Use of Secretory Leukoprotease Inhibitor to Augment Lung Antineutrophil Elastase Activity*

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Physiologically, secretory leukoprotease inhibitor (SLPI) is the major antiprotease of the epithelium of the upper respiratory tract providing protection against neutrophil elastase (NE). The recombinant form of SLPI (rSLPI) has several advantages compared with α1-antitrypsin that make it interesting as potential therapy. In vitro, rSLPI proves to be an excellent inhibitor of NE. When administered as an aerosol in vitro and in vivo, the structure and function of rSLPI remain intact. Using the aerosol route, the half-life of rSLPI in respiratory epithelial lining fluid is 12 h; thus, giving it twice daily should guarantee satisfactory levels in the lung. Following inhalation, rSLPI moves from the epithelium in an intact form into the interstitium of the lung. Following on from these in vitro and in vivo experiments, a short-term study in patients with cystic fibrosis was performed with aerosolized rSLPI. Promising results relative to NE level reduction and the consequences for the inflammatory process in the bronchi were achieved. rSLPI not only induced an increase of the anti-NE protective screen, but also improved the antioxidant protection by raising glutathione levels in the lung in sheep. rSLPI may therefore provide a unique opportunity for protecting the lung from the damage caused by inflammatory processes by giving a single drug.

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Acid-stable protease inhibitors have been demonstrated in a variety of glandular secretions such as seminal fluid, cervical mucus, and synovial fluid, as well as in secretions from the parotid gland, nose, and bronchi. It has recently become clear that all these molecules are identical and are split products of a single native inhibitor.1,2 For historic reasons, this protease inhibitor has various names, including mucus protease inhibitor, antileukoprotease, bronchial mucus inhibitor, human seminal inhibitor I, and secretory leukoprotease inhibitor (SLPI).3

SLPI is a 12-kd, 2-domain, single-chain protein with 107 amino acid residues. The tertiary structure of the molecule resembles a boomerang with each arm carrying one domain. The domains show about 35% homology. The enzyme activity is restricted to the COOH-terminal domain with the active center being formed by the Leu72-Met73 residues.8,10 SLPI has 16 cysteine residues that form disulfide bridges with each other, thereby connecting the polypeptide segments of the molecule11-14 (Fig 13). The SLPI protein is coded by a single gene consisting of 4 exons and 3 introns and spans approximately 2.6 kb.10 Until now, no polymorphism of the SLPI gene and no SLPI-deficiency state has been found.

SLPI inhibits a variety of proteases, such as cathepsin G, trypsin, chymotrypsin, chymase, tryptase, and neutrophil elastase (NE). Based on enzyme kinetic studies, its major physiologic function is probably the inhibition of NE.17-21 In contrast to α1-antitrypsin (α1-AT), SLPI is a local inhibitor; as mentioned above, it is synthesized and secreted in several glandular organs, including the lung. Using immunohistochemical techniques, SLPI is observed in the human lung, mainly in the serous cells of submucosal tracheal and bronchial glands and in nonciliated cells of the bronchial and bronchiolar epithelium.22-26 Lavage studies targeted at the physiologic role of the molecule in the normal lung suggest that SLPI provides the major anti-NE protective screen at the epithelial surface of the upper respiratory tract.27-29

Of the therapeutic options to increase anti-NE activity in the human lung to date, plasma-derived α1-AT has been used mainly.30-34 SLPI has several theoretical advantages compared with α1-AT that may be beneficial for its potential use as a drug: (1) The isoelectric point of SLPI (>9) is very similar to that of NE (10.8); in addition—in contrast to α1-AT—SLPI is able to inhibit elastin-bound NE.35-37 SLPI may therefore target the same molecules in the interstitium as
NE, e.g., elastin, and may even inhibit NE that is already bound to elastin; (2) SLPI is not glycosylated, therefore, recombinant SLPI (rSLPI) that is synthesized in Escherichia coli using a synthetic SLPI gene is absolutely identical to SLPI in terms of structure and function; in contrast, recombinant \( \alpha_1 \)-AT (r\( \alpha_1 \)-AT) does not have the carbohydrate side chains that the natural form of \( \alpha_1 \)-AT possesses; as a result of this, when the IV route is used for administration, \( \alpha_1 \)-AT is excreted into the urine within minutes; in addition, the physical stability of the molecule is hampered. (3) While \( \alpha_1 \)-AT provides more than 90% of the anti-NE defense of the lower respiratory tract, SLPI is the quantitatively dominating NE inhibitor of the bronchi—at least on the epithelial surface. rSLPI may therefore be the most natural form of therapy for disorders of the pulmonary epithelium such as cystic fibrosis and bronchitis, where it may provide effective protection for the epithelium against NE.

Our initial experiments with rSLPI were directed at the evaluation of the anti-NE activity of this molecule. The ability of rSLPI to inhibit NE was tested in two ways: the determination of the time-independent and of the time-dependent inhibition. The evaluation of the time-independent inhibition of NE by rSLPI showed an activity of 96±1%, ie, nearly all of the rSLPI molecules were capable of inhibiting NE in vitro. As a parameter for the velocity of NE inhibition, the association rate constant (K association) was determined by a time-dependent assay based on the approach of Beatty et al and Straus et al. The K association of rSLPI with NE was determined to be 7.1±0.1 \( \times \) 10^9 M^−1s^−1—a value that is about 20% lower than that for the most common normal \( \alpha_1 \)-AT variant (M1M1[Val^123]) and very similar to that of r\( \alpha_1 \)-AT (6.8±0.8 \( \times \) 10^9 M^−1s^−1). Thus, rSLPI inhibits NE with about the same velocity as \( \alpha_1 \)-AT and a little slower than natural \( \alpha_1 \)-AT. Combining the results of the time-dependent and the time-independent titration, rSLPI is shown to be an excellent inhibitor of NE.

In vitro and in vivo evaluation of rSLPI after aerosolization demonstrated that the aerosolization process had no significant effect on the ability of rSLPI to inhibit NE (Fig 2); in an in vitro aerosol experiment, the anti-NE activity of the aerosolized rSLPI was 90±2%. This activity was not significantly different from the activity of the native material (Fig 2A, p>0.07, compared with baseline). Hence, the aerosol procedure itself does not inactivate rSLPI. As a next step, rSLPI (50 mg) was aerosolized in sheep; to evaluate possible structural changes caused by in vivo aerosolization, BAL was performed 3 h postaerosol and the BAL fluid (BALF) was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting, using an anti-SLPI antibody. This analysis demonstrated that the rSLPI recovered from the lung was an intact molecule, identical to rSLPI before aerosolization (Fig 2B). To assess the antiprotease function of rSLPI following in vivo aerosolization, the anti-NE capacity was quantified in sheep BALF obtained 3 h postaerosol and compared with the corresponding values before aerosolization. The anti-NE capacity rose from 1.2±0.5 pmol/L to 5.0±0.8 pmol/L (Fig 2C, p<0.001, compared with baseline). Thus, rSLPI does not lose its structural integrity or antiprotease function in the first hours following aerosol, suggesting that a considerable increase of the anti-NE capacity of the respiratory tract should be possible using this approach.

Analyzing rSLPI concentrations in BALFs obtained at several time points up to 48 h after the aerosol (50 mg) enables the half-life of rSLPI in epithelial lining fluid (ELF) of the sheep to be calculated. The volume of ELF was determined using the urea method. The half-life was calculated to be 12 h. To evaluate if rSLPI reaches the interstitium of the lung after aerosol application, the caudal effervent lymph ducts of sheep were cannulated with a catheter to collect lung lymph. BALF, lung lymph, plasma, and urine were obtained at different time points after a single rSLPI aerosol (50 mg) and analyzed for rSLPI levels (Fig 3). As expected, the rSLPI level in ELF rose sharply. One hour after the aerosol, rSLPI could be detected in lung lymph, with the concentration in-
increasing over the following hours. In plasma and urine, no rSLPI could be found. The immunoblot analysis of lung lymph that had been collected 3 h after the aerosol showed that rSLPI which had crossed the epithelium was intact.42

To summarize these data, rSLPI given as an aerosol is deposited in the respiratory tract without alteration in its function or form. After a single administration considerable quantities of rSLPI can be found in ELF. Taking into account the half-life of rSLPI in ELF, it should be possible to maintain adequate ELF levels by administering two aerosols per day. The aerosolized rSLPI significantly increased the anti-NE capacity of ELF. Not only did rSLPI reach the epithelial surface of the lung, but in part, it also moved across the epithelium into the interstitium as an intact molecule. These findings lead to the conclusion that the application of rSLPI by aerosol is a promising way of increasing the anti-NE defense of the lung, at least in the pulmonary epithelium, and possibly also in the interstitium.

This conclusion is in agreement with studies showing that instillation of rSLPI into the trachea of hamsters protects the animals from lung destruction and bronchial epithelial cell metaplasia development following the application of NE.47,48 rSLPI was also effective in reducing the damage in an emphysema model with lipopolysaccharide as the emphysema-

![Figure 2](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21741/)  
**Figure 2.** Characterization of rSLPI before and after in vitro and in vivo aerosolization. A: inhibition of NE by rSLPI before (preaerosol) and after (postaerosol) in vitro aerosolization. B: immunoblot evaluation of rSLPI electrophoresis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis before aerosolization (lane 1) compared with rSLPI recovered from BALF 3 h after aerosolization of 50 mg rSLPI in sheep (lane 2). The 12-kd rSLPI is indicated. C: anti-NE capacity of lung ELF of sheep before (preaerosol) and 3 h after aerosolization of 50 mg rSLPI (postaerosol) (reprinted from Vogelmeier et al42, with permission).

![Figure 3](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21741/)  
**Figure 3.** Comparison of concentrations of rSLPI in ELF, lung lymph, plasma, and urine after aerosol administration of 50 mg rSLPI in sheep. Various fluids were collected before and at various time intervals after aerosol (reprinted from Vogelmeier et al42, with permission).
inducing stimulus.\textsuperscript{49} Instillation of rSLPI into the trachea of rats confirmed that rSLPI does not lose its antiprotease activity in the first hours following application. In this study, a half-life of 4 to 5 h was calculated.\textsuperscript{50}

As an alternative to the aerosol route, rSLPI can be given IV. Using this mode of administration, in the sheep model, a considerable increase in rSLPI levels and anti-NE capacity in ELF, as well as in lung lymph, could be observed; as expected for a 12-kd molecule, the urinary excretion was 20\% over 5 h when the rSLPI infusion (1 g) was given over 10 min. Reducing the infusion speed resulted in a parallel reduction of the urinary excretion.\textsuperscript{51}

The reported findings suggest that the administration of rSLPI represents a possible way to correct a disturbance of the protease-antiprotease balance. For the above-mentioned reasons, aerosolized rSLPI may be particularly useful for lung diseases with an increased number of neutrophils and therefore NE on the epithelial surface of the lung. Following on from the above-mentioned animal studies, patients with cystic fibrosis were treated with an rSLPI aerosol: 17 patients with cystic fibrosis received 100 mg rSLPI per aerosol twice a day for 7 days without suffering side effects. The SLPI level in ELF increased significantly during the study, while the active NE levels decreased by 68\%. It has been shown that bronchial epithelial cells of patients with cystic fibrosis express the cytokine interleukin 8 (IL-8), which is induced by exposure to NE, in high quantities. As IL-8 is a very strong chemoattractant for neutrophils, it may have an important role in the development and maintenance of the neutrophil-dominated inflammation in the lungs of these patients.\textsuperscript{52,53} Incubating human bronchial epithelial cells with BALF from rSLPI-treated patients with cystic fibrosis resulted in a much lower expression of IL-8 than with BALF obtained before initiation of therapy. As a consequence, the IL-8 concentration and the number of neutrophils in BALF from patients receiving rSLPI therapy were markedly reduced.\textsuperscript{54} These results enable us to conclude that treating patients with cystic fibrosis with an rSLPI aerosol—at least on a short-term basis—induces a considerable increase of the anti-NE defense, probably with important consequences for the inflammatory processes in the bronchi, without carrying risks for the patient.

In the course of the animal experiments described, we observed an increase of the glutathione concentration in ELF of sheep that had received rSLPI aerosol. In a systematic study that focused on the possible interaction between rSLPI application and glutathione metabolism, we found that a single application of rSLPI (100 mg) induced a 5-fold increase in glutathione concentration in ELF, with most of the glutathione being in the reduced form. While the rSLPI concentration was at its maximum immediately after the aerosol and decreased from then on, the glutathione levels reached their maximum 24 h after the aerosol (Fig 4). The functional consequences of these findings were an immediate and sharp increase in the anti-NE capacity, whereas the values for the antioxidant capacity of ELF changed according to the glutathione concentration.\textsuperscript{55} (Fig 5). While inhaled reduced glutathione has a half-life in ELF of only 2 h,\textsuperscript{56} aerosolized rSLPI increases the levels of reduced glutathione for at least 24 h; ie, rSLPI acts as an equivalent of the "slow release" form of glutathione.

This increase of glutathione is a specific phenomenon restricted to rSLPI—inhalation of buffer or rα1-AT does not influence the glutathione concentration in ELF.\textsuperscript{55} The exact mechanism by which rSLPI increases the glutathione concentration in ELF has not yet been elucidated. It is conceivable that cysteine residues released by rSLPI are of major importance: cysteine is the central component of the tripeptide glutathione. For the synthesis of glutathione, cells need exogenous cysteine.\textsuperscript{57} As described above, rSLPI is a
molecule with 8 disulfide bridges that interconnect 16 cysteine residues. When the cysteine residues are released during the physiologic metabolism of rSLPI in the lung, they can be utilized for the synthesis of glutathione. This hypothesis is supported by the finding that in patients with pulmonary fibrosis, an increase in glutathione concentration in the lung was observed following application of acetylcysteine.58

The finding that the application of rSLPI leads to a rise in glutathione concentration and consequently in the antioxidant activity of ELF is important in the context of protecting the lung epithelium against the destructive consequences of inflammatory processes. Various lung diseases are characterized by an increased burden of NE and reactive oxygen metabolites that jointly are able to overcome the antiprotease and antioxidant protection of the lung, thereby leaving the epithelium and interstitium vulnerable. Although there are other compounds available that either increase the anti-NE activity, such as α1-AT, or raise the antioxidant shield, such as glutathione, rSLPI is currently the only molecule that is obviously capable of improving both major defense components of the lung against inflammatory reactions. rSLPI may therefore be useful as a "single-drug approach" for the treatment of inflammatory lung disorders.

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