The Effect of Augmentation Therapy on Bronchial Inflammation in α1-Antitrypsin Deficiency

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α1-Antitrypsin (AAT) deficiency predisposes to bronchitis and emphysema associated with neutrophilic airway inflammation. The efficacy of augmentation therapy has not been proven clinically or by demonstrating an effect on airway inflammation. We treated 12 patients with four infusions of Prolastin (60 mg/kg) at weekly intervals and monitored both the serum and secretion concentrations of AAT as well as markers of neutrophilic inflammation, including myeloperoxidase, elastase, and the neutrophil chemoattractants interleukin-8 and leukotriene B_4. Serum AAT rose and was maintained above the protective threshold. In addition, AAT concentrations in the sputum rose from a mean of 0.17 μM (SEM ± 0.04) before therapy to concentrations similar to nondeficient subjects (0.43 ± 0.12) 1 week after the first infusion ($p < 0.01$). This was associated with a reduction in elastase activity ($p < 0.002$) and the chemoattractant leukotriene B_4 ($p < 0.02$), which fell from a median baseline value of 13.46 nM (range, 4.17–55.00) to 8.62 nM (4.23–21.59) the day following the last infusion. Although median values for myeloperoxidase and interleukin-8 also fell, the changes failed to achieve statistical significance. In summary, short-term therapy with AAT increased lung secretion concentrations and was associated with a fall in leukotriene B_4, which is thought to be central to the airway inflammation of AAT deficiency.

Keywords: α1-antitrypsin; sputum; inflammation; leukotriene B_4

α1-Antitrypsin deficiency (AATD) is associated with the early onset of rapidly progressive lung disease, especially in smokers. The condition is characterized by predominantly lower zone emphysema, the presence of chronic bronchitis (1), and bronchiectasis (2). The pathogenesis of these features is unclear but is thought to be a direct result of lung damage by neutrophil elastase (NE) released from recruited neutrophils (3). The critically low level of α1-antitrypsin (AAT) predicted to be present in the lung interstitium (3) and present in the airway (4, 5) greatly enhances the amount and area of activity of NE (6) and hence the degree of lung damage.

Factors resulting in neutrophil recruitment to the airway are only partly understood. However, studies with bronchoalveolar lavage (which samples the peripheral airways) suggested that the major neutrophil chemoattractant is leukotriene B4 (LTB4), which is released from airway macrophages by active NE as a direct consequence of the AATD (7). Indeed, recent studies have confirmed that high concentrations of LTB4 and elastase activity are also a feature of secretions obtained from the larger airways (4), where the number of neutrophils is also increased in AATD. This suggests that AAT also plays a key role in the protection of the proximal regions of the lung despite the presence of significant concentrations of the other major airway inhibitor of NE, secretory leukoproteinase inhibitor. Indeed, recent studies have suggested that acute exacerbations (a clinical feature of the larger airways) are associated with more inflammation in AATD (8), that these are associated with loss of lung function (9), and that augmentation may ameliorate these episodes (10).

Augmentation therapy for AATD has been available for many years, although to date no sufficiently powered clinical study has been performed to demonstrate its efficacy. Studies have shown that augmentation restores the concentration of AAT in the blood to protective levels (11) and normalizes the concentration in epithelial lining fluid (12). Whether this biochemical "normalization" influences the pathogenic processes thought to be of importance in the development and progression of lung disease is unknown. Unfortunately, bronchoalveolar lavage is an invasive procedure and is proinflammatory so that it is difficult to carry out frequent or sequential studies. Thus, assessing the lung concentration changes and effects of augmentation are difficult studies to implement. On the other hand, it is possible to collect airway secretions as expectorated sputum on a daily basis from patients with chronic bronchitis. Changes in these secretions may not only reflect those relevant at the alveolar level but in particular may be relevant to the significant airway component of the disease and exacerbations. We therefore studied the effect of intravenous augmentation of AAT on the airway concentration of AAT and airway inflammation measured in spontaneously expectorated sputum. In particular, we were interested in markers of neutrophil recruitment and activation and the concentrations of the neutrophil chemoattractant cytokines, interleukin-8 (IL-8) and LTB4. This study describes the results of an open study in 12 patients treated with intravenous AAT augmentation administered weekly for 4 consecutive weeks.

METHODS

Twelve patients with AATD of the Pi Z phenotype were studied. All had emphysema visualized on high-resolution computed tomography scan, and two had limited tubular bronchiectasis. The patients were selected because of their proximity to the study center and a history of chronic cough and daily sputum production. None had smoked for at least 3 months before the study or had suffered from an acute exacerbation of their lung disease within the previous 2 months. No patient had received recent steroid therapy with the exception of inhaled beclomethasone, diproprionate (400 mcg twice daily) or equivalent, and none had selective IgA deficiency. The average ± SEM forced expired volume in one second was 1.17 ± 0.22 L postbronchodilator (400 mg of Salbutamol) with an FEV_1 to forced vital capacity ratio of 34.8% ± 3.3, indicating moderate to severe airflow obstruction.

All patients were seen on two occasions before the commencement of the AAT infusions to ensure both clinical and physiologic (dynamic flow rates) stability. At these visits, 10 ml of venous blood was taken, allowed to clot, and centrifuged to obtain the serum that was stored (−70°C) for subsequent analysis. Patients also collected sputum (as free from saliva as possible) from rising over a 4-hour period into a sterile container; an aliquot was cultured for bacteria, and the

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remainder (untreated) was ultracentrifuged at 50,000 × *g* (3°C) for 90 minutes to obtain sputum sol phase, which was also stored at −70°C until analyzed.

After this control period, the patients were treated with four infusions (60 mg/kg) of human blood-derived AAT (Prolastin; Bayer Pharmaceuticals, Westhaven, CT) at weekly intervals. Serum and sputum were collected and processed, as mentioned previously here, on the morning of the first infusion before therapy (Day 1). Subjects attended the department the following day (Day 2) as well as on Day 5 and Day 8 (in the morning before the second infusion). Patients then attended on Days 15 and 22 (in the morning before the third and fourth infusions, respectively) and subsequently on Days 23, 26, and 29. Patients were then seen weekly for up to 5 weeks after the final infusion. At each visit, serum and sputum were obtained where possible and were processed as described previously here.

The serum and sputum samples from all collection times were analyzed for AAT concentration using a specific enzyme-linked immunosorbent assay (8). In addition, sputum samples obtained in the 2 weeks before the first infusion, on Day 2 (24 hours after the first infusion), on Day 23 (24 hours after the fourth and last infusion), and at 3 weeks later were assayed for myeloperoxidase (MPO), NE activity, LTB4, and IL-8 as described previously (8). Finally, the ability of samples to inhibit porcine pancreatic elastase (PPEIC) was determined to assess the function of AAT in the secretions separately from secretory leukoproteinase inhibitor as described previously (13). The two time points on treatment (i.e., Days 2 and 23) were chosen for the major analysis of the effect of treatment, as sufficient sample was available for all analyses on most patients, and they represented time points when the sputum AAT concentrations were highest.

Statistics

Data for each measurement throughout the study were analyzed using the Friedman test, and only when this proved significant were data analyzed statistically. Serum and sputum ATT concentrations are presented as mean ± SEM, and comparisons between days were made, therefore, using a paired student *t* test (single tailed).

Because sample variability for cytokine measurements is wide, the data from the 2 control days before therapy were combined to obtain an average value for each patient. This value was then compared with the data from the day after the first and fourth infusions (Days 2 and 23, when AAT sputum and serum levels were highest) and 3 weeks after the last infusion using paired Wilcoxon rank sum tests (single tailed). Data for these results are presented as median and range. The correlation between the magnitude of the LTB4 response and baseline value was assessed using Spearman ρ. The study was approved by the local university ethics review board, and all patients gave informed consent.

RESULTS

The infusions of AAT were well tolerated, and there were no side-effects during the period of the study. The serum AAT concentration rose from an average baseline value of 4.4 ± 0.6 to 25.1 ± 1.5 μm ($p < 0.001$) on the day after the first infusion (Day 2), falling to a level of 10.7 ± 0.5 before the second infusion (Day 8). The nadir concentration showed a gradual rise over the next 3 weeks to 13.2 ± 0.7, 13.9 ± 0.7, and 14.4 ± 0.4, respectively. Thereafter, the level gradually fell to 8.8 ± 0.4 and 8.7 ± 1.0 2 and 3 weeks after the last infusion, returning to the prestudy baseline (4.5 ± 0.3) 5 weeks after the last infusion. The overall data are summarized in a figure and placed in the online data supplement.

The sputum concentration showed a similar profile rising from a pretreatment value of 0.17 ± 0.04 μm before the first infusion to 0.62 ± 0.12 the following day, Day 2 ($p < 0.005$). This concentration was maintained (0.58 ± 0.12) on the fifth day and fell to 0.33 ± 0.11 before the second infusion (Day 8). Again, the nadir concentration rose progressively to 0.33 ± 0.11, 0.39 ± 0.11, and 0.43 ± 0.12 after the second, third, and fourth infusion, respectively. The concentration of AAT in sputum fell over the next 3 weeks, returning to the baseline (0.14 ± 0.05) 5 weeks after the last infusion. These data are summarized in Figure 1.

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The analysis of sputum samples demonstrated that one patient was an outlier with excessive free elastase activity (4 and 7 μM) in the control period. Because this result was 40-fold greater than that for any other patient and 10-fold greater than any predicted increase in sputum AAT, it was concluded that augmentation would be unable to influence inflammation in this milieu and hence data from this patient alone were omitted from subsequent analysis.

Table 1 summarizes the data for the remaining 11 patients obtained during the control period, during treatment when AAT concentrations should be highest—namely on Day 2 (24 hours after the first infusion of AAT), Day 23 (24 hours after the fourth intravenous infusion), and Day 42 (3 weeks after the last infusion). Data that are significantly different to the control results are indicated.

The sputum AAT concentration was increased on therapy ($p < 0.005$ for Days 2 and 23), and this was associated with a

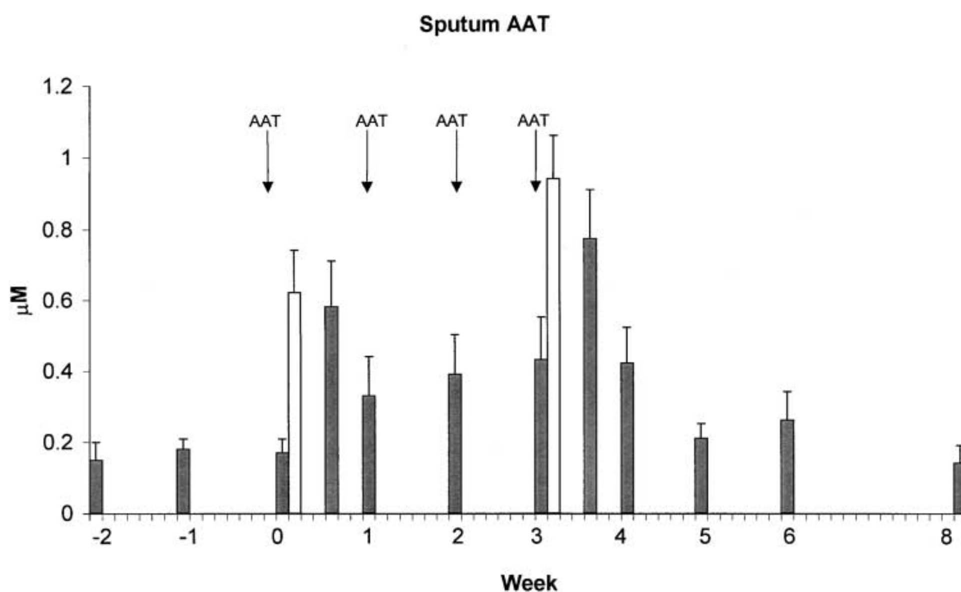

Figure 1. The average sputum AAT concentrations (*boxes* ± SEM *bar*) are shown for the 2 weeks before therapy (Weeks −2 and −1) and before infusion at Weeks 0, 1, 2, and 3, as well as during the washed-out phase. The postinfusion data for Days 2 and 23 are indicated by *open boxes*, and the AAT infusions are indicated by the *solid arrows*. The infusion increased the sputum AAT concentration above baseline ($p < 0.05$) until Week 6.

TABLE 1. MEDIAN DATA WITH TOTAL RANGES IN PARENTHESES ARE SHOWN FOR ALL SPUTUM BIOCHEMICAL MEASUREMENTS FOR THE 11 PATIENTS ANALYZED

	Control	Day 2	Day 23	Day 42
ATT, μM	0.13 (0.04–0.36)	0.57* (0.03–1.47)	0.92* (0.18–1.51)	0.18 (0.00–0.84) n = 10
PPEIC, nM	8.77 (4.2–38.4)	17.13* (5.25–187.00) n = 9	23.82* (0.02–134.76) n = 9	10.46 (4.33–35.22) n = 7
MPO, units/ml	0.69 (0.07–2.27)	0.36 (0.04–1.30)	0.37 (0.04–1.89) n = 10	0.20 (0.03–1.42) n = 10
IL-8, nM	12.02 (3.65–65.44)	9.09 (0.74–71.74)	6.47 (2.05–97.58)	14.38 (1.64–90.95) n = 10
LTB4, nM	13.46 (4.17–55.0)	5.80* (2.95–25.39)	8.62* (4.23–21.59)	9.31* (1.04–0.66) n = 10
Elastase MeSAPN, nM	1.28 (0.22–107.16)	1.03* (0–3.52)	0.58* (0–44.44)	2.81 (0–38.48) n = 10

Definition of abbreviations: AAT = α1-antitrypsin; IL-8 = interleukin-8; LTB4 = leukotriene B_4; MeSAPN = Methoxy Succinyl-ala-ala-pro-vol paranitroanilide; MPO = myeloperoxidase; PPEIC = porcine pancreatic elastase inhibitory capacity.

For data in which an insufficient sample was available from all patients, the number of patients analyzed is shown (n). Data indicated by an asterisk are significantly different from the control data (*see* text).

significant increase in PPEIC on Day 2 ($p < 0.001$) and Day 23 ($p = 0.03$). Conversely, the elastase activity detectable with the NE specific synthetic substrate, Methoxy Succinyl-ala-ala-pro-vol paranitroanilide, was reduced on Day 2 ($p < 0.02$) and Day 23 ($p < 0.002$), and these changes were associated with a small reduction in MPO activity, which failed to achieve statistical significance.

The median concentrations of IL-8 levels fell but failed to reach statistical significance on Days 2 or 23. Finally, the LTB4 concentration fell on therapy being significantly reduced on Day 2 ($p < 0.01$) and Day 23 ($p < 0.03$) but no longer different to baseline on Day 42 ($p > 0.05$). The magnitude of the fall in LTB4 between baseline and Day 23 showed a significant correlation with the initial baseline value ($r = 0.709$, $p = 0.015$).

DISCUSSION

AATD is associated with a decrease in both the serum and lung secretion concentrations of this inhibitor. This study confirms this with respect to expectorated secretions from the major airways and is consistent with our recent work comparing AATD subjects with nondeficient patients who have the same degree of airflow obstruction (4).

Infusions of Prolastin increased serum AAT, as demonstrated by others (11, 12, 14), and nadir levels rose slowly but remained, on average, greater than the proposed protective level of 11 μm. This change was matched by a rise in sputum AAT that followed the same time course, and at the end of the week after the fourth infusion, the concentration in sputum was 0.43 ± 0.10 μm, which is similar to that found in sputum from nondeficient subjects (4). Again, these data are supportive of previous studies assessing AAT concentrations in lung lavage fluids from patients before and during augmentation therapy (12).

In previous studies, the increase in AAT in bronchoalveolar lavage fluid has been mirrored by a similar increase in the elastase inhibitory capacity (12). The studies have used NE to determine elastase inhibition, as it was believed that AAT was the only inhibitor of this enzyme at alveolar level (15), although this belief has been challenged by others (16, 17). There is less controversy concerning the relevant inhibitors in the major airways where secretory leukoproteinase inhibitor is thought to be the predominant inhibitor of NE. In airway secretions, therefore, the NE inhibitory capacity measures both AAT and secretory leukoproteinase inhibitor function. On the other hand, porcine pancreatic elastase is not inhibited by secretory leukoproteinase inhibitor and has therefore been used to determine AAT function in secretions containing both inhibitors (18). This study shows a significant increase in PPEIC, which is consistent with the increase in AAT.

This observation needs further comment as the PPEIC was low compared with the total amount of AAT present (nM versus μM). In addition, NE activity was detected in most of the samples, although its activity was low. The apparent paradox of measurable porcine pancreatic elastase inhibition in the presence of detectable NE activity may be related to the method. The specific substrate used to detect elastase activity (Methoxy Succinyl-ala-ala-pro-val paranitroanilide) has a very high affinity for NE, as well as being a small chromogenic peptide. It is recognized that small peptides can detect elastase that is bound to and, therefore, effectively inhibited physiologically by α2-macroglobulin (19). Previously, studies from our group (20) have shown that sputum from AATD patients contain measurable quantities of α2-macroglobulin (approximately 0.02 μM) and probably explains why mixtures containing α2-macroglobulin fail to inhibit enzymes completely using low molecular weight substrates even in the presence of excess inhibitor (21).

The PPEIC did not equal that predicted for the amount of AAT present, and in general, this disparity is to be expected for several reasons. First, sputum is an inflamed secretion, and neutrophils are usually present together with low concentrations of released elastase (22), which can interact with and hence inactivate AAT. Indeed, previous studies have shown that approximately two-thirds of the AAT in mucoid sputum is nonfunctional even in the stable clinical state (23). Furthermore, the dose of Prolastin is administered as active and not total AAT, whereas immune assays (as used here to measure it in biologic secretions) will detect all of the antigen present. The current preparation of AAT (Prolastin), however, is approximately 90% active (data not shown), which would reduce

the expected function of the AAT relative to the absolute concentration even in serum immediately after administration (although only minimally with the current preparation). The final possibility to account for the lower AAT function observed in this study is that there is more inflammation in the larger airways where sputum is generated than in bronchoalveolar lavage fluids, which sample the lower airways and alveolar regions. Therefore, further inactivation of AAT within these sputum samples may have occurred particularly because collection of secretions occurs over a 4-hour period leaving the secretion AAT and neutrophils together. Thus, the disparity between the PPEIC and AAT concentration remains understandable, although at present unresolved. Again, unfortunately, there were insufficient samples to carry out further studies of the nature of the secretion AAT, such as Western blot analysis as described previously (16).

Previous studies of augmentation therapy have demonstrated biochemical efficacy, namely an increase in the elastase inhibitory capacity of the secretions following infusions of Prolastin. There are little data on the consequences of improving lung AAT. For instance, studies assessing elastin degradation (thought to be enhanced by AATD) have been disappointing, with no clear reduction in elastin breakdown products following augmentation (24). It is possible that the methods involved are too insensitive to detect the change expected or that elastin degradation itself may be irrelevant to the pathogenic processes in AATD. However, recent studies of the lung secretions have been more fruitful. AATD is associated with an increased number of neutrophils in lung lavage fluids (7). It is thought that this is related to chemoattraction as a result of LTB4 release from macrophages caused by a failure to inhibit elastase in the airway (7). If this theory is correct, replacing AAT should inhibit elastase *in situ* thereby leading to a reduction in LTB4 production and subsequently a reduction in neutrophil recruitment. In addition, NE has also been implicated in the production of the other neutrophil chemoattractant IL-8, with studies demonstrating that the enzyme can stimulate epithelial cells to release this chemoattractant cytokine (25).

A reduction in inflammation associated with a decrease in neutrophil chemoattractants should result in less neutrophil recruitment. This study did not quantify neutrophil numbers, again largely because of restricted sample size and the need to treat a portion of the sample with a reducing agent (which alters cytokine measurements) to isolate the cells for quantification. However, these changes were not associated with a demonstrable reduction in MPO activity, which is a marker of the neutrophil numbers and their activation (26). The failure to demonstrate a reduction in MPO activity could be related to several factors. First, a reduction in LTB4 may be insufficient to reduce neutrophil influx and activation over the relatively short period of the study. Alternatively, LTB4 may not be central to neutrophil recruitment, although recent data suggest that it is the major chemoattractant in these secretions *in vitro* (27). Finally, it is possible that a significant change in MPO activity is more difficult to detect in view of the background variability of this cytokine. Indeed, retrospective analysis indicates that it would be necessary to study between 25 and 40 patients in order that the average changes observed here would have an 80% power of achieving statistical significance. This study does, however, clearly demonstrate that the chemoattractant LTB4 (thought to be important in the inflammatory process leading to lung destruction and damage in AATD) is modified by augmentation therapy. This observation would be consistent with the hypothesis that LTB4 release (probably from airway macrophages) is related to a protease/antiprotease imbalance in the airway (7). Interestingly, the magnitude of the change was related to the baseline value, indicating the response to augmentation depends on the starting value within the range studied here, which suggests that the efficacy of AAT augmentation may be most beneficial in those with the most inflammation.

The data do not, however, support the concept that IL-8 release in the airway is the result of a protease/antiprotease imbalance. Again, it is possible to review the data in retrospect, and power calculations indicate that between 650 and 900 patients would have to be studied to confirm that the average changes seen here were significant with an 80% probability. Thus, on balance, it seems unlikely that IL-8 in sputum is a direct response to uninhibited airway NE.

The relevance of the changes reported here on the pathogenic processes in AATD needs to be taken in context. First, the biochemical changes of inflammation in the large airways are similar to those described in the peripheral alveolar region. Modification of elastase activity and LTB4 in the studies reported here indicates a mechanism that would support a potential efficacy of augmentation therapy in the alveolar inflammation and hence destruction. Of more direct importance, the results of the reduction in LTB4 release in the larger airways may be critical in the pathogenesis of exacerbations. Retrospective analysis has suggested that augmentation decreases such episodes (10), and these are also related to increased NE activity and LTB4 (8). The current data indicate a mechanism that may abrogate the effects of such episodes.

In summary, this study shows that AAT augmentation in patients with AAT deficiency restores airway concentrations of AAT to normal. This is associated with a reduction in LTB4 that is thought to be a major mediator of neutrophil recruitment and activation. The study therefore provides clearer supporting biochemical evidence than has previously been available concerning the efficacy of augmentation therapy at least in the larger airways.

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