



Lung deposition of inhaled α_1 -proteinase inhibitor in cystic fibrosis and α_1 -antitrypsin deficiency

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ABSTRACT: Individuals with α_1 -antitrypsin (AAT) deficiency and cystic fibrosis (CF) have a protease–antiprotease imbalance in their lungs, which leads to early onset progressive lung disease. Inhalation of AAT may restore protective levels in the lungs. This study aimed to determine the efficiency of delivering AAT using a novel inhalation device in subjects with AAT deficiency and CF compared with healthy subjects.

In total, 20 subjects (six healthy, seven with AAT deficiency and seven with CF) inhaled ~70 mg of radiolabelled active AAT, with controlled breathing patterns adjusted to lung function. Post-inhalation, total and regional lung deposition and extrathoracic deposition of radiolabelled AAT were measured.

Total lung deposition of AAT was ~70% of the filling dose. The magnitude of deposition was similar in all treatment groups, with no adverse effect on lung function or any influence of disease severity on total lung deposition.

Inhalation with controlled breathing patterns using the AKITA² device (lung function adapted) leads to high total lung deposition regardless of the degree of lung function impairment. Delivery of large amounts of AAT was achieved in a short period of time. This device may be an ideal option for aerosol therapy.

KEYWORDS: α_1 -Antitrypsin, α_1 -antitrypsin deficiency, controlled inhalation, cystic fibrosis, deposition, nebuliser

Cystic fibrosis (CF) and α_1 -antitrypsin (AAT) deficiency are the most frequently inherited genetic disorders in Caucasians. Approximately one in every 2,000–2,500 people born in Europe and North America is affected by these diseases [1, 2]. In patients with CF, the submucosal bronchial glands are hypertrophied, and primary viscid secretions are frequently infected. Repeated infection leads to chronic neutrophilic inflammation, which is thought to be one of the factors responsible for early lung destruction [3–5]. The most frequent pathological phenotypic mutation, PI*Z, in AAT deficiency leads to the accumulation of AAT protein in the endoplasmic reticulum of hepatocytes. This is due to dysfunctional protein export from the liver, which causes an imbalance in the protease and antiprotease levels in the lungs. This results in premature development of pulmonary emphysema and chronic obstructive pulmonary disease.

The evaluation of the lungs of AAT-deficient individuals with emphysema shows diffuse destruction of the alveoli, typically beginning in the lower lung zones and eventually throughout the entire lung [6].

Several studies have shown that the protease–antiprotease imbalance in individuals with both CF and AAT deficiency may be restored by inhalation of AAT [7, 8]. Intravenous AAT is currently licensed in several countries for augmentation therapy for AAT deficiency. However, the direct delivery of AAT to the target organ *via* nebulisation should be a more effective route of administration. The key to success for this new nebulisation route of AAT administration includes the following factors: 1) The dose that is delivered must be reproducible both inter- and intra-subject. 2) The dose deposited in the lung must be therapeutic. 3) The regional location of deposition

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within the lung must be consistent and reproducible. 4) The procedure must be able to be performed with good reproducibility in individuals with differing degrees of disease severity.

In this study, inhalation of radiolabelled AAT (Prolastin®; α_1 -proteinase inhibitor (human); Talecris Biotherapeutics Inc., Research Triangle Park, NC, USA) with a new inhalation device, the AKITA² APIXNEB® (Activaero GmbH, Gemünden, Germany), was investigated in three populations: healthy subjects, subjects with AAT deficiency and subjects with CF. The AKITA² APIXNEB combines a vibrating mesh nebuliser (PARI GmbH, Starnberg, Germany) with low drug residual volumes and the ability to control inhalations [9–11], such that both inhalation flow rate and inhaled volume are controlled by a computerised compressor. This inhalation device has been specially designed for the efficient inhalation of substances such as AAT, with the breathing pattern being normalised to the subject's lung function in order to obtain individually optimised inhalation parameters.

MATERIALS AND METHODS

Subjects

In total, 20 subjects (six healthy subjects, seven subjects with AAT deficiency and seven subjects with CF) participated in this study (table 1). Subjects were male or female and aged between 18–65 yrs with no clinically significant or uncontrolled cardiac, hepatic, renal, gastrointestinal, endocrine, metabolic, neurological or psychiatric disorder. All were nonsmokers or required to have been ex-smokers for at least 2 yrs prior to enrolment in the study. Subjects with AAT deficiency were included if they had a PiZZ, PiZ(Null), Pi(Null, Null) or PiSZ phenotype confirmed by isoelectric focusing or genotyping, and the presence of emphysema confirmed by chest radiograph or computed tomography scan. Two AAT-deficient subjects received long-term oxygen therapy prior to study entry. Subjects with CF were included if the diagnosis was established by a sweat or genetic test.

TABLE 1 Baseline characteristics and lung function parameters of the study population

Parameter	Healthy	AAT deficiency	CF
Subjects n	6	7	7
Female/male n	5/1	3/4	3/4
Age yrs	33.3±12.1	51.7±13.2	28.6±6.1
Height cm	168±6.8	169±10.7	171.3±8.7
Weight kg	70.2±16	68±16.4	60.6±10
FEV ₁ L	3.45±0.6	1.58±0.8	2.38±0.9
FEV ₁ % pred	106±14	51±15	62±15
FIV ₁ L	4.1±0.9	3.92±1.2	3.56±1.1
FVC L	4.32±0.8	3.68±1.4	3.65±1.0
FVC % pred	115±15	98±17	81±10
RV L	1.63±0.2	3.85±1.3	2.92±0.8
RV % pred	103±11	193±55	186±55

Data are presented as mean±SD, unless otherwise stated. AAT: α_1 -antitrypsin; CF: cystic fibrosis; FEV₁: forced expiratory volume in 1 s; % pred: % predicted; FIV₁: forced inspiratory volume in 1 s; FVC: forced vital capacity; RV: residual volume.

AAT deficiency and CF patients must have had an inspiratory capacity of ≥ 0.77 L and a forced expiratory volume in 1 s (FEV₁) between 25–80% predicted. AAT or CF subjects were excluded if they had a history of lung transplant, pulmonary surgery within the past 2 yrs, any pulmonary infection/exacerbation in the last month or were on any thoracic surgery waiting list. All subjects were excluded from the study if they: were pregnant or lactating; were not using reliable contraceptive methods; had a history of anaphylaxis to AAT or blood products; had known immunoglobulin A deficiency; had a history of transfusion reactions; had an infection in the last 2 months; had a history of drug abuse in the last 12 months; had participated in another investigational drug study in the last month; or were unable to provide independent informed consent. Written informed consent was obtained from each subject. The protocol was approved by the ethics committee of the Bayerische Landesärztekammer (Munich, Germany), the German Competent Drug Authority (Paul-Ehrlich-Institut, Langen, Germany) and the German Federal Office for Radiation Protection (Bundesamt für Strahlenschutz, Salzgitter, Germany).

Radiolabelling of AAT

The labelling of AAT was similar to the technique described by LAFONT *et al.* [12]. On each study day a vial of AAT was reconstituted according to the manufacturer's instructions using the kit provided by the manufacturer. All AAT used in this study was from the same batch. 0.5 mL of technetium pertechnetate (160 megabecquerel (MBq)) was incubated with 1.5 mL of AAT for at least 30 mins. Following this, 0.5 mL (<19 MBq) of radiolabelled AAT was mixed with 9.5 mL unlabelled AAT.

The specific activity of the diluted radioactive AAT solution was measured by gamma scintillation. Validation of the labelling process was performed by comparing the particle size distribution of AAT within the unlabelled commercial product with the size distribution of the drug in the radiolabelled product and the size distribution of the radiolabel (see figure A in the supplementary data). These particle size distributions were measured using a Next Generation Impactor (Copley Scientific Ltd, Nottingham, UK) with a flow of 15 L·min⁻¹ for all three particle size distributions; identical mass median aerodynamic diameters (4.0 μ m) were also measured and the geometric SD was 1.6. The fine particle fractions were found to be between 67–70% (see table A in the supplementary data). The stability of the radiolabel/protein complex was tested in *in vitro* leaching tests using a dialysis tube. Within 5 h, <10% of the radiolabel was dissociated from the protein. The radiolabelled drug was administered to all subjects within 3 h after reconstitution. Thus, the radiolabel was considered to be stable between the time of reconstitution of the drug and the end of the radiolabel measurements.

Inhalation procedure

Inhalation of AAT was performed using the AKITA² APIXNEB device. This device consists of a nebuliser handset (APIXNEB) that uses vibrating mesh technology (Touchspray; PARI GmbH) and an electronic unit (AKITA² APIXNEB) that controls and supplies air to the individual. Using this device, individually adapted breathing patterns were performed, in

which the airflow rate and inhalation volume (IV) are controlled for each subject.

In this study, the flow rate was set at $0.25 \text{ L}\cdot\text{s}^{-1}$. The IV was normalised to the subject's inspiration capacity (see figure B in the supplementary data). In every case the total IV was a maximum of 65% of the inspiration capacity. There are no data about the inhalation time with the AKITA² APIXNEB device and AAT, but from *in vitro* data it can be assumed that inhalation of the study medication takes ~10 min in healthy subjects.

The pooled radiolabelled/nonradiolabelled AAT was held in a glass vial and 2 mL was transferred into the medication chamber with a pipette immediately before inhalation. Inhalation was performed until the nebuliser automatically determined the inhaler to be totally depleted.

The total applied dose of labelled and unlabeled protein consisted of ~100 mg of total protein containing 70 mg active AAT in 2 mL solution.

Assessment of lung deposition

Gamma camera images of the lung were taken using a Siemens Diacam gamma camera (Siemens, Erlangen, Germany) with a 40-cm field of view and a low-energy parallel-hole collimator 1 min after complete inhalation of the test drug. Radioactivity was assessed for the lung region (AL) and the extrathoracic region (AET), including the oropharynx, trachea, oesophagus and stomach. The amount of radioactivity from each filled nebuliser (AO), exhalation filter (AEx) and empty inhaler (Ai) was measured by scintillation counter (Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany). From these activity data, the following parameters were calculated.

Total lung deposition relative to filled activity (DL):

$$DL = (1 - Ai/AO - AEx/AO) \times (AL/(AL + AET)) \times 100$$

Extrathoracic deposition relative to filled activity (DE):

$$DE = (1 - Ai/AO - AEx/AO) \times (AET/(AL + AET)) \times 100$$

Residues in the device (MD) (relative):

$$MD = Ai/AO \times 100$$

Exhaled drug (ME) (relative):

$$ME = AEx/AO \times 100$$

The determination of central and peripheral lung regions, the ratio of central to peripheral (C/P) counts of deposited activity and whole-lung rectangular regions of interest (ROI) for each lung were drawn at the boundaries of the krypton (Kr) ventilation scan (the boundaries were defined at 15% of the peak Kr counts for the entire lung) (fig. 1). Central ROI, with dimensions equal to half the whole lung ROI width and one half its height, were positioned on the interior boundary of the lung, centred by height; the central ROI was 25% of the area of the whole lung ROI. The peripheral region was the area lying between the central and whole lung outline. These regions were displayed over the aerosol deposition (^{99m}Tc) and Kr scan to determine the counts in each region A_{central} and $A_{\text{peripheral}}$.

Central and peripheral depositions were calculated similar to total lung deposition:

$$D_{\text{central}} = (1 - Ai/AO - AEx/AO) \times (A_{\text{central}}/(AL + AET)) \times 100$$

$$D_{\text{peripheral}} = (1 - Ai/AO - AEx/AO) \times (A_{\text{peripheral}}/(AL + AET)) \times 100$$

The C/P (deposition) ratio of the drug was determined and normalised (divided) by the C/P ratio for the Kr scan. This normalisation was calculated to account for the difference in relative lung areas and thickness between the central and peripheral regions [13]. While both the central and peripheral regions overlay alveoli and intermediate/small airways, the central region also incorporates large bronchial airways not present in the peripheral region. Thus, increases in the C/P ratio reflect an increase in large bronchial airway deposition relative to intermediate/small bronchi/bronchioles and alveolar airspaces. Hence, a difference in the distribution of drug deposition within the lung, *i.e.* the ratio of deposition in the large and small airways, can be assessed but does not necessarily reflect absolute amounts of drug in a certain anatomical region.

Tissue attenuation correction was calculated using the subject's sagittal thorax diameter and the equation given by PITCAIRN *et al.* [14]. In order to assess the lung contours for the assessment of total lung deposition with the gamma camera, an ^{81m}Kr gas ventilation scan was performed for each subject.

Lung function measurement

Lung function tests (bodyplethysmography and spirometry) were performed using a commercial device (Cardinal Health GmbH, Hoechberg, Germany). The following parameters were recorded: forced vital capacity, FEV₁, forced inspiratory volume in 1 s and residual volume. Measured lung function parameters were normalised to the reference values as described by the European Community for Steel and Coal [15].

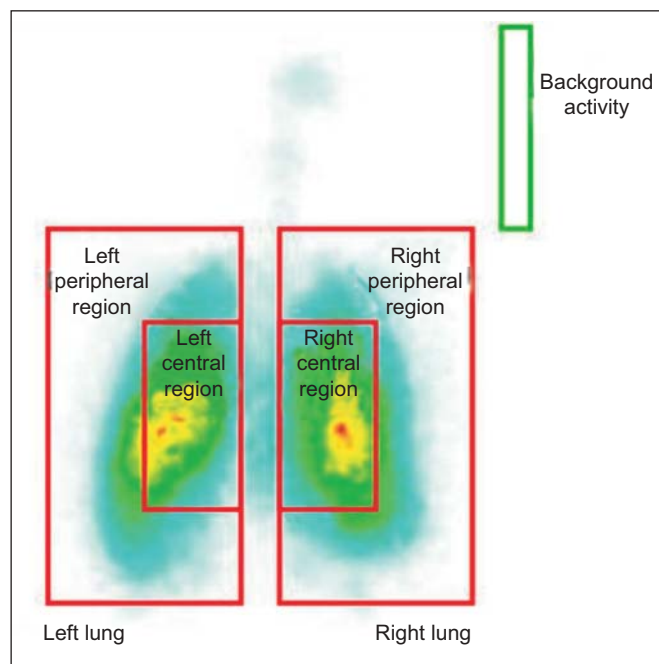


FIGURE 1. Determination of central and peripheral regions.

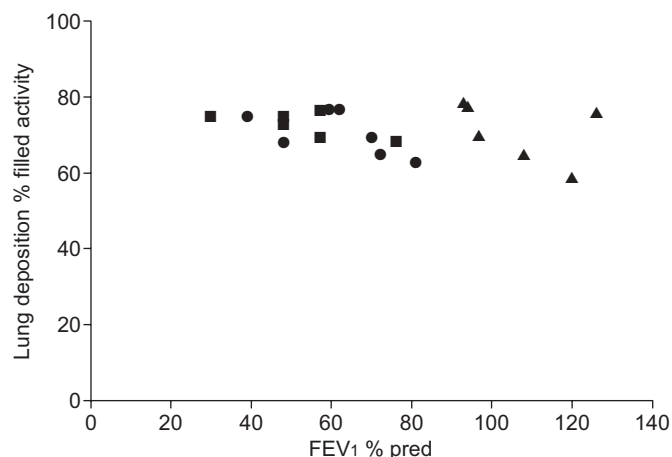


FIGURE 2. Lung deposition in relation to forced expiratory volume in 1 s (FEV₁) % predicted (% pred) for each individual subject and for each subject group. The total lung deposition exceeded 60% of the filled activity in all individuals regardless of their lung disease and lung function impairment. ■: α_1 -antitrypsin deficiency; ●: cystic fibrosis; ▲: healthy.

Statistics

Data are presented as mean \pm SD. Statistical analysis was performed using SAS software (version 9.1.3; SAS Institute, Inc., Cary, NC, USA). Deposition values in each subpopulation were summarised with descriptive methods. Differences in group averages were tested for statistical significance by calculating ANOVA (SAS Proc GLM (SAS Institute, Inc.) with the group index as an independent variable).

RESULTS

Baseline characteristics and lung function data of the study population are shown in table 1. Subjects with AAT were older than the healthy subjects and those with CF, and subjects with CF weighed less than the subjects in other groups. Healthy subjects and patients differed considerably in FEV₁% pred but the mean values in AAT-deficient and CF patients were similar ($106 \pm 14\%$, $51 \pm 15\%$ and $62 \pm 15\%$ in healthy subjects, subjects with AAT and subjects with CF, respectively). All other baseline lung function parameters measured were similar among these three groups.

A graph of the lung deposition of AAT, relative to the FEV₁% pred, for each individual subject and for each of the three subject groups is shown in figure 2. In one subject it was not possible to assess deposition parameters owing to an inhalation device failure. Total mean lung deposition of AAT in all three subject groups was between 70–73% of the amount filled into the nebuliser ($70.3 \pm 7.9\%$, $72.6 \pm 3.2\%$ and $70.6 \pm 5.8\%$ in healthy, AAT-deficient and CF subjects, respectively) (fig. 3). Peripheral deposition was $40.9 \pm 4.5\%$ of filled activity in healthy subjects, $42.3 \pm 6.6\%$ in subjects with AAT deficiency and $43.3 \pm 5.3\%$ in subjects with CF. Central deposition was $29.4 \pm 4.8\%$ of the filled activity in healthy subjects, $30.3 \pm 4.3\%$ in subjects with AAT deficiency and $27.3 \pm 4.7\%$ in subjects with CF (fig. 3). As shown in table 2, peripheral deposition was considerably higher than central deposition in all subject groups, and among groups the peripheral deposition remained similar. The C/P ratios were 1.48 ± 0.19 in healthy subjects,

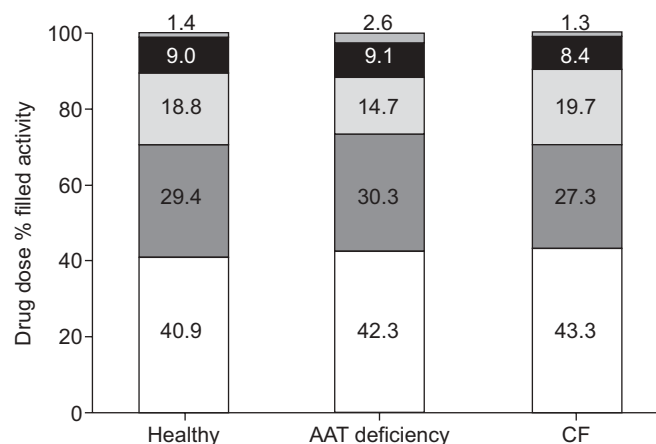


FIGURE 3. Average values of the drug doses in the peripheral lung (□), central lung (■), extrathoracic (▒), the residuals in the device (■) and the residuals exhaled (■) for the three study populations. Owing to the limited measuring precision of the deposition measurement, individual deposition values were rounded to one decimal place. Therefore, the mean deposition values in the figure do not add up to 100% for each group. AAT: α_1 -antitrypsin; CF: cystic fibrosis.

1.66 ± 0.46 in subjects with AAT deficiency and 1.37 ± 0.24 in subjects with CF (no statistically significant differences).

Extrathoracic deposition was between 15% and 20%: $18.8 \pm 6.8\%$ in healthy subjects, $14.7 \pm 3.1\%$ in AAT-deficient subjects and $19.7 \pm 4.9\%$ in subjects with CF (fig. 3). Again, there were no statistically significant differences among treatment groups. The amount of MD was $\sim 9\%$ ($9.0 \pm 1.6\%$, $9.1 \pm 1.3\%$ and $8.4 \pm 2.8\%$ in healthy subjects, subjects with AAT deficiency and subjects with CF, respectively) (fig. 3), amounting to $<200 \mu\text{L}$. No significant differences were observed among subject groups. The amount of ME was $1.4 \pm 0.7\%$ in healthy subjects, $2.6 \pm 1.7\%$ in subjects with AAT deficiency and $1.3 \pm 0.2\%$ in subjects with CF (fig. 3). The difference between subjects with CF and subjects with AAT deficiency was statistically significant ($p=0.046$). On average, the inhalation time was $6.9 \pm 3 \text{ min}$ for healthy subjects, $13 \pm 10 \text{ min}$ for subjects with AAT deficiency and $8.3 \pm 3.6 \text{ min}$ for subjects with CF; however, these differences were not statistically significant.

A comparison of ventilation and inhalation scans of all individuals with AAT deficiency demonstrated that the scans

TABLE 2 Regional deposition of inhaled α_1 -antitrypsin (AAT)

	Healthy	AAT deficiency	CF	p-value
Central deposition %	29.4 ± 4.8	30.3 ± 4.3	27.3 ± 4.7	NS
Peripheral deposition %	40.9 ± 4.5	42.3 ± 6.6	43.3 ± 5.3	NS
C/P ratio	1.48 ± 0.19	1.66 ± 0.46	1.37 ± 0.24	NS

Data are presented as mean \pm SD. CF: cystic fibrosis; C/P: central to peripheral; NS: nonsignificant.

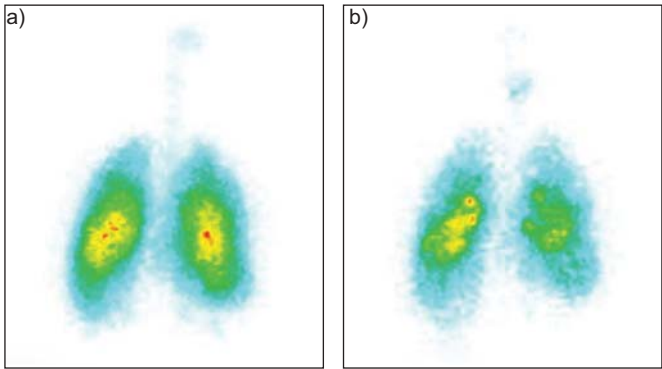


FIGURE 4. An example of a ventilation scan and drug deposition in a subject with α_1 -antitrypsin deficiency. a) IDO19 krypton, b) IDO19 drug.

were well matched, with very little extrathoracic and central deposition. Figure 4 shows an example of scans from an AAT-deficient subject. The complete set of scans for all 19 subjects for which a deposition assessment was available is included in the supplementary data.

All subjects were able to complete the inhalation of AAT, including two AAT-deficient subjects receiving long-term oxygen therapy. However, because the inhalation procedure had to be interrupted several times, inhalation in these two subjects took longer than for the other AAT-deficient subjects (between 20–30 min; fig. 5), which contributed to the longer mean inhalation time in patients with AAT deficiency. Table 3 lists all the adverse events. Only two subjects experienced adverse events for which a relationship to the study medication or the inhalation effort could not be excluded (headache in one subject with CF and tongue vesicles/dysphagia in one healthy subject).

DISCUSSION

The concept of controlled inhalation was developed after it was shown that the breathing pattern is one of the main determinants of good drug deposition with nebuliser systems [16]. In earlier studies it has been shown that the use of controlled and optimised breathing patterns (slow and deep inhalation [9, 16]) not only increases total drug deposition but also reduces its variability and reduces differences in deposition between healthy individuals and those with lung diseases. The AKITA device was developed to perform controlled inhalations in practice. This device included a modified PARI LC Star nebuliser (PARI GmbH). In the nebuliser cross-over

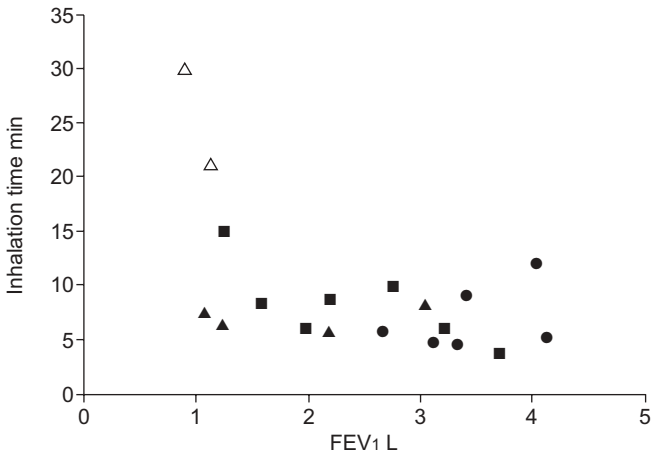


FIGURE 5. Relationship between forced expiratory volume in 1 s (FEV₁) and the inhalation time. ●: healthy; ■: cystic fibrosis; ▲: α_1 -antitrypsin deficiency; △: α_1 -antitrypsin deficiency with oxygen therapy.

study by BRAND *et al.* [10], use of this device for the inhalation of AAT in subjects with AAT deficiency has shown that peripheral deposition of AAT was highest for the AKITA device. Peripheral deposition was ~30–35% for the PARI LC Star device and HaloLite (Medic Aid, Bognor Regis, UK) device, and was 50–60% for the AKITA device; thus showing that it was possible that a high drug concentration could be effectively deposited in a target region of interest, specifically the peripheral lungs. However, in this same study, deposition was expressed as a fraction of the inhaled drug amount so that drug loss in the nebuliser system, which can be significant, was not determined [10].

The current study showed that ~70% of the AAT from the AKITA² APIXNEB inhalation system was deposited directly into the lungs. There were no significant differences in mean drug deposition among the three populations studied (healthy subjects, subjects with AAT deficiency and subjects with CF); however, it should be noted that the fractionation of the lung dose into a single central region and a single peripheral region in two dimensions is a very crude method to determine the anatomical site of deposition. A large proportion of the drug (~40%) was deposited in the peripheral lung regions. Since the central region (defined by the ROI specified in this study) contains a large number of alveolar airspaces, which was not attributed to the peripheral deposition count, it would underestimate peripheral deposition. In actuality, the peripheral

TABLE 3 Adverse events reported by all subjects			
Subject	Adverse event	Treatment group	Relationship to study drug
1	Tongue vesicles and dysphagia	Healthy	Possible
2	Mild headache	Cystic fibrosis	Possible
4	Cold symptoms	Cystic fibrosis	Unlikely
11	Diarrhoea and dizziness	Cystic fibrosis	Unlikely
12	Cold symptoms	Cystic fibrosis	Unlikely
17	Gastroenteritis	Cystic fibrosis	Unlikely

deposition should be considerably higher than 40%. Mean extrathoracic deposition was <20% suggesting a very efficient delivery of drug into the target organ (lung). Another practical consideration affecting compliance was that the average inhalation times for the different populations tested were extremely short, ~7–13 min to deliver ~100 mg of the AAT protein directly into the subjects' lungs. This short inhalation time will be of critical importance for patient acceptability and compliance. In addition, the inhalation of AAT in this short timeframe was well tolerated with few adverse events.

It was also shown that lung deposition can be converged in different populations by individualising the inhalation manoeuvre in inhalation volume and flow. Lung deposition of a given drug with a defined particle size can vary by a factor of 5 to 10 for any given patient just by changing the breathing pattern. Three components of the programming of the AKITA² APIXNEB reduce this variability. First, lowering the inspiratory flow to very low values reduces turbulent flow and thereby early impaction of particles in extrathoracic or central airways [16]. Secondly, flushing the airways with clean air after a bolus delivery of the drug minimises the amount of drug either centrally deposited or exhaled and increases peripheral deposition. Thirdly, adjusting the inspiratory volume and the length of the drug bolus inhalation to the individual lung function of each patient further optimises the total and peripheral lung deposition.

AKITA² APIXNEB was able to achieve a high delivered dose and minimise drug delivery loss by its optimised airflow geometry and high output rate, utilising its vibrating mesh technology. This device was optimised for nebulisation of substances like AAT [9, 10]. The results from the first *in vivo* performance data of this device has shown that both high lung deposition values with only minor drug losses within the nebuliser were achieved.

This study has shown that not only healthy individuals but also those with severe lung disease, *i.e.* individuals with AAT deficiency and individuals with CF, were able to perform the required breathing pattern. Even two AAT-deficient subjects with long-term chronic oxygen therapy were able to complete the inhalation, although the inhalation took longer in these two individuals than for other subjects since they had to interrupt the procedure several times.

In the present study there were no statistically significant differences in deposition between healthy subjects and those with AAT deficiency or CF. However, it should be kept in mind that, owing to the low number of subjects in each group, the statistical power of the study is relatively low. Using the data obtained in the study, it was retrospectively calculated that only differences in lung deposition of >13% (absolute deposition value) could be detected with a power of 80%. Therefore, the possibility that smaller differences were present but did not reach statistical significance cannot be excluded.

Several previous studies have investigated aerosol treatment with AAT, both in patients with AAT deficiency and in those with CF, in an attempt to restore the protease–antiprotease imbalance. It has been shown that aerosolised AAT can be deposited in the periphery of the lung and retains antiprotease activity [17]. Active AAT with an associated increase in

anti-elastase activity has also been shown after aerosolisation in individuals with AAT deficiency [8] and CF [5, 18]. However, so far aerosol treatment with AAT for either indication has not yet been approved for clinical use.

This study showed that use of the AKITA² APIXNEB inhalation system to inhale AAT (Prolastin®) was well tolerated and led to excellent lung deposition of AAT of approximately ≥70% of the drug amount loaded into the nebuliser. These results were obtained by controlling the breathing pattern of the subjects and by individualising inhaled volume based on the lung function of the subjects while minimising left-over medication in the nebuliser. The inhalation time was judged to be short and convenient by the majority of subjects (data not shown). All subjects, even those receiving long-term oxygen therapy, were able to perform the required breathing patterns. This new technology should improve inhalation delivery of AAT in individuals with AAT deficiency as well as in those with CF.

CLINICAL TRIAL

This study, carried out between July 1 and September 30, 2006, was registered with the European Union Drug Regulatory Authorities (EUDRA): clinical trial number 2005-003190-24.

SUPPORT STATEMENT

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A poster based on this study was presented at the 2007 European Respiratory Society Annual Congress, the 2008 International Congress of the American Thoracic Society and at the 2008 Canadian Respiratory Conference. The abstract has been published in the corresponding publications.

STATEMENT OF INTEREST

Statements of interest for all authors and the study itself can be found at www.erj.ersjournals.com/misc/statements.dtl

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