LUNG DEPOSITION OF INHALED ALPHA-1-PROTEINASE INHIBITOR (ALPHA1-PI) – PROBLEMS AND EXPERIENCE OF ALPHA1-PI INHALATION THERAPY IN PATIENTS WITH HEREDITARY ALPHA1-PI DEFICIENCY AND CYSTIC FIBROSIS

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Abstract
Alpha-1-proteinase inhibitor (α1-PI) is the most relevant protease inhibitor in the lung. Patients with hereditary deficiency of α1-PI suffer from an impaired hepatic synthesis of α1-PI in the liver and in consequence an insufficient concentration of the protease inhibitor in the lung followed by development of lung emphysema due to an impaired protease antiprotease balance and a local relative excess of neutrophil elastase (NE). In contrast, patients with cystic fibrosis (CF) are characterised by a normal synthesis of α1-PI and a severe pulmonary inflammation with a strong excess of NE in the lung followed by progressive loss of lung function. In principle, both patient groups may benefit from an augmentation of α1-PI. Intravenous augmentation, which is established in patients with α1-PI deficiency only, is very expensive, subject to controversial discussions and only about 2% of the administered protein reaches lung interstitium. Inhalation of α1-PI may serve as an alternative to administer high α1-PI doses into the lungs of both patient groups to restore the impaired protease antiprotease balance and to diminish the detrimental effects of NE. However, prerequisites of this therapy are the reproducible administration of sufficient doses of active α1-PI into the lung without adverse effects. In our review we describe the results of studies investigating the inhalation of α1-PI in patients with α1-PI deficiency and CF. The data demonstrate the feasibility of α1-PI inhalation for restoration of the impaired protease antiprotease balance, attenuation of the inflammation and neutralisation of the excess activity of NE. Likely, inhalation of α1-PI serves as cheaper and more convenient therapy than intravenous augmentation. However, inhalation will be further optimised by use of novel nebulisers and optimised breathing techniques.

Key words: α1-proteinase inhibitor, α1-proteinase deficiency, cystic fibrosis, inhalation, deposition, nebuliser

INTRODUCTION
Neutrophils play a crucial role in acute inflammation of the lung and other tissues, e.g., by means of phagocytosis, respiratory burst and release of neutrophil elastase which serves as a potent protease [1-4]. Therefore, in focus on pulmonary diseases it has been suggested that an inhibition of neutrophil elastase may be an important therapeutic principle in at least three distinct categories of pulmonary diseases. In brief, these are hereditary emphysema due to deficiency of α1-PI protease inhibitor (α1-PI, α1-antitrypsin (α1-AI)), diseases with predominantly neutrophilic inflammation (e.g., non-hereditary chronic obstructive lung disease (COPD), cystic fibrosis (CF) and bronchiectasis) as well as acute lung injury [2, 4-6]. However, the prevention of protein degradation by means of inhibition of neutrophil elastase is only one aspect. Other also important actions of the serin protease are the inhibition of mucociliary clearance (e.g., by increase of mucin secretion and decrease of ciliary beat frequency), its role in airway remodelling (e.g., induction of goblet/mucus cell metaplasia, increase of epithelial permeability and degradation of extracellular matrix), its proinflammatory effects (e.g., degradation of α1-PI, increase of interleukin-8 (IL-8) expression and activation of pro-matrix metalloproteinase-9 (pro-MMP-9)) as well as the inhibition of innate and adaptive immunity (e.g., degradation of lactotransferrin and cleavage of opsonins (surfactant proteins SP-A and SP-D and immunoglobulin G (IgG)) [4]. A number of physiological (α1-PI, secretory leukoprotease inhibitor (SLPI)) and synthetic compounds may serve as inhibitors of neutrophil elastase [2, 4, 6]. However, in our review we focussed exclusively on α1-PI and its use in patients with hereditary α1-PI deficiency and cystic fibrosis.

The relation between α1-PI and pulmonary emphysema has been firstly described more than 45 years ago [7, 8]. The glycoprotein (MW 52000 Da) is encoded on the long arm of chromosome 14 (14q31-32.1) and is predominantly secreted by hepatocytes into the blood [9-11]. Only about 2% of the plasma protein passes into the lung interstitium, where it serves as the predominant inhibitor of neutrophil elastase protecting more than 90% of the anti-neutrophil elastase protection of the lower respiratory tract [9, 12-14]. Up to now, more than 120 protein variants (so called Pi phenotypes) have been described which are termed according to their electrophoretic migration behaviour [5, 10, 15, 16]. Plasma concentrations of α1-PI strongly depend on its genotype but there is also some influence of other factors (e.g., increase due to acute phase...
response or estrogens). In brief, the P_in allele is the wild type (>90% of the population) and homocytote individuals (PiMM phenotype) have the highest ('normal') plasma concentrations [10, 11, 15, 16]. Severe decreases of the α₁-PI plasma concentrations below the protective threshold level of 11 µmol/l (80 mg/dl; normal range: 20–53 µmol/l) are found in individuals homocytote for the Z- or the null-allele, whereas intermediate deficiency includes subjects with PiMz, PiSS and PiSZ phenotypes having plasma concentrations between 20 and 60 % of normal [5, 11, 16-18]. Hereditary α₁-PI deficiency is a frequent disease affecting about one in every 2000–2500 people born in Europe and North America. However, there are relevant differences in the allele frequency between different countries and ethnic groups [17-19]. About 96% of the patients with hereditary lung emphysema have a ZZ-phenotype and the remaining 4% mostly belong to SZ- and MZ-phenotypes, whereas the others (e.g., the null phenotype) are very rare [15, 17, 19]. The affected patients have a largely increased risk to develop lung emphysema especially if they are cigarette smokers as oxidants reduce α₁-PI activity to 1/2000 and 70% of them die of lung disease by the age of 50 years [10, 16]. However, there is a mean delay of about 8 years between first symptoms and diagnosis and only 5-10% of the patients are correctly diagnosed [10, 16].

Current therapeutic principles to prevent lung emphysema due to hereditary α₁-PI deficiency are population screening in order to reduce the number of not yet identified individuals at risk, cessation of smoking and antiobstructive treatment in identified patients and augmentation of α₁-PI (by means of weekly infusions in order to restore the protease-antiprotease balance and to reduce the progression of the disease) [16]. The results of the first experiments with intravenous augmentation of α₁-PI were published about 30 years ago and the clinical therapy is available in the USA and in Germany since 1988 and 1989, respectively [9, 16]. Currently, a number of α₁-PI preparations of purified plasma are in the market (Prolastin (Talecris Biotherapeutics, Research Triangle Park, NC, USA), Aralast (Baxter, Deerfield, IL, USA), Trypsone (Grifols, SA, USA) and Zemaira (CSL-Behring, King of Prussia, Pennsylvania, USA [5]). However, a large drawback of this therapy is the only causal one is its very high price. In detail, there are estimated annual costs of up to 70000 Euro and 150000 Dollar in the USA [20, 21]. Furthermore, the obtained data are conflicting as study designs are often very different and the study outcomes are biased. In addition, not all studies have shown a slowed decrease of FEV₁ or loss of density in high resolution computer tomography and an effect on the mortality which serves as a primary endpoint has not been shown. In consequence, some authors recommend this therapy, whereas others question its use or do not recommend it [5, 11, 16, 20-22]. Inhalation of α₁-PI instead of its intravenous administration seems to be an interesting approach to reduce the high cost of this therapy and to increase the convenience of the patients (needle-free mode of drug administration, treatment at home and not in a medical ward) to be treated. However, a number of prerequisites should be fulfilled (sufficient and reproducible dosage of α₁-PI into the deep respiratory tract, stability and functional activity of the administered α₁-PI, penetration of deposited α₁-PI from alveolar surface into lung interstitium and safety of treatment) for this type of therapy [13, 23].

Cystic fibrosis is another common hereditary disorder in Caucasians with about 30000 recognised patients in the US population and the same number in Europe [24-26]. The autosomal hereditary disease has a frequency of 1:2000–1:4000 live births which is similar to that of α₁-PI deficiency described before [24, 27]. All racial populations and ethnic groups are affected. However, there are strong differences of the frequency within different populations [26]. The disease is caused by mutations in a single gene of chromosome 7 (α₁-PI, encoding the cystic fibrosis transmembrane conductance regulator (CFTR) which is a membrane bound cAMP-regulated chloride channel also regulating other ion channels [4, 24, 26]. Up to now, more than 1600 mutations have been characterised. However, the vast majority of cases (30-80% depending on the ethnic group) are caused by deletion of phenylalanine in the amino acid position 508 of the protein [24, 26]. The mutation is followed by an impaired epithelial ion and water transport in many tissues (e.g., the respiratory tract which is most severely affected (causing 95% of morbidity and mortality) but also hepatobiliary tract, intestine, pancreas and urogenital tract) and in consequence by the production of viscous secretions [4, 24, 26]. In the respiratory tract, dyscrinia and impaired mucociliary clearance cause the development of recurrent and almost chronic infections [24, 28-30]. At early age, Staphylococcus aureus and Haemophilus influenzae can be detected, rapidly followed by a colonisation with Pseudomonas aeruginosa (frequency rapidly increasing with age) which plays a crucial role for the further course and the long-time prognosis of the pulmonary disease [28-30]. At a later stage the respiratory tract is colonised by further bacteria (e.g., Burkholderia cepacia, Stenotrophomonas maltophilia) and fungi (e.g., Candida spp., Aspergillus spp.) from which some serve as relevant pathogens [28-30]. Due to their chronic infection and frequent exacerbations, cystic fibrosis patients develop an ongoing lung destruction resulting in respiratory failure and required lung transplantation [4, 28]. The treatment of cystic fibrosis patients focuses on their pulmonary symptoms. In detail, the intensive physical therapy is supported by systemic treatment and inhalation therapy for improvement of secretoly- sis and treatment of respiratory exacerbations [26, 29, 31]. However, even though lifespan and quality of life were largely improved in the last decades by improved medical treatment, cystic fibrosis patients, up to now, show a significant reduction of lifespan and quality of life when compared to normal controls with only about 2% of the patient population older than 45 years [25, 28]. Likely, modern and emerging treatment with a large number of pharmacological compounds to be administered systemically or by inhalative means and even gene therapy vectors will cause a further decrease of morbidity and mortality in cystic fibrosis patients [31]. One approach is the augmentation of α₁-PI by inhalative means to neutralise the large excess of...
neutrophil elastase in the respiratory tract of the affected patients [4, 27]. However, administration of α1-PI not only restores the impaired balance between α1-PI and neutrophil elastase but also neutralises the other detrimental effects of neutrophil elastase described before [4]. Thus, sufficient augmentation of α1-PI may diminish airway hyperresponsiveness and bronchoconstriction, increased secretion of airway glands, mucociliary clearance and frequency of exacerbations [4, 24, 27].

**Inhalation of α1-PI in Patients with Hereditary α1-PI Deficiency**

The first studies on inhalation of α1-PI in patients with hereditary α1-PI deficiency were performed more than 20 years ago by Hubbard et al [12, 32]. However, prior to these investigations a number of studies were performed in animals in order to demonstrate the feasibility and safety of α1-PI aerosol therapy [14, 33, 34]. The first studies in humans with α1-PI deficiency were performed shortly after introduction of the intravenous augmentation therapy into clinical treatment [35]. Below, α1-PI inhalation studies performed in patients with hereditary α1-PI deficiency and healthy controls are compiled which investigate the pulmonary deposition and the biochemical or pharmacological effects of inhaled α1-PI.

In the first study performed in humans (n = 16 patients) with α1-PI deficiency (10 men, aged 39 ± 2, FEV1 78 ± 28 %pred) were included [32]. To investigate a potential immunisation caused by recombinant α1-PI produced by yeast (Cooper Biochemicals, Mountain View, CA, USA and Ciba-Geigy Corp., Summit, NJ, USA), a subgroup of 6 patients received subcutaneous injections of α1-PI for 6 months. Aerosolisation was performed by means of a compressed air driven nebuliser (Mallinckrodt, Maryland Heights, Missouri, USA) generating droplets with an aerodynamic diameter < 3 µm. The aerosol inhalation study was done in two phases. In the first, escalating single doses (10 mg, 50 mg, 100 mg and 200 mg; n = 13) of α1-PI were administered and in the second, 3 patients inhaled 200 mg to determine the time dependency of biologic parameters in the lung. In all patients, two bronchoalveolar lavages were performed before and 4 h or 24 h after α1-PI inhalation in order to assess levels of α1-PI and anti-neutrophil elastase activity in epithelial lining fluid (ELF). Furthermore, blood samples were taken for determination of safety parameters and antibodies against yeast as well as measurement of penetration of inhaled α1-PI into the blood after inhalation. The investigators observed a strong dose-dependent increase of α1-PI level and anti-neutrophil elastase activity in ELF 24 h after inhalation of α1-PI when compared to baseline values. The observed increases were completely reversed to pretreatment levels 1 month after end of the inhalation. Single-dose inhalation of 200 mg α1-PI was followed by a strong increase of the levels of α1-PI (> 40-fold over the baseline level) and anti-neutrophil elastase capacity which were both higher than the normal range in ELF. 24 h later levels of both parameters were below normal values again. However, even at this time levels of α1-PI were fivefold higher than before treatment. The investigators also performed immunological analyses for α1-PI in ELF and serum and were able to demonstrate that inhaled α1-PI was the dominant or the only one type of α1-PI (ZZ-homozygous and Null-allele homozygous patients, respectively) in ELF and passed from the alveoli into blood within 24 h after administration. Finally, analysis of adverse events and safety parameters demonstrated that subcutaneous and inhalative administration of α1-PI were well tolerated and not followed by an immunisation against the used recombinant α1-PI (see Table 1) [32].

Based on the results of their first study [32], Hubbard et al [12] performed another study with a different design also in patients with α1-PI deficiency and lung emphysema. In that study, the patients (n = 12, n = 8 males, aged 42 ± 2, FEV1 65 ± 16 %pred) were treated with aerosolised α1-PI 100 mg (Prolastin; i.e., lyophilised preparation from human plasma, Cutter Biological, Berkeley, California) twice daily for 7 days. The aerosol with a mass median aerodynamic diameter (MMAD) of 2.8 ± 1.3 µm was administered by means of an air pressure driven nebuliser (Ultravent, Mallinkrodt, Maryland Heights, Missouri, USA) within an inhalation time of 20 min. Measurements of α1-PI concentration and anti-neutrophil elastase activity were performed in epithelial lining fluid (ELF) sampled by three bronchoalveolar lavages (before the first inhalation, just before the second inhalation and after the last inhalation). The investigators observed a rapid increase of α1-PI concentration and anti-neutrophil elastase activity in ELF. Normal ranges of both parameters were reached after 2 and 3 days and at the end of the study the obtained values were at the upper limit of the reference range or higher, respectively. Additionally, blood samples from the patients showed that α1-PI administered by means of aerosol inhalation was detectable in blood demonstrating once again the alveolar absorption of the protein. The inhalative administration of α1-PI was well tolerated and no adverse events (e.g., bronchospasm, dyspnea, changes of pulmonary function tests and blood laboratory parameters) were observed demonstrating the safety of this mode of drug administration (see Table 1) [12].

Vogelmeier et al [36] investigated the intrapulmonary half-life of aerosolised α1-PI (Prolastin) to evaluate the duration of action. The study included 30 healthy volunteers (n = 20 males, aged 25.6 ± 4.2) from which 29 were further investigated after the screening phase. The participants received a single dose of 200 mg α1-PI which was administered by means of a baffled venture type nebuliser (Pari Master, Pari, Starnberg, Germany) within inhalation times of 20–30 minutes. The MMAD of the administered aerosol particles was 3.3 ± 2.7 µm. Two bronchoalveolar lavages were performed in each individual, the first 3–7 days before and the second 0.5 h, 6 h, 12 h, 24 h, or 36 h after aerosol inhalation. Inhalation of α1-PI was followed by a strong increase of its concentration in epithelial lining fluid compared to the baseline values. However, the increase showed a time dependent decline and was no more significant 24 h after inhalation. Correspondingly, the anti-neutrophil elastase (NE) activity showed also a strong increase after α1-PI
Table 1. Clinical trials of α₁-PI inhalation in patients with hereditary α₁-PI deficiency and healthy individuals. Data of studies investigating patients with cystic fibrosis are also compiled in Table 2.

| Study                        | Study design                                                                 | Results                                                                                                                                                                                                                                                                                                                                 |
|------------------------------|------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Hubbard et al [32]           | Single dose inhalation of different doses of recombinant α₁-PI from yeast in 16 α₁-PI deficient patients. Pilot study with subcutaneous injection of recombinant α₁-PI in 6 α₁-PI deficient patients for 6 months. | Strong dose-response relationship between administered α₁-PI dose and levels of α₁-PI and anti-neutrophil elastase activity in ELF. Levels of α₁-PI and anti-neutrophil elastase activity in ELF higher than the normal range 4 h and lower than the normal range 24 h after inhalation. Detection of the inhaled α₁-PI in the serum of the patients. No immunisation against administered recombinant α₁-PI. Inhalation of α₁-PI well tolerated. |
| Hubbard et al [12]           | Inhalation of α₁-PI twice daily for 1 week in 12 α₁-PI deficient patients.    | Rapid increase of α₁-PI concentration and anti-neutrophil elastase activity in ELF. Values of α₁-PI concentration and anti-neutrophil elastase activity in ELF at least in the upper normal limit at the end of the study. Detection of inhaled α₁-PI in the serum of the patients. Inhalation of α₁-PI well tolerated.                                                          |
| Vogelmeier et al [36]        | Inhalation of α₁-PI in 29 healthy individuals.                               | Strong increase of α₁-PI concentration and anti-neutrophil (NE) activity in ELF after inhalation with half-life times of 65.2 h and 53.2 h, respectively. No change of the concentration of the α₁-PI/NE complex in ELF. No change of the α₁-PI/NE complex uptake by macrophages. Inhalation of α₁-PI well tolerated.                                             |
| Kropp et al [37]             | Inhalation of ¹²³I labelled α₁-PI in 18 α₁-PI deficient patients grouped according to their individual values of FEV₁. | Percentage uptake values and degradation of α₁-PI depending on individual values of FEV₁ with a greater peripheral deposition and longer half-life times of inhaled α₁-PI in patients with mildly impaired lung function compared to patients with severely impaired lung function.                                                       |
| Brand et al [38]             | Inhalation of ⁹⁹ᵐTc labelled α₁-PI in 6 α₁-PI deficient patients by means of different nebulisers. | Significantly higher values of total and peripheral deposition after α₁-PI inhalation by means of nebuliser systems based on individualised breathing manoeuvres. Significantly shorter inhalation times required by nebuliser systems based on individualised breathing manoeuvres.                                                              |
| Geraghty et al [39]          | Inhalation of α₁-PI powder aerosol for 14 days in 11 α₁-PI deficient patients, 9 patients with community acquired pneumonia, and 9 healthy controls. | Strong decrease of neutrophil elastase activity which was no more detectable in ELF of α₁-PI patients after end of the treatment. No effect on the percentage of neutrophils in BAL fluid of α₁-PI patients. Significant decrease of cathepsin B and metalloproteinase-2 (MMP-2) in BAL fluid of α₁-PI patients. Significant increase of the lactoferrin level but only minor increase of the secretory leukoprotease inhibitor (SLPI) level in BAL fluid of α₁-PI patients. |
| Brand et al [40]             | Inhalation of ⁹⁹ᵐTc labelled α₁-PI by means of an individualised breathing technique in 7 α₁-PI deficient patients, 7 cystic fibrosis patients, and 6 healthy controls | Homogenous pulmonary deposition of α₁-PI in all groups. Higher peripheral (about 42% of the filled activity) than central (about 29% of the filled activity) deposition in all groups. Strong similarity of α₁-PI deposition to ⁸¹Kr ventilation scan. No effect of individual FEV₁(%pred) on the deposition. Inhalation of α₁-PI well tolerated.                     |

Abbreviations: BAL - Bronchoalveolar lavage fluid; ELF - Epithelial lining fluid.

Inhalation which however remained significant until end of the observation period. Calculated half-life times of α₁-PI and anti-neutrophil elastase activity were 69.2 h and 53.2 h, respectively. However, these values should serve as estimates only because direct measurement cannot be performed in interstitium and lymph of humans and there might be relevant differences between healthy individuals and patients with lung diseases. The investigators also studied the concentration of the α₁-PI/NE complex and the α₁-PI uptake by macrophages and found neither a trend toward an increased concentration of the complex nor a trend towards an increased load of lavage cells indicating that there was no effect of α₁-PI inhalation on
neutrophils to secrete neutrophil elastase and only very little phagocytosis of the administered protein. Finally, the authors studied the safety of inhalative administration of α₁-PI. Inhalation of α₁-PI was well tolerated and no relevant side effects except such caused by the bronchoscopy (e.g. nausea due to premedication, intermittent decrease of FEV₁ after bronchoscopy) were reported (see Table 1) [36].

Pulmonary deposition of ¹²⁵I labelled α₁-PI (Bayer AG, Leverkusen, Germany) was also determined by Kropp et al [37]. These investigators studied 18 patients with severe α₁-PI deficiency (n = 15 males; aged 49.8 ± 8.4 years, FEV₁ 50.1 ± 23.8 %pred). The included patients were grouped according to their initial values of FEV₁ (group 1 – ≤40 %pred, group 2 – 40-60 %pred, group 3 – > 60 %pred). Demographic data of age and FEV₁ (in parentheses) were 51.8 ± 9.9 years (1.1 ± 0.2 l, 29.7 ± 5.2 %pred), 44.0 ± 7.4 years (2.1 ± 0.3 l, 52.2 ± 4.5 %pred) and 51.0 ± 5.7 years (2.7 ± 0.8 l, 81.0 ± 15.4 %pred) in the patients included in groups 1, 2, and 3, respectively. α₁-PI aerosol with a median mass aerodynamic diameter (MMAD) of 3.9 ± 2.5 µm was administered by means of an air-pressure driven nebuliser (Master LL, Pari, Starnberg, Germany). In all patients pulmonary deposition was assessed by means of a gamma camera before (T₀) and 1 h (T₁), 2 h (T₂), 4 h (T₃) and 24 h (T₄) after inhalation in two different regions of interest (ROI: 1 - whole right lung; 2 – central activity in trachea and main bronchi). Depending on the times after inhalation (T₁, T₂, T₃, T₄) the absolute percentage uptake values were 12.4, 7.3, 4.6, and 1.2% in group 1, 13.0, 9.6, 6.2, and 2.0% in group 2, and 14.6, 11.4, 6.5, and 3.6% in group 3, respectively, indicating a higher availability of α₁-PI in the lung after inhalation than after intravenous injection (14.6 ± 2.0% [12-14]). However, the evaluation of the regions of interest (ROI) demonstrated that the lung function influenced the distribution of α₁-PI deposition in the lung. In detail, the C/T ratio between central (C) and total (T) region was significantly lower for patients with mild disease than for those with moderate and severe disease (1.38 ± 0.21, 1.67 ± 0.30, and 1.69 ± 0.24 in groups 3, 2, and 1, respectively). The obtained values of radioactivity in urine also corresponded to lung function and were higher in patients with normal or mildly impaired lung function than in those with moderately or severely impaired lung function. The investigators also calculated the half-life times of the inhaled α₁-PI. However, due to different techniques and influence factors the obtained half-life times of 9.9, 11.5, and 17.3 h (in groups 1, 2, and 3, respectively) were largely shorter than the half-life time of 69.2 h reported by Vogelmeier et al [36]. Finally, analysis of safety parameters and adverse events revealed that α₁-PI inhalation was well tolerated. However, headache, increased coughing, tickling of the throat and sore throat were reported in one patient each (see Table 1) [37].

Different types of commercial inhalation devices were performed in a small pilot study of Brand et al [38] performed in 6 patients with hereditary α₁-PI deficiency and mildly to severely impaired lung function (n = 4 males; aged 58 ± 12, FEV₁ 1.6 ± 0.8 l, FEV₁ - 54 ± 22 %pred). The patients inhaled ⁹⁹mTc labelled α₁-PI (Prolastin, Bayer Vital, Leverkusen, Germany) by means of the nebuliser systems Pari-LC Star Jet nebuliser with a Turbo Boy Compressor (Pari, Starnberg, Germany), a HaloLite (Medic Aid, West Sussex, UK) and an AKITA (Inamed, Germindien, Germany) combined with a Pari-LC Star or a Sidestream nebuliser (Pari, Starnberg, Germany). Corresponding median mass aerodynamic diameters (MMAD) were 4.4 ± 2.0, 3.7 ± 2.0, 3.8 ± 2.0, and 3.9 ± 2.0 µm, respectively. Different inhalation manoeuvres were performed for aerosol inhalation. In case of the Pari-LC Star, patients were asked to inhale normally and to activate the nebuliser during inhalation. Even in case of the computer-controlled device HaloLite patients were advised to inhale normally while an aerosol bolus is emitted for a certain period within the first 50% of the inhalation. In contrast, an individualised breathing pattern was used for α₁-PI administration by means of the AKITA devices in combination with a Pari-LC Star or a Sidestream nebuliser. In detail, the flow rate was 200 ml/s with an inhalation volume of 1.76 ± 0.29 l with an aerosol administration until 200 ml before end of inhalation and an end-inspiratory breath-hold time of 2 s. In all patients values of total deposition (D₁) and peripheral deposition (D₂) were measured by means of a lung counter. The obtained values of D₁ and D₂ (in parentheses) were significantly higher for AKITA/Sidestream and AKITA/LC Star (75 ±8% (53 ±11%) and 80.0 ±7% (57 ±9%), respectively) than for HaloLite and LC Star (59 ±7% (33 ±10%) and 52 ±17% (34 ±19%), respectively). In addition, the calculated times for administration of 50 mg α₁-PI were much shorter for AKITA/Sidestream and AKITA/LC Star (24 ±13 and 18 ±6 min, respectively) than for HaloLite and LC Star (99 ±41 and 44 ±25 min, respectively). Both, the rates of drug deposition and the inhalation times indicated a superiority of inhalation techniques based on an individualised breathing manoeuvre (see Table 1) [38].

One more study with α₁-PI inhalation which strongly differs to the other studies described before regarding the administered type of aerosol was performed by Geraghty et al [39]. The investigators studied the effect of α₁-PI inhalation (augmentation study with α₁-PI doses ranging from 6 to 96 mg once daily for 14 days) on various in vivo and in vitro parameters in patients with hereditary α₁-PI deficiency (total group n = 100, n = 60 males, aged 52 ±8 years, FEV₁ - 60.9 ±12.4 %pred), community acquired pneumonia (n = 9, n = 5 males, aged 54 ±1.6 years) and healthy controls (n = 9). However, in the analysis of the α₁-PI group only 11 individuals with free neutrophil elastase in their bronchoalveolar lavage fluid (BAL) prior to aerosol augmentation and access to post-aerosolised augmentation BAL samples (6 mg α₁-PI) were included. Plasma purified α₁-PI (Aventis Behring, later on ZLB Behring, King of Prussia, PA, USA) was converted to dry powder by spray drying and packed into unit dose blisters prior to delivery by means of a powder inhalation system (Nektar, formerly Inhaled Therapeutics, San Carlos, CA, USA). The mass median aerodynamic diameter (MMAD) of the administered powder aerosol was 2 µm. In patients with hereditary α₁-PI deficiency, BAL was performed 13–14 days before en-
tering the study and 24 h (4 h) after inhalation of the last dose. The augmentation was followed by a strong decrease of neutrophil elastase activity which was no more detectable in the epithelial lining fluid (ELF) after end of the treatment. However, there was no effect of α1-PI inhalation on the percentage of neutrophils in BAL fluid. Compared to healthy controls, the activities of cathepsin B and metalloproteinase 2 (MMP-2) were largely higher in patients with α1-PI deficiency. Inhalation of 6 mg α1-PI was followed by a significant decrease of the activities of both proteases in BAL of α1-PI deficient patients. The observed strong decrease of protease activities was correlated with a significant increase of the lactoferrin level, whereas the minor increase of the secretory leukoprotease inhibitor (SLPI) failed significance (see Table 1) [39].

The latest study in this field was once again performed by Brand et al [40] investigating the lung deposition of inhaled α1-PI (Prolastin, human α1-PI inhibitor, Talecris Biotherapeutics Inc., Research Triangle Park, NC, USA) in patients with cystic fibrosis (n = 7) and α1-PI deficiency (n = 7) compared to healthy controls (n = 6). The results obtained in patients with cystic fibrosis are described in detail below. α1-PI deficient individuals and controls were aged (mean±SD) 51.7 ±13.2 and 33.3 ±12.1, had the values of FEV1 of 1.58 ±0.81 l (51 ±15 %pred) and 3.45 ±0.61 l (106 ±14 %pred), and the values of residual volume (RV) of 3.85 ±1.3 1 (193 ±55 %pred) and 1.63 ±0.21 l (103 ±11 %pred), respectively. All individuals received a 81mKr pulmonary ventilation scan for the determination of central and peripheral regions of interest (ROI) and 99mTc labelled α1-PI (Prolastin, human α1-PI inhibitor, Talecris Biotherapeutics Inc., Research Triangle Park, NC, USA) in patients with cystic fibrosis and healthy controls (n = 6). The results obtained in patients with cystic fibrosis treated with intravenously administered α1-PI and 12 healthy controls (aged 29). Cystic fibrosis patients were treated with intravenously administered α1-PI (Prolastin, Cutter Biological) once a week for four weeks (n = 5) or α1-PI aerosol (1.5 – 3.0 mg/kg) every 12 hours for 1 week. Parameters under study were the levels of α1-PI, active neutrophil elastase and anti-neutrophil elastase capacity in respiratory epithelial lining fluid (ELF) as well as the capacity of ELF for P. aeruginosa killing by neutrophils. The investigators observed similar levels of α1-PI in the ELF of patients and controls (3.6 ±1.5 µmol/l vs. 4.4 ±0.5 µmol/l) and large amounts of neutrophil elastase in patients (8.2 ±5.9 µmol/l) but no neutrophil elastase in controls. Intravenous administration of α1-PI was not followed by a suppression of the respiratory neutrophil elastase burden. In contrast, the well tolerated aerosolisation of 1.5 mg/kg α1-PI every 12 hours for 1 week resulted in a strong increase of the α1-PI concentration in ELF and even nadirs were significantly higher than pretreatment levels. Neutrophil elastase in ELF was suppressed and anti-neutrophil elastase activity in ELF was restored when concentrations of 8 µmol/l α1-PI in ELF were reached. Furthermore, inhalation of α1-PI improved the killing of P. aeruginosa by neutrophils which was impaired before treatment (see Table 2) [41].

The next study in this field was performed by Griese et al [42, 43]. In this study 8 patients with cystic fibrosis (n = 4 males, aged 16 ±1.2, FEV1 79 ±8 %pred) inhaled 100 mg α1-PI (Prolastin, Bayer, Elkhart, IN, USA) twice daily for a period of 8 weeks. The aerosol was administered by means of a LC Plus nebuliser driven by a Pari Master compressor (Pari, Starnberg, Germany). Bronchoalveolar lavages were performed before and 12 hours after the last inhalation. The investigators observed a reduction of the free human leukocyte elastase activity in BAL samples. However, a surplus of elastase activity remained present. Prior to inhalation there was a strong correlation between the concentrations of α1-PI and neutrophils in BAL which was lost after treatment (r = 0.855, P = 0.0068 vs. r = 0.647, P = 0.0823). Total protein, the number and amount of proteins with a protein mass <20 kDa, the total phospholipid content as well as phospholipids in small surfactant aggregates (SA) and large surfactant aggregates (LA) were also re-
duced. However, there were no effects on the im-
paired minimal surface tension and the conversion of
LA to SA (see Table 2) [42, 43].

Another study investigated patients with cystic fi-
brosis was performed by Cantin et al [44]. In this
study 19 patients with cystic fibrosis were included
from whom 17 (n = 11 males, aged 27.7 ±6.4, FEV1,
61.9 ±16.2 %pred) completed the study. The included
patients were assigned to three sequential treatment
periods (period 1 - 5 ml normal saline aerosol therapy
twice daily, period 2 - 5 ml 50 mg/ml α1-PI (Prolastin)
twice daily, period 3 - no intervention except usual
therapy) with duration of 10 days each. The inhalation
therapy was performed by means of a Pari-LC Plus
nebuliser (Pari, Starnberg, Germany) and sputum sam-
pies were collected immediately prior to aerosol ther-
apy at days 8, 9, and 10 of each study period for mea-
surement of α1-PI, elastase activity, interleukin 8 (IL-
8), and taurine. The level of α1-PI in sputum showed a
strong (P = 0.002) increase at the end of phase 2 (α1-
PI inhalation) compared to phase 1 (saline inhalation)
which was completely reversed at the end of phase 3
(wash out period; 2.41 ±0.53 μM, 8.07 ±1.88 μM, and
1.98 ±0.36 μM in phases 1, 2, and 3, respectively).
However, the high values of the elastase activity were
not significantly affected by the inhalation therapy
(5.24 ±0.86 μM, 5.00 ±1.24 μM, and 5.91 ±1.69 μM
in phases 1, 2, and 3, respectively). The concentration of
taurine which serves as a surrogate marker of neu-
trophil density in sputum, trended to decrease within
the study period (1.05 ±0.20 μmol/g, 0.79 ±0.12
μmol/g, and 0.69 ±0.004 μmol/g at phases 1, 2, and 3,
respectively; phase 2 vs. phase 1 - P = 0.052, phase 3
vs. phase 1 - P = 0.020). In contrast, no significant
changes were observed for the concentration of IL-8
in sputum. No significant changes were also observed
for FEV1, demonstrating the safety of the aerosol
therapy. However, two patients were unable to com-
plete the study protocol (respiratory exacerbation
while receiving α1-PI and acute respiratory exacerba-
tion with fever and hemoptysis during the trial) (see
Table 2) [44].

A major double-blinded, randomised, placebo-con-
trolled parallel-group trial was performed by Martin et
al [45]. In this study 39 patients with cystic fibrosis
(from which 38 completed the study) were included
who were divided into 4 treatment groups (500 mg -
10 patients; 250 mg - 10 patients; 125 mg - 9 patients,
and placebo - 10 patients in groups 1, 2, 3, and 4 with
inhalations once daily) regarding the administered
dose of recombinant α1-PI (PPL Therapeutics, Roslin,
Scotland, UK). Corresponding anthropometric data of
the patients were as follows: 9 males, aged 24.9 ±5.2,
FEV1 2.28 ±0.96 l (group 1), 9 males, aged 27.4 ±8.4,
FEV1 2.26 ±0.87 l (group 2), 7 males, aged 31.8 ±15.0,
FEV1 1.92 ±0.83 l (group 3), and 7 males, aged 31.2
±10.4, FEV1 1.40 ±0.88 l (group 4). However, the ran-
domisation process resulted in an inclusion of older
patients with poorer lung function in the placebo
group (FEV1 <40 %pred, FEV1 41-60 %pred, FEV1
61-80 %pred, and FEV1 >80 %pred; n = 2, n = 2, n =
6 and n = 0; n = 2, n = 5, n = 3 and n = 0; n = 2, n =
6, n = 1 and n = 0 as well as n = 6, n = 2, n = 1 and n =
1, respectively). The recruited patients were investi-
gated 8 times within a study period of 16 weeks. After
patient screening (visit 1) there was a 4 week treatment
period (visits 2-5) followed by a period of 2-4 weeks
without treatment, a 2 week rechallenge phase for
safety and immunogenic analysis, and a last visit about
2 weeks after the last dosing. At all visits physical in-
vestigation, pulmonary function tests, sputum analysis,
blood and urine analysis and the measurement of safet-
ty parameters were carried out. Inhalation was per-
formed by means of a Pari-LC Star nebuliser with a
Turbo Boy compressor (Pari GmbH, Starnberg, Ger-
many) and inhalation times of 20–30 min duration.
The obtained activities of free neutrophil elastase
(NE) activity were largely different within and be-
between the study groups (0.72 ±1.88 μg/ml, 2.81 ±4.94
μg/ml, 0.83 ±1.66 μg/ml, and 2.63 ±3.78 μg/ml in
groups 1, 2, 3, and 4, respectively). α1-PI inhalation
was followed by a small, but insignificant reduction of
free neutrophil elastase in sputum (visit 5 vs. visit 1).
However, different results were observed within the
treatment groups (500 mg vs. placebo, P = 0.21; 250
mg vs. placebo, P = 0.09; and 125 mg vs. placebo, P =
0.31). In addition, the concentrations of α1-PI/NE
complexes in sputum showed a decrease in the treat-
ment groups of 125 mg and 500 mg (visit 5 vs. visit 1,
125 mg placebo, P = 0.05). Therapy induced de-
creases were also observed for the activity of myeloperoxidase (MPO) in sputum in the patient
groups treated with 125 mg and 500 mg α1-PI (visit 5
vs. visit 1, 500 mg vs. placebo, P = 0.04). However,
there was only a trend towards lower sputum concen-
trations of interleukin-8 (IL-8) in one of the patient
groups (visit 5 vs. visit 1, 500 mg α1-PI, P = 0.009)
and no effect of α1-PI inhalation on the concentra-
tion of tumour necrosis factor (TNF) p55 receptors.
Microbiological analysis in sputum revealed also no sig-
nificant changes in bacterial ecology (most commonly
Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus viridans and Burkholderia cepacia) and colony
counts under therapy. Within the observation period,
the investigators observed no notable changes in phys-
ical examination or vital signs and no significant
changes of pulmonary function tests. In total, 24 pa-
patients reported a pulmonary exacerbation (8, 6, 3, and
7 patients in groups 1, 2, 3, and 4, respectively). How-
ever, these events were considered to be drug related
in only 3 cases (1 in group 1 and 2 in group 4). The
development of antibodies against the administered
recombinant α1-PI occurred in 2 patients (in treat-
ment groups 1 and 2), but was not correlated with
changes in pulmonary function tests and laboratory
data which were indicative of an allergic response (see
Table 2) [45].

Another major randomized prospective study was
performed by Griese et al [46]. The aim of their study
was to investigate the optimal deposition region for in-
haled α1-PI and to examine the effect of inhaled α1-
PI on lung function, pulmonary protease-antiprotease
balance and airway inflammation. 52 Caucasian pa-
patients (aged ≥8, 25 ±9 years (mean ±SD), FEV1,
-67 ±25 %pred) were included in the study and received
25 mg per day α1-PI (Prolastin, Bayer Corporation,
Clayton, NA, USA) for 4 weeks targeting their periph-
eral or bronchial compartment. Inhalation therapy was
performed by means of an AKITA inhalation device (Activaero GmbH, Gemünden, Germany) combined with a Pari-LC Star nebuliser (mass median aerosol diameter (MMAD): 3.5 µm) or a Pari LC Plus (MMAD: 5.0 µm) (Pari, Starnberg, Germany), respectively. Corresponding breathing manoeuvres were a slow inhalation flow (200 ml/s) and an individualised inhalation volume calculated from the individual inspiratory capacity [47] as well as a very low inhalation flow and a low inhalation volume (100 ml/s and 60% of the individual inspiratory capacity) [47].

Table 2. Clinical trials of α₁-PI inhalation in patients with cystic fibrosis and healthy individuals. Data of studies investigating patients with α₁-PI are also compiled in Table 1.

| Study                  | Study design                                                                 | Results                                                                                                                                                                                                 |
|-----------------------|-----------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| McElvaney et al [41]  | Inhalation of α₁-PI twice daily for 1 week in 12 cystic fibrosis patients, i.v. administration of α₁-PI once weekly for 4 weeks in 5 cystic fibrosis patients and 12 healthy controls. | No difference of α₁-PI concentration in ELF of cystic fibrosis patients and controls prior to treatment. Large amounts of neutrophil elastase in ELF of cystic fibrosis patients, but not in controls prior to treatment. Inhalation followed by a strong increase of α₁-PI concentration in ELF. Inhalation of α₁-PI followed by suppression of neutrophil elastase in ELF up to a restoration of the anti-neutrophil elastase activity. Inhalation of α₁-PI improved the killing of *P. aeruginosa* by neutrophils. No effect of intravenous administration of α₁-PI on the respiratory neutrophil elastase burden. Inhalation of α₁-PI well tolerated. |
| Griese et al [42, 43] | Inhalation of α₁-PI in 8 cystic fibrosis patients twice daily for 8 weeks.   | Reduction of free elastase in BAL, however a surplus of elastase activity remained. Correlation between α₁-PI concentration and neutrophils in BAL before, but not after inhalation. Reduction of total protein, number and amount of proteins with a molecular mass <20 kDa, total phospholipid content and phospholipids in small and large surfactant aggregates (SA/LA). No effect on minimal surface tension and the conversion of LA to SA. |
| Cantin et al [44]     | Saline inhalation twice daily (phase 1), α₁-PI inhalation twice daily (phase 2), and wash out (phase 3) in 17 cystic fibrosis patients. | Increase of α₁-PI in sputum at end of phase 2 compared to phases 1 and 3. No changes of elastase and IL-8 in sputum. Slight decrease of taurine in sputum in phases 2 and 3. No effect of α₁-PI inhalation on FEV₁. |
| Martin et al [45]     | Double-blinded, randomised, placebo-controlled parallel-group trial with 4 groups and inhalation of 3 different doses of recombinant α₁-PI once daily in 39 cystic fibrosis patients for 16 weeks. | Decrease of free neutrophil elastase (NE) concentration in sputum in one group. Decrease of the concentrations of α₁-PI/NE complexes in sputum in two groups. Decrease of the activity of myeloperoxidase (MPO) in sputum in two groups. Trend towards lower sputum concentration of interleukin-8 (IL-8) in one group. No effect on the concentration of tumour necrosis factor (TNF) p55 receptors in sputum. No changes in bacterial ecology and colony counts in sputum. No effect of α₁-PI inhalation on pulmonary function tests. Inhalation of α₁-PI well tolerated. |
| Griese et al [46]     | Prospective, randomised study with inhalation of α₁-PI for bronchial and peripheral deposition in 52 cystic fibrosis patients for 4 weeks. | No differences between peripheral and bronchial deposition of inhaled α₁-PI. Increase of α₁-PI level and 54kDa IgG fragments in induced sputum. Decrease of neutrophils, IL-8, IL-1β, TNF-α and LTβ levels in induced sputum. Reduction of *P. aeruginosa* load in induced sputum. Effects more pronounced after 4 than after 2 weeks of treatment. No effect of α₁-PI inhalation on FEV₁. |
| Brand et al [40]      | Inhalation of 99mTc labelled α₁-PI by means of an individualised breathing technique in 7 α₁-PI patients, 7 cystic fibrosis patients, and 6 healthy controls. | Homogenous pulmonary deposition of α₁-PI in all groups. Higher peripheral (about 42% of the filled activity) than central (about 29% of the filled activity) deposition in all groups. Strong similarity of α₁-PI deposition to 81mKr ventilation scan. No effect of individual FEV₁ (%pred) on the deposition. Inhalation of α₁-PI well tolerated. |

Abbreviations: BAL - Bronchoalveolar lavage fluid, ELF - Epithelial lining fluid.
individual inspiratory capacity with a maximum value of 0.5 l for peripheral and bronchial deposition, respectively. At begin of the study (visit 1), 72 patients were included and randomised for peripheral and bronchial deposition (37 and 35 patients, respectively). Patients received inhalation of isotonic saline for a run in period of 2 weeks. At the end of the run in period baseline measurements were taken and 30 and 29 patients were included into the consecutive 4 week treatment period (visits 3 and 4 after two and 4 weeks of treatment, respectively) from whom 28 and 24 finished the study. Parameters under study were the levels of free elastase activity, 54kDa IgG fragments, α1-PI, neutrophils and proinflammatory cytokines (interleukin-8 (IL-8), tumour necrosis factor α (TNF-α), interleukin 1β (IL-1β), leukotriene B4 (LTB4)) and their corresponding mRNA levels (IL-8 mRNA, TNF-α mRNA, and IL-1β mRNA) and the numbers of P. aeruginosa in induced sputum as well as lung function tests. For all parameters no significant differences were observed between the groups treated with peripheral and bronchial deposition of inhaled α1-PI, which might be caused by deposition of α1-PI in lung periphery by both inhalation manoeuvres. In consequence, both patient groups were combined for the analysis of treatment induced effects on lung inflammatory burden and lung function. In this analysis, Griese et al [46] observed that inhalation of α1-PI was followed by an increase of α1-PI levels and the proportion of 54kDa IgG fragments in induced sputum. Furthermore, they found a decrease of the levels of free elastase activity, neutrophils, proinflammatory cytokines (IL-8, TNF-α, IL-1β, and LTB4) (also the corresponding mRNA levels), and P. aeruginosa load. The observed effects were more pronounced after 4 weeks of treatment than after 2 weeks demonstrating a therapy-induced reduction of the lung inflammatory burden. However, there was no significant effect of the performed short time inhalation of α1-PI on FEV1. The authors also investigated gender and type of adverse events. Mild or moderate adverse events were observed in 6 patients (bronchial deposition group – 3, fatigue (in the run in phase), fatigue and haemoptysis (under treatment); peripheral deposition group – 3, pruritus, influenza-like illness, gastro-intestinal pain) (see Table 2) [46].

The most recent study investigating the inhalation of α1-PI in patients with cystic fibrosis was published by Brand et al [40]. In this study, the lung deposition of inhaled α1-PI (Prolastin human α1-PI inhibitor, Talecris Biotherapeutics Inc., Research Triangle Park, NC, USA) was determined in patients with cystic fibrosis (n = 7) and α1-PI deficiency (n = 7) compared to healthy controls (n = 6). The results obtained in patients with hereditary α1-PI deficiency were described before. Patients with cystic fibrosis and controls were aged (mean ±SD) 28.6 ±6.1 and 33.3 ±12.1 years and had the values of FEV1 of 2.38 ±0.91 (62 ±15 % pred) and 3.45 ±0.61 (106 ±14 % pred) and the values of residual volume (RV) of 2.92 ±0.81 (186 ±55 % pred) and 1.63 ±0.21 (105 ±11 % pred), respectively. Prior to the inhalation of radioactively labelled α1-PI, all individuals received an 81mKr pulmonary ventilation scan for the determination of central and peripheral regions of interest (ROI). α1-PI was administered using an AKITA2 APIXNEB device consisting of a nebuliser handset (APIXNEB) based on vibrating mesh technology (Touchspray; Pari GmbH, Starnberg, Germany) and an electronic unit (AKITA2 APIXNEB, ActivAero GmbH, Gemünden, Germany) by means of an individualised inhalation volume and a flow rate of 250 ml/s (required inhalation time in healthy controls about 10 min). The investigators observed a total lung deposition of about 70% of the activity filled into the nebuliser (healthy controls 70.3 ±7.9 %), patients with cystic fibrosis - 70.6 ±5.8% which was not significantly affected by the individual values of FEV1% (given in %pred). The obtained values of central deposition 29.4 ±4.8 and 27.3 ±4.7% of the filled activity, peripheral deposition (40.9 ±4.5 and 43.3 ±5.3% of the filled activity), extrathoracic deposition (18.8 ±6.8 and 19.7 ±4.9% of the filled activity), and the values of the residuals in the device and the residuals exhaled showed no significant differences between healthy controls and patients with cystic fibrosis, respectively. In both groups, the peripheral deposition of α1-PI was considerably higher than the central deposition. Furthermore, the scans of the 81mKr ventilation scintigraphy and the α1-PI deposition in all individuals were well matched and showed only little amounts of extrathoracic and central deposition. All healthy individuals and patients with cystic fibrosis were able to inhale α1-PI. The inhalation was well tolerated. However, as mentioned before two adverse events possibly related to the medication were reported (tongue vesicles and dysphagia in one individual of the control group and mild headache in one patient with cystic fibrosis) (see Table 2) [40].

**Conclusions**

The history of α1-PI inhalation in patients with hereditary α1-PI deficiency and cystic fibrosis is about 20 years and almost as long as the history of intravenous α1-PI supplementation in emphysema patients. Clinical experience regarding the intravenous augmentation therapy in α1-PI deficient patients has demonstrated that this therapy is safe and well tolerated. However, the clinical efficiency of intravenous augmentation is up to now subject of controversial discussions, because of the small numbers of studies and patients, the biased outcomes and the very high price of this therapy [5, 11, 16, 20-22]. In these patients, inhalative administration of α1-PI may serve as a therapeutical alternative and has a number of advantages. Firstly, the required amount of drug can be reduced, because high protein doses can be selectively administered directly into the lung by inhalation, whereas only about 2% of the administered α1-PI reach the lung after intravenous injection. Secondly, inhalation (at home, twice a day, duration some minutes only) is a more convenient mode of administration than intravenous injection (weekly, in a medical ward). In contrast to patients with hereditary α1-PI deficiency, intravenous augmentation of α1-PI plays no role in patients with cystic fibrosis, because these patients have no impaired hepatic synthesis of the protein, but suffer from increased pulmonary inflammatory burden with an excess of neutrophil elastase. However, even in these patients in-
halation may serve as a potent therapy to neutralise the excess of inflammatory proteins and neutrophil elastase by administration of high α1-PI doses. Up to now, only a few short studies with inhalation of α1-PI in patients with hereditary α1-PI deficiency and cystic fibrosis were performed. Therefore, further studies with longer observation periods and a higher number of included patients should be performed to investigate the protective effect of α1-PI inhalation therapy. In addition, modern nebulisers and inhalation techniques will further improve inhalative administration of α1-PI due to a reduction of the required drug doses and the costs of the therapy, a reduction of the inhalation time and a reproducible administration of sufficient drug doses [48, 49].

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