Inhalation of [^{123}I]α_1-Protease Inhibitor: Toward a New Therapeutic Concept of α_1-Protease Inhibitor Deficiency?

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The α_1-protease inhibitor (α_1-Pi) is separated from human serum and is therefore extremely expensive. Because only 2%–3% concentrates in the lung after intravenous administration, inhalational therapy for α_1-Pi deficiency would seem likely to be better. The aims of this study were therefore to determine the pattern of deposition of inhaled α_1-Pi labeled with ^{123}I and measure the amount deposited in the lungs. **Methods:** Eighteen patients with congenital severe α_1-Pi deficiency were enrolled in the study. The low-specific-activity ^{123}I-labeled α_1-Pi aerosol (median particle size ± SD, 3.9 ± 2.5 μm) was generated by an air pressure–driven nebulizer. The patients inhaled for an average of 23.6 ± 8.9 min. Static scintigrams in two projections were acquired immediately after (T_1) and 1 (T_2), 4 (T_3), and 24 h (T_4) after inhalation. The patients were divided into the following three groups according to their forced expiratory volume in 1 s (FEV_1): group I, ≤40% of predicted normal (n = 8); group II, 40% < FEV_1 ≤ 60% of predicted normal (n = 4); group III, >60% of predicted normal (n = 6). **Results:** The absolute percentage uptake values of α_1-Pi in group I were 12.4 for T_1, 7.3 for T_2, 4.6 for T_3, and 1.2 for T_4; in group II the values were 13.0, 9.6, 6.2, and 2.0, respectively; and in group III, 14.6, 11.4, 6.5, and 3.6, respectively. Differences between the groups were generally statistically significant. Between T_1 and T_2, the probability value was <0.05 for group I versus group II, <0.006 for group I versus group III, and <0.39 for group II versus group III. Between T_1 and T_3, the probability value was <0.29 for group I versus group II, <0.22 for group I versus group III, and <0.94 for group II versus group III. Retention (between T_1 and T_4) was also dependent on the grade of the disease: $P < 0.2$ for group I versus group II, $P < 0.001$ for group I versus group III, and $P < 0.02$ for group II versus group III. Grading of the uptake pattern by three independent experienced investigators (87% agreement) revealed a peripheral deposition that was group dependent. We found that greater peripheral deposition corresponded with lower lung functional impairment: $P < 0.5$ for group I versus group II, $P < 0.01$ for group I versus group III, and $P < 0.08$ for group II versus group III. Degradation also corresponded with functional impairment: $P < 0.05$ for group I versus group II, $P < 0.006$ for group I versus group III, and $P < 0.3$ for group II versus group III. **Conclusion:** The results of this study show that sufficient amounts of α_1-Pi can be deposited in the periphery of the lung by inhalation at least in patients with low-grade disease. Inhalation of α_1-Pi may thus represent a new and more convenient route of drug administration.

Key Words: α_1-protease inhibitor; antitrypsin; Prolastin; inhalation therapy; ^{123}I-α_1-Pi

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The α_1-protease inhibitor (α_1-Pi, or α_1-antitrypsin; molecular weight, 52 kDa) is the most important inhibitor of the neutrophil elastase, a broad-spectrum protease that can degrade structural proteins composing the tissue matrix (*1*,*2*). α_1-Pi protects the lung from these harmful effects of protease stress (*3*). α_1-Pi deficiency (normal serum level, 1.5–2.0 g/L) is an autosomal codominant hereditary disease based on more than 90 known deficient alleles of the α_1-Pi gene on chromosome 14 (*4*). The genetic heterogeneity with 33 variants of the molecule is remarkable, resulting in as many phenotypes. The most frequently observed form is the PiZ glycoprotein, predominantly produced in hepatocytes, in which substitution of Glu^{342} by Lys^{342} (*5*) results in a structural change. The PiZ then accumulates in the cells, and a low α_1-Pi serum level is observed (*6*). In phenotypes with a severe serum deficiency of α_1-Pi, an imbalance between proteases and antiproteases arises and leads to lung emphysema (*7*), mainly in the fourth and fifth decades of life. Therapy for these patients consists of prevention and avoidance of irritation of the lung, bronchodilatation, physiotherapy, and substitution of the missing protein to prevent degradation of lung parenchyma by surplus proteases (*8*). Apart from liver transplantation, three routes of application of α_1-Pi are theoretically available. The well-established intravenous substitution of α_1-Pi increases plasma α_1-Pi to an assumed protective level in heterozygote individuals (*9*–*11*). Inhalation of aerosolized α_1-Pi has also been used to increase local α_1-Pi levels in the lung (*12*,*13*). The admin-

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istration of α_1-Pi–producing cell lines using genetic engineering is still an experimental procedure to increase local or systemic production of α_1-Pi (*14*).

Intravenous substitution has been evaluated in several large studies (*15–17*). However, the amount of α_1-Pi entering the lung and the epithelial lining fluid (ELF) is approximately only 2%–3% (*18*). To increase this value, inhalation of aerosolized α_1-Pi would be an attractive alternative. Animal studies showed increased amounts of active α_1-Pi in the ELF as well as in lymphatic fluid of the lung after inhalation, indicating an improvement in the local antiprotease shield and the antielastase capacity in the interstitium (*12,19*). Increased α_1-Pi in the ELF of humans after inhalation was confirmed in healthy volunteers (*20*) and patients with α_1-Pi deficiency (*12*). However, these studies did not address the influence of the grade of functional impairment.

The amount of peripherally deposited aerosol is influenced by its physical properties (e.g., droplet size) and by the anatomy and function of the airways. Patients with severe α_1-Pi deficiency frequently have severe, mostly irreversible airway obstruction and chronic bronchitis. The influence of these factors on aerosol deposition is not predictable and needs to be determined. In addition, clearance of the deposited particles, both in the airways and in the alveoli, may be abnormal in patients with severe α_1-Pi deficiency.

The goals of our study were to measure the pattern and amount of aerosolized ^{123}I-labeled α_1-Pi in the lungs, to measure the dependence on lung function, to estimate the clearance rates of the involved compartments, and to estimate the half-life of peripherally deposited α_1-Pi. These measurements can be achieved with an easy-to-use nebulizer that the patients themselves can handle.

MATERIALS AND METHODS

Radiolabeling of α_1-Pi

The sterile lyophilized substrate α_1-Pi (Bayer AG, Leverkusen, Germany) was 95% pure. All other chemicals were of standard purity. Approximately 100 μL ^{123}I (750 MBq [20 mCi]) dissolved in NaOH were combined with 50 μL 0.1N phosphate buffer, pH 7.5. To this reaction mixture, 1 mg (16.6 nmol) α_1-Pi dissolved in the same phosphate buffer but containing 2 mol uric acid was added. The radioiodination reaction was initiated by an additional 10 μL (0.22 μmol) chloramine-T solution. The reaction was allowed to continue for 2 min while the reaction vessel was gently shaken. The reaction was stopped by adding 10 μL (2 μmol) sodium-metabisulfite solution. The radiolabeled compound was then purified by a Sephadex G-25 M PD-10 column (Pharmacia, Uppsala, Sweden) equilibrated with phosphate-buffered saline (PBS) buffer. The column was washed before the purification procedure with 50 mL PBS buffer. After the compound was applied, the column was eluted with PBS buffer and 0.5-mL fractions were collected.

Radiochemical Stability of ^{123}I-α_1-Pi

The chemical purity and stability of the radiolabeled compound was tested by an isocratic high-performance liquid chromatography (HPLC) method (*21*), which was performed on a 300 × 7.8 mm Bio-Sil SEC-250 column (Bio-Rad, Hercules, CA) eluted with a 0.05 mol/L sodium phosphate solution at a pH of 7.0 in 0.15 mol/L sodium chloride and 0.05% sodium azide. The flow rate was 0.5 mL/min, and the chromatogram was monitored at 280 nm with an L-4250 ultraviolet (UV) monitor (Merck, Whitehouse Station, NJ). The peak was measured along with molecular weight standards (Sigma Chemical, St. Louis, MO). HPLC analysis was performed 1 and 30 h after radiolabeling. During this period, the radiolabeled compound was stored in the original PBS solution.

Biologic Activity

The biologic activity of the ^{123}I-α_1-Pi was evaluated by a Nor-Partigen α_1-antitrypsin radial immunodiffusion plate (Behring, Marburg, Germany). A protein standard plasma was applied into three wells with various concentrations of α_1-antitrypsin; a control serum was placed into a single well, and into eight others our radiopharmaceutical was placed with various concentrations of α_1-antitrypsin. The plate was developed over 24 h and evaluated. In addition, the plate was placed on a small-field-of-view gamma camera (CX 250; Picker, Cleveland, OH) and a static scintigram was acquired with a total of 500,000 counts.

Study Protocol

Patients with severe α_1-Pi deficiency (plasma levels < 0.8 g/L) and older than 20 y were eligible to enter the study. Patients excluded from the study were those with an infectious disease of the lungs, a known allergy against proteins, or congestive grade III or IV heart failure according to the criteria of the New York Heart Association. Premenopausal female patients without definite sterilization were also excluded.

Patients underwent a detailed medical examination at the beginning and end of the study. Whole-body plethysmography and blood and urine tests were performed on days one and three. The inhalation of ^{123}I-labeled α_1-Pi and measurements of radioactivity were performed on days two and three. Blood samples were drawn 1, 4, and 24 h after inhalation, and urine was collected over a 24-h period. The patients used a Wright peak flow meter (Clement Clarke International, Essex, U.K.) during the study to monitor changes in lung function caused by the inhalation procedure.

The study protocol followed the declaration of Helsinki and was approved by the in-house ethical committee. All patients gave written informed consent.

Patient Characteristics

Eighteen patients (15 men, 3 women) with α_1-Pi deficiency (phenotype PiZZ) were included in the study. They were grouped according to their initial forced expiratory volume in 1 s (FEV_1): group I, ≤40% of predicted normal; group II, 40% $< FEV_1 \leq$ 60% of predicted normal; group III, >60% of predicted normal. Fifteen patients received weekly intravenous therapy with 60 mg/kg of body weight human α_1-Pi (Prolastin HS; Bayer). The patient data are summarized in Table 1.

Inhalation

The particle size was determined by repeated measurement of the aerosol in a 12-stage cascade impactor. The median mass aerodynamic diameter was 3.9 fm, with a geometric SD of 2.5. The fraction of particles < 5 μm was 61%, and the fraction < 3 μm was 39%. The influence of different air flow rates (10, 20, and 30 L/min) on median mass aerodynamic diameter was negligible.

Before inhalation began, a calibration factor between the NaI autogamma counter (Berthold, Wildbad, Germany), the dose cal-

TABLE 1
Demographic Data of 18 Patients with Severe α_1-Pi Deficiency Receiving Aerosolized Radiolabeled [^{123}I]α_1-Pi

Parameter	All	Group I	Group II	Group III
n	18	8	4	6
M/F	15/3	7/1	4/0	4/2
Age (y)	49.8 ± 8.4	51.8 ± 9.9	44.0 ± 7.4	51.0 ± 5.7
Weight (kg)	76.5 ± 11.8	75.6 ± 14.6	78.8 ± 7.0	76.2 ± 11.8
Height (cm)	177.1 ± 9.6	176.6 ± 9.0	180.3 ± 8.1	175.5 ± 12.2
Broca index	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.2
FEV_1 (L/s)	1.8 ± 0.8	1.1 ± 0.2	2.1 ± 0.3	2.7 ± 0.8
FEV_1 % predicted (%)	50.1 ± 23.8	29.7 ± 5.2	52.2 ± 4.5	81.0 ± 15.4

ibrator (Messelektronik Dresden, Dresden, Germany), and the gamma camera was determined. With this calibration and with phantom measurements mimicking the attenuation of the lungs, we could calibrate the number of counts in the scintigrams to the number of megabecquerels of ^{123}I-α_1-Pi deposited. The net amount in the air pressure–driven nebulizer (Master LL; Pari, Starnberg, Germany) was 100 mg α_1-Pi combined with 200 ± 26.2 μg ^{123}I-α_1-Pi containing 252 ± 98.2 MBq (6.7 ± 2.62 mCi) ^{123}I.

Inhalation was performed with the patients sitting upright with the nose blocked. The average time for inhalation was 23.6 ± 8.9 min (range, 12–45 min). At the end of the inhalation, the remaining radioactivity in the nebulizer and expiration filter was determined.

Evaluation of Deposition

Static scintigraphy was performed using a large-field-of-view gamma camera (Prism 2000; Picker) equipped with a high-resolution collimator; ventral and dorsal views were acquired into a 128 × 128 matrix up to 500 kcts. Scans were obtained before and immediately after inhalation (T_1) and after 1 (T_2), 4 (T_3), and 24 h (T_4). Because of low counting rates, T_4 images could not be obtained for 2 patients of group I. Mean values of T_4 images were calculated for 16 patients in the whole group and for 6 patients of group I. The geometric means of the counts were used for further analyses.

Two experienced nuclear medicine physicians and one pneumologist analyzed the pattern of deposition using the 24-h distribution. Interference from swallowed radioactivity in the esophagus and stomach was avoided by analyzing only the right lung. Regions of interest (ROI) were marked with the agreement of all three investigators. Reproducibility was tested by masking the data of each patient and evaluating the data twice.

One ROI covered the whole right lung, whereas a second region comprised exclusively central activity, that is, activity in the trachea and main bronchi. The ratio of central counts to total counts is inversely related to the amount of peripheral deposition (*22*). All values for ^{123}I were half-life corrected, as were the T_1 values for the fraction of free ^{123}I measured in the HPLC analyses.

Mathematic and Statistical Methods

Analyses were performed with the SAS 6.08 statistical package (SAS Institute, Cary, NC). The influence of lung function impairment was addressed by grouping the patients according to their initial FEV_1. This group definition was used as a fixed factor in the statistical models. For some specific analyses, the percentage of predicted initial FEV_1 was used as a continuous covariable.

An ANOVA for repeated measurements was used to assess the time profile of deposition measured on multiple occasions. The time constants derived from these models (representing the clearance rates per time unit) were the time slope for the logarithmically transformed deposition of radioactivity (i.e., ln[deposition] = $\alpha + \beta \times$ time). The half-life of α_1-Pi was analyzed by performing a regression analysis after appropriate logarithmic transformation. For the analysis of the half-life of the peripherally deposited α_1-Pi, only the relevant interval between T_3 and T_4 was considered.

Descriptive data are presented as mean ± SD. The data for deposition assessment are presented as either geometric means ± SD or percentage of initial values, where appropriate. The type I error rate α was fixed at a value of 0.05, and no adjustment was performed for cases of multiple testing. Probability values below this threshold thus indicate statistical significance on a comparisonwise level.

RESULTS

Radiolabeling

The labeling yield was 33.6% ± 13.1%, with a radiochemical purity of 96% ± 1.8%.

Stability

Over 30 h, the UV peak area of the labeled α_1-Pi decreased from 95.6% to 77.0% (Fig. 1) compared with total peak area. An unidentified peak increased from 4.4% to 20.7%. The area of the radioactive peak corresponding by UV to α_1-Pi (Fig. 2) remained stable, with 70.0% and 69.2%, as well as the peak, corresponding to free ^{123}I (five runs; 12.5% ± 2.7% and 15.4% ± 3.4%; $P > 0.1$) 1 and 30 h, respectively, after labeling.

Biologic Activity

A ring of the immunoprecipitate was observed in all wells, with the diameters correlating to the amount of protein applied ($r = 0.98$). Scintigraphic analysis of the immunodiffusion plates revealed various amounts of radioactivity in wells 5 through 12, where the radiolabeled compound was administered.

Patient Studies

At T_1 the uptake of ^{123}I-labeled α_1-Pi (Fig. 3) in the right and left lungs was similar (7.0% ± 3.9% and 6.3% ± 3.4%, respectively), with a tendency toward higher values in the right lobe reflecting the larger volume. The distribution of radioactivity in the ventral and dorsal views showed an

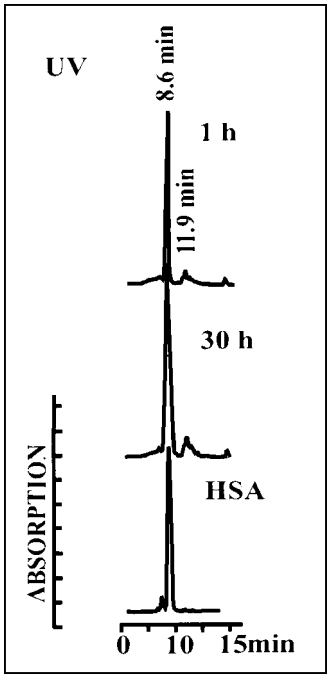

FIGURE 1. UV absorption of HPLC of radiolabeled ^{123}I-α_1-Pi with cold iodinated α_1-Pi added as standard 1 and 30 h after labeling. Bottom shows HPLC of human serum albumin (HSA), revealing same retention time as for α_1-Pi, as expected.

almost equal distribution in patients with severe emphysema, but patients with moderate or mild disease showed higher counts in the dorsal parts of the lungs (6.1% ± 2.3% and 6.3% ± 3.3%, respectively, in group I, compared with 7.2% ± 3.5% and 5.8% ± 3.8%, respectively, in group II and 8.1% ± 5.3% and 6.4% ± 4.9%, respectively, in group III). However, these differences were not statistically significant. At T_1 the mean amount of α_1-Pi deposited in the lungs as a percentage of the net initial material in the nebulizer was 13.2% ± 7.2%.

Time strongly influenced the observed disappearance rate of radioactivity. The influence of lung function on the decline of radioactivity was illustrated by also calculating the values as a percentage of the immediate postinhalation value. The uptake of α_1-Pi decreased rapidly between T_2 and T_3 and more slowly between T_3 and T_4. The absolute radioactive material deposited at T_1 for the different groups differed slightly (Table 2). At T_2 the uptake of α_1-Pi differed significantly between group I and group II and between group I and group III (Table 2). Analysis of the values at T_4 also showed lung function to have a statistically significant influence on retention. Although the absolute amount of retained α_1-Pi was not statistically different between the groups, the mean value of retention depended significantly on the postinhalation value. After adjustment of values to this factor, the retention was significantly lower with increasing severity of lung function impairment (Table 2). The difference was statistically significant between group III and those patients with either moderate or severe emphysema (groups I and II), whereas the difference between group I and group II did not reach statistical significance.

The evaluation of α_1-Pi clearance showed an exponential curve. A rapid decline in α_1-Pi between T_1 and T_2 was seen in all individuals. The decline became less steep between T_2 and T_3 and decreased even further between T_3 and T_4. Although this decline in uptake was similar in all patients, the three groups showed differences in slope. The clearance rates were more rapid in patients with advanced emphysema. Separate analysis of the time constants for the slow decline showed a borderline difference between group I and group III ($P = 0.058$). The resulting half-life of α_1-Pi between T_3 and T_4 was longer for patients with mild disease (17.2 h) than for patients with moderate or severe impairment of lung function (11.5 and 9.9 h, respectively; Table 3).

Regional Distribution of Deposition

Statistical analysis showed excellent agreement among the ROIs drawn by the three physicians. Analysis of regional distribution 24 h after inhalation showed a slightly smaller total area for the ROIs in group III, but the counts per pixel were similar in all groups (Table 4).

The number of and counts per pixel in the central region was higher in group I (Table 4). The analysis showed the impairment of lung function to influence the deposited activity in the central region ($P = 0.009$). The probability value for the influence of pulmonary function on the counts per pixel was 0.066.

The relative size of the central region compared with the total region (C/T) was not different for the three subgroups (Table 4). However, lung function influenced the radioac-

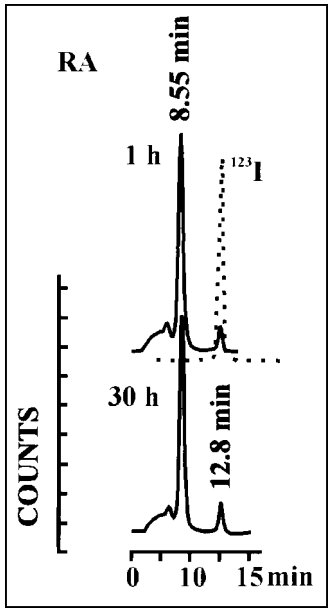

FIGURE 2. Same analysis as in Figure 1, but data of flow through radioactivity (RA) detector are shown along with ^{123}I. Same retention time as shown on UV scan is obvious for radioiodinated α_1-Pi, indicating stable radioiodinated compound.

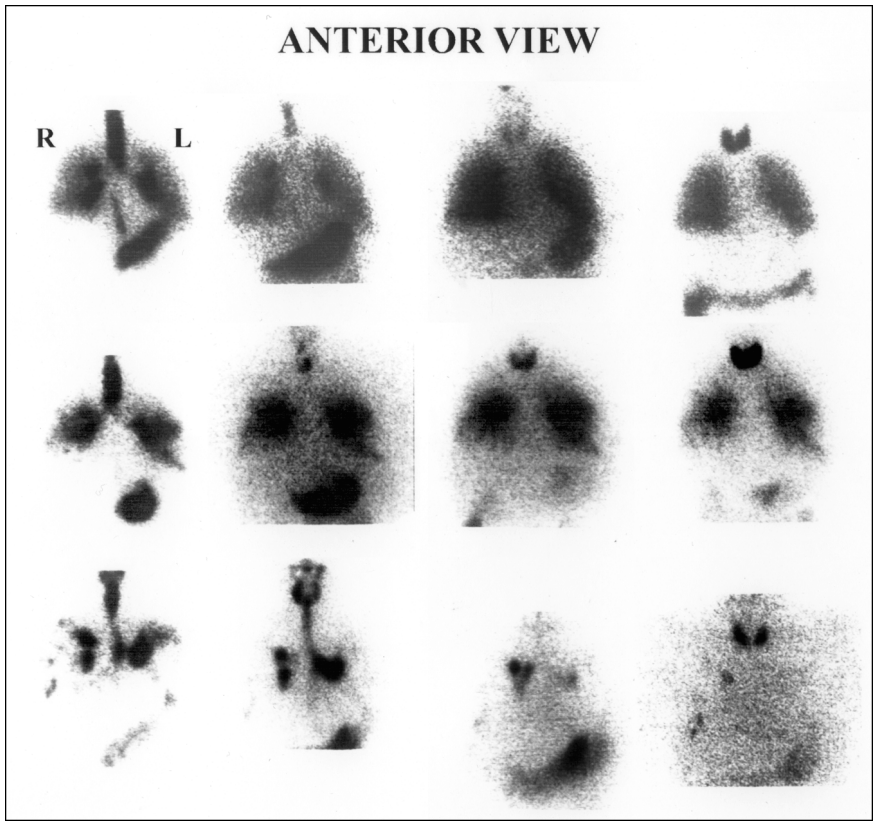

FIGURE 3. Gamma camera scintigrams immediately after inhalation and 1, 4, and 24 h after inhalation (columns from left to right, respectively) for one patient from each of groups I, II, and III (rows from bottom to top, respectively).

tivity of C/T to a statistically significant degree. C/T was significantly lower for patients with mild disease (1.38 ± 0.21) than for those with moderate or severe disease (1.67 ± 0.30 and 1.69 ± 0.24, respectively; $P = 0.01$), as illustrated by the scintigrams of three patients with different degrees of lung function impairment (Fig. 3). At T_4 radioactivity in the central region was greater in patients with moderate and severe compromised lung function than in the patient with mild lung disease. The difference in C/T between mild and moderate emphysema had a probability value of 0.08 (not statistically significant).

Safety Data

All patients completed the protocol. Inhalation of α_1-Pi did not change blood pressure or heart rate. One patient each complained about a headache, increased coughing, tickling of the throat, and sore throat. No changes in urine or blood values were detected in any patient. Inhalation of α_1-Pi did not change lung function or peak flow values.

Radioactivity in Urine and Blood

The mean radioactivity was highest at T_2, with a mean value of 0.0022%/dL, which decreased to 0.00145%/dL at T_3 and to 0.00031%/dL at T_4. The mean amount of radioactivity, expressed as a percentage of the initial lung radioactivity found in the urine, was 45.8% for the whole group and depended on the impairment of lung function. The values were higher in patients with normal lung function (57.5% ± 19.9%) than in patients with moderate impair-

TABLE 2
Absolute Amount of Radiolabeled α_1-Pi Deposited in Lung and Amount Relative to First Value After Inhalation

Time after inhalation	All (μg)	Group I		Group II		Group III	
		μg	%	μg	%	μg	%
Immediate	26.5 ± 14.4	24.8 ± 10.1	100	25.9 ± 14.6	100	29.1 ± 20.4	100
1 h	18.3 ± 9.2	14.6 ± 4.8*,†	59	19.2 ± 6.0	74	22.8 ± 13.8	78
4 h	11.2 ± 4.4	9.2 ± 2.0	37	12.4 ± 4.6	48	13.0 ± 5.8	45
24 h	4.6 ± 3.2	2.4 ± 1.0*	10	4.0 ± 1.2‡	16	7.2 ± 3.8	25

*Difference between group I and group II ($P < 0.05$).
†Difference between group I and group II ($P < 0.01$).
‡Difference between group II and group III ($P < 0.05$).

TABLE 3
Time Coefficients β for Clearing of α_1-Pi from Whole Lung

Coefficient β interval	Group I	Group II	Group III
0–4 h	−0.19	−0.14	−0.15
4–24 h	−0.07	−0.06	−0.04
Half-life (4–24 h)*	9.9	11.5	17.3

*Half-life of inhaled α_1-Pi in periphery of lung.
Data are calculated as ln(deposition in %) = $\alpha + \beta \times t$ (t in hours).

ment (44.5% ± 24.0%) or severe impairment (33.2% ± 13.5%).

DISCUSSION

Deficiency of α_1-Pi can cause panacinar emphysema in affected patients. The only rational treatment available consists of a weekly intravenous infusion of the deficient protein to reverse the protease–antiprotease imbalance. Recent data indicate that intravenous treatment with α_1-Pi may be able to slow the decline in FEV_1 (*15,16*). However, this therapy is costly and, because it causes discomfort, is demanding for both patients and physicians. Because the lung is the main target organ for α_1-Pi and only 2%–3% of the administered drug is effective, an attempt at direct delivery of the drug to the lungs makes sense.

Aerosolized α_1-Pi has been shown to retain its antielastase activity, and the presence of α_1-Pi in the luminal surface of the alveoli 2 h after administration was proven by histochemical staining (*19*). Bronchoalveolar lavage recovered intact α_1-Pi in animals (*19*), in healthy humans (*20*), and in individuals with α_1-Pi deficiency (12), and a consecutive increase in antielastase activity was noted. Aerosolized α_1-Pi has also been shown to diffuse from the alveoli through the interstitium into the lung lymph in sheep (*12,19*). The theoretic basis for α_1-Pi augmentation through aerosol is therefore favorable. This study shows that significant amounts of α_1-Pi are deposited in the lungs and that the deposition pattern is influenced by the degree of airway obstruction.

With a minor modification of the chloramine-T method, we easily radiolabeled α_1-Pi with ^{123}I; the labeling yield was moderate, but the radiochemical purity was excellent in all preparations. This labeling method, although sometimes causing denaturation of the protein, does not compromise ^{123}I-α_1-Pi, as shown by our biologic activity studies; the method is easy to use, and our attempts to apply a more gentle method were unsuccessful. The radiopharmaceutical was stable over 30 h, indicating a specific site of the label (Fig. 2). For HPLC analysis, free radioiodine was added and could be clearly separated from the radiolabeled compound. In addition, the UV peak matched the retention time of human serum albumin, where α_1-Pi is expected. With an immunodiffusion method, we could substantiate the biologic activity of the labeled α_1-Pi.

Aerosol particles ≤ 5 μm must be inhaled for peripheral deposition because otherwise they are retained in the extrathoracic airways (*23*). We used a nebulizer with a polydispersoid spectrum that provided a high percentage of respirable particles, thus achieving pulmonary deposition.

The rate of drug elimination from the lungs depends on the mucociliary clearance rate, the rate of drug transport from the airways to blood, and the rate of proteolytic degradation of α_1-Pi (*24*). Particles deposited in the central airways up to the seventh generation are rapidly cleared from the ciliated airways through the mucociliary escalator and coughing (*25*). The rate of removal of inert particles from the smaller airways and the alveoli depends on the particle size and may take much longer (*26*) because these structures may not be ciliated.

Gamma camera imaging qualitatively and quantitatively assesses the deposition of a radioaerosolized drug, and the fraction of the radioaerosol remaining in the lungs after 24 h represents deposition in the small airways and alveoli. In our study, clearance of radiolabeled α_1-Pi after inhalation mimicked an exponential decline. Up to T_3 a rapid decline in α_1-Pi caused by fast clearance from the central airways

TABLE 4
Quantification of Deposition Patterns: Ratio of Central to Total Deposition 24 Hours After Inhalation

Deposition	Parameter	Group I	Group II	Group III
Total	Pixels	8,009 ± 971	8,084 ± 5,674	7,272 ± 0
	Counts	142,598 ± 162,005	114,057 ± 311,273	127,388 ± 22,205
	Counts per pixel	17.98 ± 2.63	14.21 ± 3.83	17.52 ± 3.05
Central	Pixels	1,824 ± 281	1,753 ± 343	1,582 ± 0
	Counts	54,855 ± 11,304	42,403 ± 17,084	39,085 ± 12,487
	Counts per pixel	30.53 ± 7.25	23.84 ± 7.33	24.71 ± 7.89
Central-to-total ratio	Pixels	0.23 ± 0.02	0.22 ± 0.00	0.22 ± 0.02
	Counts	0.38 ± 0.05	0.36 ± 0.07	0.30 ± 0.05
	Counts per pixel	1.69 ± 0.24*	1.67 ± 0.30†	1.38 ± 0.21

*Difference between group I and group III ($P < 0.01$).
†Difference between group II and group III ($P < 0.05$).

was found in the lungs of all individuals. Beyond T_4 the decline was less steep. This portion of the clearance curve presumably represents mucociliary clearance from the more peripheral ciliated airways. At T_4 we still found material in the periphery of the lungs representing uptake in the alveoli. Because of the relatively low concentration of radiolabeled α_1-Pi and the short half-life of the radioisotope used, measurements beyond 24 h after inhalation were not possible.

The calculations were modeled for an inert particle without any disintegration of the radioactivity from the inhaled particle. These assumptions are not entirely true for radiolabeled α_1-Pi, because α_1-Pi diffuses into the interstitium of the lung and subsequently is detected in the blood (*12*). Some α_1-Pi is also metabolized, leading to increasing uptake in the thyroid (Fig. 3). The presence of radioactivity in the urine showed that considerable deiodination of the α_1-Pi occurs because of endogenous deiodases. The higher amount of radioactivity found in the 24-h urine of group III most probably reflects longer retention in the lung of larger amounts of radiolabeled α_1-Pi, more of which can thus be deiodinated. All these factors lead to an underestimation of the true peripheral deposition and of the half-life of α_1-Pi. These data are consistent with the findings of Vogelmeier et al. (*20*), who showed a much longer half-life—69.2 h—of aerosolized α_1-Pi calculated through bronchial lavage of healthy individuals; in comparison, we found 17.3 h in patients with mild impairment of lung function. To obtain the true half-life of aerosolized α_1-Pi in patients with α_1-Pi deficiency, the α_1-Pi in bronchial lavage fluid will need to be studied.

Delivery of proteins to the alveolar surface requires that obstacles be overcome, including the tortuosity of the upper air passages and the arborization of the bronchial tree. Such obstacles lead to substantial deposition of inhaled substances in the pharynx and proximal large airways rather than in the alveoli. The amount of deposited material also depends on the inspiratory flow and the resistance of the lungs. These factors are altered in chronic obstructive pulmonary disease, and the turbulent airflow may increase central deposition of radioactivity. Consistent with these observations, we found that lung function impairment (as determined by FEV_1) influenced the amount of deposited material and the deposition pattern. In patients with low FEV_1 values, the amount of retained material in the periphery after 24 h was significantly lower than in patients with mild lung disease, because the total amount of material deposited was adversely influenced by a low FEV_1 and showed a more central deposition pattern with decreasing FEV_1.

Elimination between T_1 and T_3 increased in group I compared with groups II and III, obviously because of the more quickly removed material centrally deposited. Elimination between T_3 and T_4 also increased in group I; the significance compared with group III was borderline (Table 2). These results denote either that mucociliary clearance from the lower parts of the lungs is increased in patients with low FEV_1 or that clearance of particles from the larger airways is prolonged in groups II and III.

The half-life of drug remaining in the lungs was inversely influenced by the degree of lung function impairment. This result is surprising, because one would expect impaired mucociliary clearance in advanced chronic obstructive pulmonary disease with a slower mucociliary transport. An explanation might be found in the smaller amount of radioaerosol deposited in the alveolar compartment in patients with advanced chronic obstructive pulmonary disease; that is, the deposition is shifted toward the central and peripheral ciliated airways, leading to removal of a greater proportion of the drug over the first 24 h (*25*). This assumption is supported by the analysis of the regional distribution of deposition. The larger the ratio, the larger the relative amount of activity in the central region. The statistically significant lower ratio for patients with mild emphysema proves that peripheral deposition of aerosolized α_1-Pi is better in them than in patients with moderate or severe emphysema.

The performance of the nebulizer used in this study allowed deposition of significant amounts of α_1-Pi—up to 24.2%—in the alveoli of only patients with an $FEV_1 > 40\%$. With our special setup, only these patients would likely be considered for inhalation therapy; patients with an $FEV_1 < 40\%$, showing decreased uptake in the alveoli, would probably not benefit. This situation may change with different nebulizers or inhalation techniques; for example, Hubbard et al. (*12*) showed that the amount of α_1-Pi in the ELF was higher after repeated aerosol application than after a single dose.

Other diseases, such as cystic fibrosis, also disrupt the protease-to-antiprotease ratio. Although patients with cystic fibrosis have normal or increased α_1-Pi plasma levels, the α_1-Pi in sputum or bronchoalveolar lavage fluid is overwhelmed by the amounts of neutrophil elastase. Application of α_1-Pi aerosol in vivo inactivated neutrophil elastase, restored antineutrophil elastase capacity in the ELF (*13*), and significantly reduced the viscosity of the sputum (*27*). Aerosol application may also become important for patients with α_1-Pi deficiency, because some have bronchiectasis (*28*) and may benefit if deposition of α_1-Pi in the larger airways can reduce the viscosity of the sputum.

CONCLUSION

Inhalation of α_1-Pi for therapy of α_1-Pi deficiency may be beneficial for patients with mild to moderate impairment of lung function. Significantly more α_1-Pi was deposited in the lungs through the inhalational route than through the intravenous route (14.6% vs. 2%). This improved deposition may reduce costs and open resources for treating more patients, possibly including those with other diseases, such as cystic fibrosis. In addition, patients would be freed from intravenous injections and thus would enjoy improved mobility and quality of life.

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REFERENCES

1. Janoff A, White R, Carp H, Harel S, Dearing R, Lee E. Lung injury induced by leukocytic proteases. *Am J Pathol*. 1979;97:111–117.
2. Gader JE, Fells GA, Wright DH, Crystal RG. Neutrophile elastase functions as a type III collagenase. *Biochem Biophys Res Commun*. 1980;95:1815–1824.
3. Gadek JE, Zimmerman RL, Fells GA, Rennard SI, Crystal RG. Antielastases of the human alveolar structures: implications for the protease-antiprotease theory of emphysema. *J Clin Invest*. 1981;68:889–898.
4. Faber JP, Poller W, Weidinger S, et al. Identification and DNA sequence analysis of 15 new α_1-antitrypsin variants, including two PI*Q0 alleles and one deficient PI*M allele. *Am J Hum Genet*. 1994;55:1113–1121.
5. Nukiwa T, Satoh K, Brantly ML, et al. Identification of a second mutation in the protein coding sequence of the Z-type alpha 1-antitrypsin gene. *J Biol Chem*. 1986;261:15989–15994.
6. Massi G. Pathogenesis and pathology of liver disease associated with α1-antitrypsin deficiency. *Chest*. 1996;110:251S–255S.
7. Eriksson S. Pulmonary emphysema and alpha$_1$-antitrypsin deficiency. *Acta Med Scand*. 1964;177:175–182.
8. Gadek JE, Klein H, Holland PV, Crystal RG. Replacement therapy of alpha 1-antitrypsin deficiency: reversal of protease-antiprotease imbalance within the alveolar structures of PiZZ subjects. *J Clin Invest*. 1981;68:1158–1165.
9. Wewers MD, Casolaro MA, Sellers SE, Swayze SC, McPhaul KM, Crystal RG. Replacement therapy for alpha 1-antitrypsin deficiency associated with emphysema. *N Engl J Med*. 1987;316:1055–1062.
10. Schmidt EW, Rasche B, Ulmer WT, et al. Replacement therapy for alpha-1-protease inhibitor deficiency in PiZ subjects with chronic obstructive lung disease. *Am J Med*. 1988;84(suppl 6A):63–69.
11. Schwaiblmair M, Vogelmeier C, Fruhmann G. Long-term augmentation therapy in twenty patients with severe alpha-1-antitrypsin deficiency: three year follow-up. *Respiration*. 1997;64:10–15.
12. Hubbard RC, Brantly ML, Sellers SE, Mitchell ME, Crystal RG. Anti-neutrophil-elastase defenses of the lower respiratory tract in α1-antitrypsin deficiency directly augmented with an aerosol of α1-antitrypsin. *Ann Intern Med*. 1989;111:206–212.
13. McElvaney NG, Hubbard RC, Birrer P, et al. Aerosol α1-antitrypsin treatment for cystic fibrosis. *Lancet*. 1991;337:392–394.
14. Knoell DL, Wewers MD. Clinical implications of gene therapy for alpha 1-antitrypsin deficiency. *Chest*. 1995;107:535–545.
15. Seersholm N, Wencker M, Banik N, et al. Does α_1-antitrypsin augmentation therapy slow the annual decline in FEV1 in patients with severe hereditary α1-antitrypsin deficiency? *Eur Respir J*. 1997;10:2260–2263.
16. Wencker M, Banik N, Buhl R, Seidel R, Konietzko N. Long-term treatment of α_1-antitrypsin deficiency-related pulmonary emphysema with human α_1-antitrypsin. *Eur Respir J*. 1998;11:428–433.
17. McElvaney NG, Stoller JK, Buist AS, et al. Baseline characteristics of enrollees in the national heart, lung and blood institute registry of alpha 1-antitrypsin deficiency. *Chest*. 1997;111:394–403.
18. Hubbard RC, Crystal RG. Strategies for aerosol therapy of alpha 1-antitrypsin deficiency by the aerosol route. *Lung*. 1990;168(suppl):565–578.
19. Smith RM, Traber LD, Traber DL, Spragg RG. Pulmonary deposition and clearance of aerosolized alpha-1-proteinase inhibitor administered to dogs and to sheep. *J Clin Invest*. 1989;84:1145–1154.
20. Vogelmeier C, Kirlath I, Warrington S, Banik N, Ulbrich E, du Bois RM. The intrapulmonary half-life and safety of aerosolized α1-protease inhibitor in normal volunteers. *Am J Respir Crit Care Med*. 1997;155:536–541.
21. Hein RH, van Beveren SM, Shearer MA, Coan MH, Brockway WJ. Production of alpha$_1$-proteinase inhibitor (human). *Eur Respir J Suppl*. 1990;9:16s–20s.
22. Smaldone GC, Messina MS. Flow limitation, cough, and patterns of aerosol deposition. *J Appl Physiol*. 1985;59:515–520.
23. Hubbard RC, Casolaro MA, Mitchell M, et al. Fate of aerosolized recombinant DNA-produced α1-antitrypsin: use of the epithelial surface of the lower respiratory tract to administer proteins of therapeutic importance. *Proc Natl Acad Sci USA*. 1989;86:680–684.
24. Ma JKH, Bhat M, Rojanasakul Y. Drug metabolism and enzyme kinetic in the lung. In: Hickey AJ, ed. *Inhalation Aerosols: Physical and Biological Basis for Therapy*. New York, NY: Marcel Dekker Inc.; 1996:155–195.
25. Pavia D. Lung mucociliary clearance. In: Clarke SW, Pavia D, eds. *Aerosols and the Lung: Clinical and Experimental Aspects*. London, U.K.: Butterworths; 1985:127–155.
26. Scheuch G, Stahlhofen W, Heyder J. An approach to deposition and clearance measurements in human airways. *J Aerosol Med*. 1996;9:35–41.
27. Hansen G, Schuster A, Zubrod C, Wahn V. Alpha 1-proteinase inhibitor abrogates proteolytic and secretagogue activity of cystic fibrosis sputum. *Respiration*. 1995;62:117–124.
28. Shin MS, Ho KJ. Bronchiectasis in patients with alpha 1-antitrypsin deficiency: a rare occurrence? *Chest*. 1993;104:1384–1386.