.9 (16.0–23.9)	20.7 ± 2.7 (17.9–27.2)	20.1 ± 2.1 (17.7–23.9)
Weight (kg)	62.5 ± 7.6 (49.0–73.7)	62.3 ± 10.4 (46.3–77.3)	59.2 ± 7.7 (43.0–70.6)	58.4 ± 9.2 (47.0–75.0)
Height (cm)	167.9 ± 8.1 (149.0–178.0)	175.7 ± 7.2 (157.0–182.0)	169.1 ± 7.0 (155.0–179.0)	170.0 ± 1.4 (156.0–180.0)
Resting respiratory rate (breaths/min)	19.8 ± 4.2 (14.0–28.0)	19.3 ± 3.5 (15.0–24.0)	20.1 ± 3.8 (14.0–26.0)	19.2 ± 2.7 (16.0–24.0)
FEV ₁ (l)	2.28 ± 0.96 (0.7–3.6)	2.26 ± 0.87 (1.3–3.9)	1.92 ± 0.83 (0.7–3.1)	1.40 ± 0.88 (0.6–3.4)
FVC (l)	3.43 ± 1.26 (1.6–5.9)	3.39 ± 1.18 (1.7–5.6)	2.92 ± 1.09 (1.2–4.8)	2.36 ± 1.08 (1.1–4.6)
VC (l)	3.47 ± 1.38 (1.9–6.4)	3.50 ± 1.08 (1.8–5.6)	2.92 ± 0.97 (1.1–4.2)	2.67 ± 1.09 (1.0–4.9)
FEV ₁ (% predicted)				
≤40%	2 (20%)	2 (20%)	2 (22%)	6 (60%)
41–60%	2 (20%)	5 (50%)	6 (67%)	2 (20%)
61–80%	6 (60%)	3 (30%)	1 (11%)	1 (10%)
>80%				1 (10%)
Sputum free neutrophil elastase activity (µg/ml)	0.72 ± 1.88 (0.004–6.05)	2.81 ± 4.94 (0.005–16.16)	0.83 ± 1.66 (0.004–5.15)	2.63 ± 3.78 (0.004–8.59)
Frequency of <i>Burkholderia cepacia</i> complex	30%	30%	22%	20%

¹Results are expressed as mean ± SD (range), with exceptions of FEV₁ (% predicted) and *Burkholderia cepacia* complex frequency.

31.2 ± 10.4 years, respectively) in comparison to the other groups (24.9 ± 5.2 years and 27.4 ± 8.4 years in the 500-mg and 250-mg rAAT groups, respectively). Thirty-two (82%) patients were male, and 7 (18%) were female. The mean height was 170 cm (range, 149.0–182.0 cm), the mean weight was 61.2 kg (range, 43.0–78.6 kg), and the mean body mass index was 21.0 kg/m² (range, 16.0–27.2 kg/m²). The mean value for each of the pulmonary function tests was found to be similar between groups, but was lowest consistently in the placebo group. The highest mean baseline concentration of NE activity was seen in the 250-mg rAAT and placebo groups, and the lowest mean concentration was seen in the 500-mg rAAT group. The majority of patients did not have BCC (31 (76%) patients), and there were similar proportions of patients with BCC across the treatment groups. Only one patient (placebo group) was on low-dose prednisolone at the start of the study.

Safety

There were no notable changes in physical examination or vital signs throughout the duration of the study. There was a non-significant decrease in FVC and FEV₁ 30 min post-dose in some treatment groups with some of the doses. However, none of these declines were symptomatic. At this time, the placebo, 125-mg, and 500-mg treatment groups showed minimal change in FEV₁, whereas the 250-mg group fell by 5.3%. By 2 hr post-dose, the 125-mg and 500-mg doses had increased FEV₁ of +4.9% and +2.3% from baseline, respectively, the 250-mg group had recovered slightly to -4.3%, and the placebo group showed a decrease of -6%. The change in FVC ranged from -5.6% to no change 30 min post-dose, to -5% (placebo group) and to +3.4% (500-mg group) 2 hr post-dose. Twenty-four patients reported a pulmonary exacerbation (8, 6, 3, and 7 in the 500-mg, 250-mg, 125-mg, and placebo groups, respectively). Only 3 of these patients had events that were considered to be related to the study drug (1 patient in the 500-mg rAAT group, and 2 in the placebo group). Two patients showed quantifiable concentrations of antibodies to rAAT (IgG): one in the 500-mg rAAT group, and one in the 250-mg rAAT group. These antibodies developed after rechallenge with rAAT. Neither of these patients had any adverse events during the study, and there were no changes in pulmonary function tests or laboratory data which were indicative of an allergic response. In addition, skin-prick testing during the rechallenge phase also showed no reaction to rAAT. A development-phase assay was used to quantify rAAT in plasma, and the concentrations were broadly dose-dependent although variable between individuals during the study. These results also provided a fair indication of patient compliance. There were no discontinuations due to abnormal laboratory test results,

although there were some changes in median concentrations of hematological and biochemical parameters, but these were of no consistent pattern, and the changes were thought likely to be related to the underlying condition.

Efficacy Results

The mean change in sputum free NE activity for the treatment groups at baseline and visit 5 is shown in Figure 1. The data were initially expressed as a geometric mean calculated by log transformation of the data, as the baseline concentrations of sputum free elastase were very variable both within and between groups. Analysis showed a small reduction in sputum free NE concentrations with active treatment. However, statistical significance was not reached. Treatment comparisons between the 500-mg rAAT and placebo groups resulted in a *P*-value of 0.21, whereas the 250-mg and 125-mg groups vs. placebo generated *P*-values of 0.09 and 0.31, respectively (Wilcoxon two-sample test). Concentrations of sputum NE/AAT complexes were reduced from baseline in the 500-mg and 125-mg groups in comparison with placebo (Fig. 2). The difference between the 125-mg rAAT group and the placebo group was found to reach statistical significance (*P* = 0.05). There was no significant change in visit 5/baseline differences between treatment groups in relation to concentrations of plasma NE/AAT complexes. A trend toward lower sputum IL-8 concentrations was observed with the 500-mg rAAT dose; however, statistical significance was not reached (*P* = 0.09) (Fig. 3). A decrease in sputum MPO was seen with the 500-mg and 125-mg rAAT groups. The difference between the 500-mg rAAT and placebo groups reached statistical significance (*P* = 0.04) (Fig. 4). There were no significant differences between treatment groups in relation to concentrations of TNF p55 receptors (data not shown).

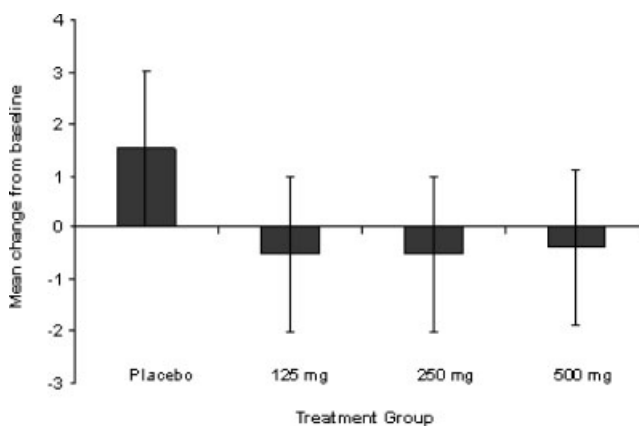


Fig. 1. Change in sputum neutrophil elastase activity 4 weeks after daily dosing with rAAT (visit 5), expressed as mean change (SD) in log-transformed data for sputum NE (visit 5 – visit 1). A statistically significant difference was not observed between treatment groups.

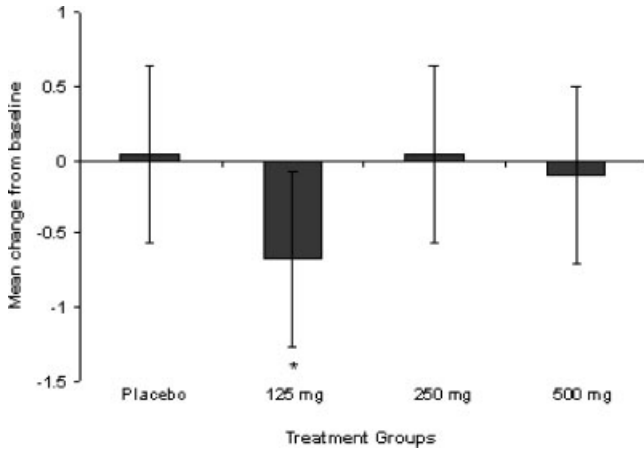


Fig. 2. Mean change (SD) in log-transformed data for sputum neutrophil elastase/α₁-antitrypsin complexes between baseline (visit 1) and 4 weeks after daily dosing with rAAT or placebo (visit 5). *Statistically significant difference obtained between 125-mg rAAT and placebo groups (P = 0.05).

Pulmonary Function and Microbiology

Trends toward a decrease in resting respiratory rate and an increase in VC, FVC, and FEV₁ were observed in the 500-mg and 250-mg rAAT treatment groups between baseline and visit 5 as compared to placebo. However, these differences were not statistically significant (Table 2). Microbiology, including identification of organisms and total viable counts, was carried out on a cough sputum sample at each visit. The four most commonly reported bacteria were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus viridans*, and *Burkholderia cepacia*. No significant change in bacterial ecology or colony counts was observed between treatment groups (data not shown).

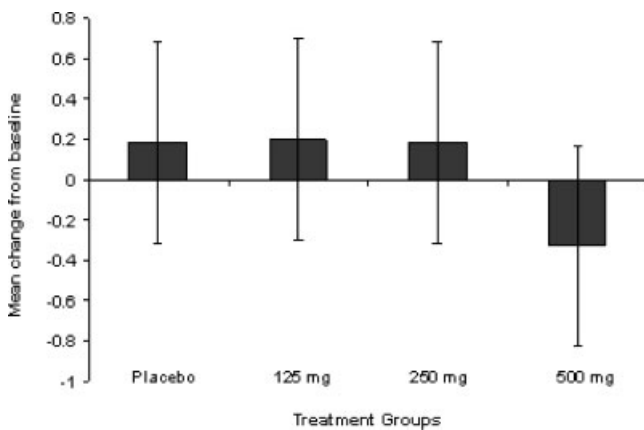


Fig. 3. Mean change (SD) in log-transformed data for sputum IL-8 (ng/ml) between baseline (visit 1) and 4 weeks after daily dosing with rAAT or placebo (visit 5). Statistically significant difference was not observed between treatment groups.

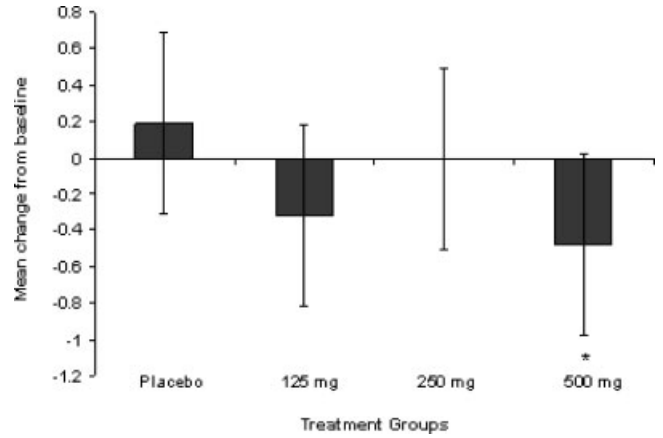


Fig. 4. Mean change (SD) in log-transformed data for sputum myeloperoxidase (µg/ml) between baseline (visit 1) and 4 weeks after daily dosing with rAAT or placebo (visit 5). *Statistically significant difference obtained between 500-mg rAAT and placebo groups (P = 0.04).

DISCUSSION

Transgenic technology enables the high-volume production of active recombinant proteins, which would be difficult and costly to produce using alternative methods. rAAT is a single-chain glycoprotein (44.3 kDa) and is identical to native AAT, with the exception of several side-chain sugars on the molecule.⁹ The recombinant gene technology involves the fusion of the human gene with a specific milk gene promoter. Purification of rAAT from the milk of transgenic sheep results in 40% recovery of the protein at >99% purity. It is unlikely that rAAT would be toxic due to immune responses. However, given the abundance of NE in the CF lung, the dosage of rAAT required to neutralize activity was a potential cause for concern.

This pilot study is the first randomized, placebo-controlled trial of recombinant alpha₁-antitrypsin augmentation therapy in patients with CF. Assessment of safety data showed no intolerance or allergic effects up to doses of 500 mg rAAT/day. The primary efficacy variable was the level of sputum free neutrophil elastase activity, with secondary variables being other reported markers of inflammation in the lung and pulmonary function.¹²⁻¹⁴

TABLE 2—Changes in Pulmonary Function Data at Baseline and Visit 5¹

Parameter	500 mg rAAT	250 mg rAAT	125 mg rAAT	Placebo
RRR (breaths/min)	-0.2	-0.3	+1.0	+0.3
VC (L)	+0.11	+0.16	+0.05	+0.01
FVC (L)	+0.18	+0.05	-0.21	+0.14
FEV ₁ (L)	+0.12	+0.13	-0.25	-0.02

¹RRR, resting respiratory rate.

The three doses used in this study (500 mg, 250 mg, and 125 mg rAAT) were similar to the doses used by McElvaney et al. (7.1 mg/kg, 3.6 mg/kg, and 1.8 mg/kg, respectively, based on a patient weighing 70 kg).⁸ McElvaney et al.⁸ performed the first preliminary trial of plasma-derived AAT (Prolastin[®], Bayer Corp.) in patients with CF. Aerosolized AAT (1.5–3 mg/kg) was administered to 12 patients with moderate respiratory impairment twice daily for 1 week, and the effect on NE activity and anti-NE capacity in epithelial lining fluid (ELF) was measured. It was observed posttreatment that ELF had suppressed NE levels when ELF AAT levels reached 8 $\mu\text{mol/l}$, and significantly, an excess of fully active AAT was also detected. In this study, using a transgenic, recombinant protein, it was not possible to demonstrate statistically significant differences between the rAAT treatment groups and placebo for sputum free NE activity. This may have been due in part to the early termination of the study because of difficulties in recruitment approaching a second winter. In addition, by chance, the randomization process resulted in the placebo group being older and having poorer lung function compared to the three treatment groups. This resulted in large variability between patients, and was confounded by the patients randomized to receive the 500-mg rAAT dose having the lowest sputum elastase concentrations at baseline. These results are somewhat disappointing compared to the previous study,⁸ but it should be noted that there were major differences in study design. The first study was small and uncontrolled, whereas this study is the first reported double-blinded, randomized, placebo-controlled, parallel-group trial of rAAT in CF patients. In addition, measurements were previously made in ELF obtained by bronchoalveolar lavage, compared to sputum obtained from expectorated sputum. The results of this study illustrate once again the inherent variability of samples obtained from patients with CF, and that this contributes to difficulty in detecting any given effect of antiprotease augmentation therapy. It should also be noted that the elastase activities measured in this study were much lower than early descriptions of high NE load in CF airways.¹⁵ This could be due to several variables, including differences in sputum processing and different patient characteristics.

There was also large intra- and interpatient variability in the secondary outcome variables. At the 125-mg dose, a significantly large reduction in NE/AAT complexes was observed, but this was not consistent across doses. Indeed, this was a surprising result, as one might have expected a rise in this measurement. However, it should be noted that the mechanism of clearance of these complexes from the lungs is not known, and therefore increased clearance may account for the observed reduction. Myeloperoxidase was generally lower on active treatment in comparison with placebo, with a statistical difference observed for the

500-mg group. There was also an indication of lower IL-8 concentrations with the 500-mg rAAT group. However, as there were no continual trends across dosing, it is likely that the mild significant differences achieved in some groups may have occurred by chance. It should be noted that every effort was made to limit variability due to collection at different study sites, and all analyses were carried out as a single batch at one site.

This study failed to demonstrate a benefit of rAAT on inflammatory markers in patients with CF. This could be due to the variability that was observed both between and within patients for most of the endpoints. Sputum is known to be a difficult matrix with which to work, and a wide variability is observed between cough samples. It was hoped that some of these problems would be overcome by collecting a pooled sputum sample over a 30-min physiotherapy session and processing the sample within 1.5 hr. However, after this study was conducted, there were several reports validating induced sputum as a safe and useful medium for assessing response to therapies in CF.^{16,17} Therefore, it is assumed that further larger-scale studies involving rAAT would include the collection of an induced sputum sample.

Although the intrapulmonary half-life of aerosolized AAT (Prolastin[®], Bayer Corp.), given as a single 200-mg dose, was determined in healthy volunteers as being 69.2 hr, and results in a sustained antielastase protection of the lung over a 36-hr period,¹⁸ the deposition of AAT in patients with airway obstruction and impaired pulmonary function remains to be determined, and will be dependent on the physical properties of the aerosol and the characteristics of the nebulizer.¹⁹ It is therefore difficult to assess whether the dosing regimens carried out in this study delivered sufficient drug to the most diseased areas of the lung. Another potential problem is that AAT may be inactivated within the oxidative and proteolytic milieu of the CF lung. The methionine residue at the active site of the AAT molecule (Met³⁵⁸) can be easily oxidized, with a resulting marked loss of activity.^{20–22} Similarly, it is vulnerable to degradation by both granulocyte and bacterial proteases.^{23,24}

Other endogenous inhibitors of NE are secretory leukoprotease inhibitor (SLPI) (12 kDa) and elastase-specific inhibitor (Elafin) (6 kDa). Both are expressed constitutively in the lung, and have the ability to inactivate other neutrophilic serine proteases (namely, cathepsin G and proteinase-3), and also have similar antimicrobial properties.^{25–27} Short-term studies using recombinant SLPI (rSLPI, Synergen, Boulder, CO) in the treatment of patients with CF showed that inhaled rSLPI significantly reduced airway NE activity *in vivo*.^{28,29} In addition, SLPI has a different reactivity from AAT; therefore, the two antielastases may have complementary action. It is postulated that future studies to combat airway elastase in inflammatory lung disorders may take a dual approach,

utilizing rAAT in partnership with rSLPI or indeed elafin. In addition, several synthetic small-molecule elastase inhibitors are in preclinical and clinical development, and these may prove to be more proteolytically stable and resistant to oxidation.¹⁹

In conclusion, rAAT was delivered safely to the airways of patients with CF without intolerance or allergic effects. With the advent of new delivery systems, it is envisaged that the problems surrounding deposition will be surmounted in the near future, resulting in sufficient doses of AAT reaching the most diseased areas of the lung. This may assist the additional larger-scale studies that are necessary to establish whether rAAT has biochemical and clinical efficacy as an effective treatment for CF.

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