Effect of human antileucoprotease on experimental emphysema

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ABSTRACT: Antileucoprotease (ALP), a potent inhibitor of human neutrophil elastase (HNE), may be a modulating factor in the pathogenesis of emphysema. Investigating the clearance of intratracheally-instilled ALP in hamsters, we observed a rapid clearance from the airway lumen within 60 min, whereafter the remaining 40% slowly decreased with a calculated half-life (T_1/2) of 2.8 h. Lung tissue-associated ALP showed a peak at 40 min and slowly decreased (T_1/2 approximately 3 h).

In vivo efficacy of ALP on HNE-induced pulmonary lesions was studied by instillation of either 365 μg or 730 μg ALP, followed after 1 h by 420 μg HNE. Emphysema, haemorrhage and secretory cell metaplasia (SCM) were quantified 21 days after instillations. ALP was found to be able to inhibit emphysema and haemorrhage in a dose-related way, the decrease of haemorrhage being less pronounced. SCM was minimally affected. These results show that ALP inhibits efficiently the development of HNE-induced emphysema and, to a lesser extent, haemorrhage. We speculate that tissue-associated ALP might be responsible for this protection.

A now commonly accepted hypothesis on the development of pulmonary emphysema is the local imbalance between proteolytic enzymes and their inhibitors [1-3]. It has been suggested that elastase from neutrophils degrades the interstitial elastic fibres in the lung, resulting in destruction of alveolar walls and distortion of lung architecture [2, 3].

The most important inhibitors of human neutrophil elastase (HNE) in the lung are plasma derived α1-protease inhibitor (α1PI) [2, 3] and locally produced antileucoprotease (ALP), also called secretory leucocyte proteinase inhibitor [4-6]. Individuals with a severe genetic deficiency of α1PI have an increased risk of developing emphysema [7]. Moreover, several lines of evidence suggest that cigarette smoking, which is the major risk factor for getting emphysema, induces a local elastase/elastase-inhibitor imbalance by oxidative inactivation of α1PI [1-3]. However, neither a severe α1PI deficiency nor smoking cigarettes are invariably associated with the development of emphysema, suggesting that additional factors have an effect on alveolar wall destruction. Such a factor may be ALP, which is a potent reversible inhibitor of HNE in vitro [6, 8-10] and which is produced in bronchial and bronchiolar epithelial cells [11, 12]. Consequently, ALP may play an important physiological role in the protection of proximal and distal airways of the human lung [13, 14]. In addition, the recent observation that ALP is associated with the amorphous elastin fibres in the parenchymal matrix of the alveolar walls of human lungs [15, 16], also strongly suggests that this inhibitor plays a modulating role in the development of emphysema.

Induction of emphysema in laboratory animals, with human neutrophil elastase (HNE), has been described by several authors [13, 17-19]. In addition to emphysematous lesions, HNE also induces bronchial secretory cell metaplasia (SCM) as shown in hamster lungs [20-22]. It has been suggested that, based on this HNE-induced bronchial injury, an imbalance between HNE and its inhibitors may also play a role in chronic bronchitis [13]. In addition to these above mentioned effects intratracheal HNE instillation induces haemorrhage, which is a major acute effect [19, 23].

In the present study we investigated the potency of ALP to inhibit HNE induced emphysema, secretory cell metaplasia and haemorrhage in hamsters. Prior to this we looked at the distribution of intratracheally instilled ALP over several body compartments at several intervals of time.

Based on the results obtained, we conclude that ALP is able to protect alveolar tissue efficiently against HNE-induced destruction. We speculate that it is the tissue-associated ALP-fraction which might be responsible for this protection. ALP, which will be available in the near future, may be an inhibitor with therapeutic potential in emphysema.
Materials and methods

Body-compartment distribution of intratracheally instilled ALP

ALP (molecular mass: 12 Da) was isolated from mucoid sputum by a specific immunoadsorbent [24, 25]. The functional activity of the purified inhibitor was determined using active-site titrated HNE and the synthetic substrate pyro-glutamyl-prolyl-valine-p-nitroanilide (Kabi Vitrum, Stockholm, Sweden) [26]. The ALP preparations used in this study were at least 85% active as measured against HNE. The quantities of HNE and ALP as mentioned in this study refer to active protein.

In order to investigate the distribution of intratracheally instilled ALP in the various organs, ALP was labelled with 125I according to McCosney and Dixon [27]. For this purpose 100 ml ALP (85 mmol·l-1) in phosphate buffered saline (PBS) was mixed with 100 μl phosphate buffer (0.5 mol·l-1, pH 7.2) and 5 μl 125I (500 μCi; Nen Dupont USA). Hereafter, 40 μl chloramine-T (9 mmol·l-1 in 0.25 mol·l-1 phosphate buffer pH 7.5) was added. After 10 s the reaction was stopped by adding 100 μl sodium-metabisulphite (50 mmol·l-1 in 0.25 mol·l-1 phosphate buffer, pH 7.5). Free radioactivity was separated from protein-bound radioactivity using a Sephadex G25 column (0.8 x 16 cm; Pharmacia, Fine Chemicals AB, Uppsala, Sweden). Radio-labelled ALP fractions were then pooled and dialysed against PBS.

Functional activity of 125I-ALP was measured by testing its capacity to bind HNE essentially as described previously [25]. In short, 1.2 mmol non-labelled ALP, to which 13 μCi 125I-ALP (specific activity 25 μCi·nmol-1) had been added, was mixed with 3.5 mmol HNE (molecular mass: 30 kDa) in a final volume of 0.5 ml buffer (0.5 mol·l-1 NaCl, 0.01 mol·l-1 phosphate, pH 7.2, 0.1% gelatin, 0.02% azide). Consequently, the HNE and ALP concentrations in the mixture amounted to 7 and 3.4 μmol·l-1, respectively. After 30 min at room temperature, the mixture was analysed by gel-filtration on a Sephadex G75 column (1.6 x 100 cm; Pharmacia, Fine Chemicals AB, Uppsala, Sweden) in the same buffer. The column was calibrated using ovalbumin (45 kDa), HNE (30 kDa), ALP (12 kDa) and cytochrome c (12.4 kDa). Fractions of 1.5 ml were collected, in which radioactivity was measured in a Packard Minaxi-y-5000 gamma counter (Packard Instrument Company Inc. Downer Grove, Ill., USA). It was observed that the eluted peak which appeared at the position of the HNE-ALP complex (molecular mass: approximately 42 kDa) contained 80% of all radioactivity, whereas the remaining 20% eluted at the position of free ALP. Control experiments were performed by analysing a mixture of non-labelled and 125I-labelled ALP without the addition of HNE or by analysing a mixture of non-labelled ALP, 125I-labelled ALP, HNE and human serum (200 μl) containing 0.5 mg α,PI. Final concentrations of ALP, α,PI and HNE in the latter mixture amounted to 3.4, 19 and 7 μmol·l-1, respectively. In the presence of serum, HNE will irreversibly bind to α,PI and not to ALP, which is a reversible inhibitor. In these two control experiments all radioactivity was eluted from the Sephadex G75 column at the position of free ALP. It is concluded from these series of experiments that at least 80% of the labelled ALP is able to form a complex with HNE and thus functionally active. Due to dilution of the reversible HNE-ALP complex (K = 3.1010 mol·l-1) [6] during gel-filtration, some dissociation of the complex might have occurred resulting in underestimation of the percentage as found. Thus, any significant inactivation of functionally active ALP did not occur as a result of the labelling procedure.

Several series of in vivo experiments with radio-labelled ALP were performed with a total of 21 male, Syrian golden hamsters (Mesocricetus auratus), 14-16 wks of age, average weight 135 g (range 100-171 g). After the animals were anesthetized with CO2, 100 μl radio-labelled ALP (=6 μmol·l-1 in PBS, specific radioactivity 120 μCi·ml-1) together with 100 μl unlabelled ALP (85 μmol·l-1 in PBS) was given by intratracheal instillation. At twelve different time points after instillation (15 min to 24 h) hamsters were anesthetized with an overdose of sodium pentobarbital (0.4 ml, 60 mg·ml-1). The abdomen was opened, followed by opening of the thoracic cavity by cutting the diaphragm. Blood was obtained with a puncture in the right ventricle of the heart. Thereafter, the hamster was exsanguinated by cutting the left renal artery. A lavage of total lung was performed using 5 x 5 ml 0.6 mmol·l-1 edetic acid (EDTA) in PBS of 4°C. Cells in the lavage fluid were recovered by centrifugation (10 min at 500 g). Urine was obtained by a bladder puncture. Subsequently, lungs, stomach, liver, kidneys and thyroid gland were removed and weighed. These organs and samples taken from blood, lavage-fluid and urine were counted for radioactivity in a Packard Minaxi-y-5000 gamma counter. Occasionally, radioactivity of the stomach and its content was counted separately.

Assuming the total blood volume in a hamster to be 7.5% of its body weight [28], we calculated radioactivity of the total blood volume. Radioactivity was also calculated for the total lavage volume. Half-life during the elimination phase from the airway lumen was calculated using the formula lnAt-lnAt-kt in which A represents the concentration at time point one and A, the concentration at time point two. The interval between the two time points is t, and the elimination rate constant is k. Radioactivity in urine was expressed per ml because total urine production during the in vivo incubation was not known. We determined the distribution pattern of ALP in the different body compartments by calculating radioactivity in an organ- or fluid-compartment as the percentage of total instilled radioactivity.

In an additional experiment, body-compartment distribution was also determined after instillation of non-labelled ALP. This experiment was performed in order to verify whether distribution patterns of radio-labelled and non-labelled ALP are similar. Six male, Syrian golden hamsters were used. Four hamsters...
received 730 µg ALP in 200 µl PBS intratracheally. The other two hamsters served as controls. The hamsters were anaesthetized 40 min after instillation with an overdose of sodium pentobarbital (0.4 ml, 60 mg·ml\(^{-1}\)). Blood, lavage fluid, urine, lungs, kidneys, stomach and liver were obtained as described above. ALP was quantitated in this experiment with a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) [29]. Before performing the immunoassay, tissues were weighed, homogenized in 10 ml of buffer (0.07 mol·l\(^{-1}\) phosphate, 0.5 mol·l\(^{-1}\) NaCl, pH 6.0) using an Ultra-Turrax homogenizer (Janke & Kunkel IKA-work, Staufen) and subsequently treated with perchloric acid as described previously [14, 29]. In addition, the inhibitory activity in the lavage fluid of the hamsters was measured against HNE, using the substrate pyro-glutamyl-prolyl-valine-p-nitroanilide according to a method described previously [26].

Effect of intratracheally instilled ALP on HNE-induced lung injury

HNE was isolated from purulent sputum by extraction as described by TWUMAHI and LIENER [30] followed by affinity chromatography and gel-filtration [31, 32]. The enzyme was active-site titrated using N benzoyloxycarbonyl-alanyl-alanyl-prolyl-aza-alanine-p-nitrophenylester (Enzyme Systems Products, Livermore, California, USA) as described by POWERS et al. [33]. ALP was isolated and titrated as described above.

The in vivo study was performed in duplicate. The experiments were carried out with five groups of six male, Syrian golden hamsters aged 16 wks, average weight 140 g (range 106–172 g) and later with five groups of eight female, Syrian golden hamsters, aged 17 wks, average weight 149 g (range 118–183 g). After being anaesthetized with CO\(_2\), both groups of hamsters were given two intratracheal instillations according to the following schedule: Group 1 (PBS/HNE): PBS followed by 420 µg HNE (14 nmol); Group 2 (ALP365/HNE): 365 µg ALP (30 nmol) followed by 420 µg HNE; Group 3 (ALP730/HNE): 730 µg ALP followed by 420 µg HNE; Group 4 (PBS/PBS): PBS followed by PBS; Group 5 (ALP730/PBS): 730 µg ALP followed by PBS.

Each animal received two instillations of 200 µl each. In all cases the first instillation was given 1 h before the second. After 21 days the animals were anaesthetized with an overdose of sodium pentobarbital (0.4 ml, 60 mg·ml\(^{-1}\)) followed by cutting the diaphragm to make a pneumothorax. Next, the left renal artery was cut and after exsanguination the lungs were carefully removed. The lungs were then inflated with a solution containing 4% formaldehyde and 1% glutaraldehyde [34] at a pressure of 25 cmH\(_{2}\)O [35] for 15 min. The lungs were stored in the fixative for 48 h. Two transversal slices were cut from the left lung. One slice was taken at the position where the left main bronchus enters the lung and the second slice was taken 0.5 cm more distally. The slices were dehydrated in graded series of ethanol and embedded in paraffin. From these slices, sections of 6 µm thickness were cut and stained with haematoxylin-eosin (HE), with periodic acid-Schiff (PAS) or with potassium ferrocyanide in hydrochloric acid [36].

Emphysema was measured by calculating the mean linear intercept (L\(_m\)) according to DUNN [37] and TRUETTER [35] in HE-sections of each slice. L\(_m\) was expressed as the average of these two measurements for each animal. The mean L\(_m\) of each group was calculated by using the L\(_m\) of each animal. Secretory cell metaplasia (SCM) was evaluated by scoring PAS-positive cells in the central bronchi using a six point scale, ranging from 0 (=normal) to 5 (=severe) essentially as described by CHRISTENSEN et al. [38]. Scoring was performed in sections taken from both slices. The mean of these two scores (called SCM-score) was the overall score of each animal. A mean SCM-score of each group was calculated by using the score of each animal. Alveolar haemorrhage was quantified by scoring positive grains in ferrocyanide/hydrochloric acid stained sections. A six point scale was used for quantification. These scores were calculated in a similar way to the SCM-scores and expressed as Fe-scores.

To be able to compare the three different parameters of destruction, inhibition by ALP on HNE-induced damage was expressed as percentage.

Statistical analyses were performed using the non-parametric Kruskal-Wallis test in which a p-value ≤0.05 was considered to be significant. When significance was found by this test, further analysis was performed between data of mutual treatment groups within an experiment using the non-parametric Mann-Whitney test. A p-value ≤0.02 was considered to be significant.

Results

Body-compartment distribution of intratracheally instilled ALP

The clearance of ALP from the airway lumen was determined by measuring the radioactivity in the lung lavage fluid obtained at different times after instillation. As can be seen in figure 1, clearance from the airway lumen occurs in a biphasic fashion. During the first phase approximately 60% of the instilled dose is rapidly cleared. The second phase starts approximately 50 min after the instillation, showing a calculated half-life of 2.8 h.

The lung tissue-associated fraction of ALP, defined as the fraction which could not be washed out by lavage, was found to reach its maximum approximately 40 min after instillation. At that time about 15% of the instilled dose is lung tissue-associated. Thereafter, its decrease occurs as slowly as the decrease of ALP in the airway lumen (fig. 1).

As can be seen in figure 2, in the urine ALP shows a steady increase in time. In the blood, 40 min after instillation, about 8% of the instilled dose is present and this level remains constant for several hours (fig. 2).
Figure 1. — Clearance of $^{125}$I-ALP from airway lumen (open circles) and lung tissue uptake (asterisks) after intratracheal instillation of a single dose. ALP-levels are expressed as percentage of the instilled dose. Lines are drawn by curve-fitting. ALP: antileucoprotease.

Fig. 2. — ALP-levels in blood (open circles) and urine (asterisks) at different time intervals after intratracheal instillation of $^{125}$I-ALP. Each point, except that at 24 h, represents mean of two experiments. Blood levels are expressed as percentage of instilled dose per total blood volume. Urine levels are expressed as percentage of the instilled dose per ml. Based on water intake, at the most 10 ml urine was produced in 24 h. Lines are drawn by curve-fitting. ALP: antileucoprotease.

Figure 3 shows that part of the instilled dose rapidly enters the stomach. Analysing the stomach and its content separately, almost all radioactivity is present in the latter (fig. 3).

The amount of ALP reaches its maximum in both kidneys 40 min after instillation, and after that decreases steadily. The liver never contains more than 3% of the instilled dose, while the amount of ALP in the thyroid gland never reaches values higher then 0.5% of the instilled dose (data not shown).

Additional experiments were performed in which non-labelled ALP was instilled intratracheally and measured 40 min later by immunosorbent assay in several organs. The results of these experiments are presented in table 1. A similar organ distribution of ALP was found compared to that obtained with $^{125}$I-labelled ALP (figs 1–3). Measuring the elastase inhibitory activity in the lavage fluid of the ALP-treated hamsters, we observed that this activity amounted to 34±8% of the instilled dose (table 1). This percentage was similar to the value obtained using radio-labelled ALP (approximately 40%) (fig. 1), which indicates that during 40 min after instillation no or minimal inactivation of the inhibitor occurred in the airway lumen. Under identical test conditions, no inhibitory activity in the lavage fluid of the untreated hamsters was detectable.

Table 1. — Distribution of intratracheally instilled ALP* (730 µg) in hamsters over several body-compartments 40 min after instillation

<table>
<thead>
<tr>
<th>% of instilled dose (n=4)</th>
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<tr>
<td>Blood</td>
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<td>Urine % expressed per ml</td>
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<td>Liver</td>
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Data are given as mean±so. *: ALP was quantified by ELISA; ELISA results obtained with samples from control hamsters did not exceed baseline values; **: based on activity measurements against elastase, the percentage in BAL fluid of the instilled dose amounted to 34.2±8.1 (mean±sd). ALP: antileucoprotease; ELISA: enzyme-linked immunosorbent assay; BAL: bronchoalveolar lavage.
Effect of intratracheally instilled ALP on HNE-induced lung injury

We investigated whether ALP was capable of inhibiting the HNE-induced injuries in the hamster lungs. We therefore instilled ALP, in two different amounts, 1 h before the instillation of elastase. As can be seen in figure 4, HNE-induced emphysematous lesions, as measured by $L_m$, were inhibited by ALP in a dose-related manner. Both doses of ALP resulted in a significant inhibition of the HNE-induced lesion. The HNE-induced increase in $L_m$ was significantly inhibited by approximately 35 and 70%, respectively, when 365 µg or 730 µg of ALP had been instilled (p<0.01 by Mann-Whitney test). An effect of ALP was also observed on the HNE-induced Fe-score (haemorrhage) (fig. 5). ALP in a dose of 365 µg and 730 µg was able to inhibit haemorrhage by 22 and 44%, respectively. However, the HNE-induced haemorrhage in the presence of ALP was not significantly different from that in the absence of ALP (p=0.06 by Mann-Whitney test). In contrast to the effect on HNE-induced emphysema and haemorrhage only a minimal non-significant effect of ALP was observed on the HNE-induced bronchial secretory cell metaplasia (fig. 6). SCM was inhibited by approximately 14% in both doses of ALP. No lung injury, as measured by $L_m$, SCM-score and Fe-score, was observed after consecutive instillations of PBS followed by PBS, or ALP followed by PBS. However, in some animals from the control groups in which ALP was instilled followed by PBS, a minimal SCM and haemorrhage was induced (figs 5 and 6).

Fig. 4. - Effect of intratracheally instilled ALP on neutrophil elastase-induced emphysema in hamsters. Results shown represent mean $L_m$ (±SEM), obtained from two sets of experiments performed with male or female animals, respectively. Hatched bars represent the first set, open bars represent the second set. The numbers of evaluated lungs in each treatment group are indicated between parentheses. ALP (365 µg or 730 µg) or PBS was instilled 1 h before the instillation of HNE (420 µg). Lungs were examined 21 days after treatment. ALP: antileucoproteinase; PBS: phosphate buffered saline; HNE: human neutrophil elastase.

Fig. 5. - Effect of intratracheally instilled ALP on neutrophil elastase-induced haemorrhage (Fe-score) in hamsters. Individual data of two sets of experiments (closed circles: exp. 1; asterisks: exp. 2) are depicted. Mean of each treatment group is depicted with an interrupted line in experiment 1 and with a dotted line in experiment 2. For experimental details and further abbreviations see legend to figure 4.
Fig. 6. - Effect of intratracheally instilled ALP on neutrophil elastase-induced secretory cell metaplasia (SCM-score) in hamsters. Individual data of two sets of experiments (closed circles: exp. 1; asterisks: exp. 2) are depicted. Mean of each treatment group is depicted with an interrupted line in experiment 1 and with a dotted line in experiment 2. For experimental details and further abbreviations see legend to figure 4.

Discussion

The present study shows that purified human ALP is able to inhibit efficiently the development of HNE-induced emphysema and, to a lesser extent, pulmonary haemorrhage. In contrast, HNE-induced SCM was not, or was only minimally, effected by ALP.

Prior to the investigation of the capacity of ALP to inhibit HNE-induced pulmonary injuries in hamsters, we performed experiments in which the body compartment distribution of intratracheally instilled ALP was studied. The latter experiments show that ALP is cleared from the airway lumen by a biphasic mechanism (fig. 1). The first phase, which is a rapid clearance taking place within 60 min, can best be explained by a relatively rapid diffusion into the surrounding tissues and, in addition, by an overflow to the gastro-intestinal tract (figs 1–3). Pilot experiments, in which the instilled ALP was dissolved in 500 μl instead of 200 μl, showed a larger overflow to the gastro-intestinal tract suggesting that the overflow is proportional to the instilled volume (data not shown). In the experiments described, we used a relatively small volume of 200 μl to minimize this overflow to the stomach.

The second phase of the biphasic clearance of ALP from the airway lumen was characterized by a relatively slow disappearance with a half-life of 2.8 h (fig. 1). Clearance during this second phase is probably attributable to excretion of ALP. Our data suggest that this occurs mainly by the renal route (fig. 2). Clearance by the liver or the gastro-intestinal tract is negligible.

It was observed that part of the instilled ALP could not be washed out from the lung by 5 × 5 ml of buffer (lung-tissue-associated fraction). The highest level of this lung-tissue-associated fraction was found 40 min after instillation and amounted to 15% of the instilled dose (fig. 1). In a recent light- and electron-microscopic investigation on human lung tissue we were able to demonstrate ALP in the parenchymal matrix of the alveolar walls exclusively in association with elastin fibres [15, 16]. There, ALP may function as an inhibitor of elastin-bound elastase and protect the elastin-fibres quite efficiently against the destructive action of HNE [4, 8, 9]. By performing an ALP-specific immunostaining on tissue sections of lavaged lungs of ALP-treated hamsters we observed that the tissue-associated ALP fraction was, at least partly, associated with interstitial elastin fibres, similar to our findings in the human lung [15, 16] (manuscript in preparation). It might be this elastin-associated fraction which is of major importance in protecting the hamster lung from HNE-induced emphysema. Based on the results of the ALP clearance study, a time interval of 1 h was chosen between the instillation of ALP and HNE to investigate the in vivo effect of ALP on HNE-induced emphysema. After 1 h, the first phase during which ALP is rapidly cleared from the airway lumen, has passed whereas the tissue associated fraction in the lung has reached its maximum value.

To verify that the radioactivity, as measured in the clearance study, is indeed ALP-associated, we also performed an experiment with non-labelled ALP in which the inhibitor concentrations in body compartments were
measured by ELISA 40 min after instillation. Similar results were obtained when the two experiments were compared (figs 1–3; table 1), suggesting that the radioactivity, as measured in the different body compartments, indeed represents ALP and that ALP is not rapidly metabolized. The stability of ALP during 40 min after instillation was also confirmed by the measurements of the elastase inhibitory activity of lavage-fluid samples, taken from hamsters treated with non-labelled ALP and using a synthetic low-molecular weight substrate [26] (table 1).

Investigating the in vivo potency of ALP to inhibit HNE-induced pulmonary injuries, we observed a dose-related inhibition of the emphysematous lesions as measured by *L* ~w~ (fig. 4). Instilling 365 μg and 730 μg ALP, and taking into account that 60% has been cleared, molar ratios of ALP over HNE in the airway lumen, at the moment that HNE was administered, were calculated to be 0.86 and 1.74, respectively. Under these conditions the HNE-induced emphysematous lesions were inhibited by 35 and 70%, respectively. Comparing the in vivo and in vitro potency of ALP, the inefficiency could be calculated, as described by Lucey and co-workers [39]. Calculated inefficiency of ALP was 1.74/0.7 = 2.5. In vivo studies performed by Lucey and co-workers [39, 40] with eglin-c and with an oligopeptide chloromethyl ketone (CMK) revealed inefficiency ratios of 2.5 and 2.9, respectively, which are comparable with that of ALP. According to Lucey and co-workers [40] the relatively low potency of instilled inhibitors may reflect unequal distribution of both the inhibitor and HNE in the lungs. Based on the fact that ALP, in contrast to eglin-c and CMK, is a reversible inhibitor of HNE, we expected to find a higher inefficiency ratio for ALP as compared to the values found for eglin-c and CMK. Investigating the effect of meo-suc-al-a-lala-pro-ambo-boro-val, which is a reversible inhibitor, Stone et al. [41] even observed a potentiating effect on HNE-induced emphysema. The relatively high potency of ALP to inhibit HNE-induced emphysema, as observed in our study, can be explained by assuming that ALP is able to operate as an inhibitor of elastin-bound elastase. This role of ALP was suggested by Gaethier et al. [4] and evidence for this has been obtained by more recent studies [8, 9, 16]. If so, this will mean that the most active fraction of the instilled ALP is represented by that part which is tissue-associated.

ALP was also found to inhibit the HNE-induced haemorrhage (fig. 5). Instillation of 365 μg and 730 μg ALP, 1 h before HNE was given, resulted in inhibition of the haemorrhage as measured by Fe-score, by 22 and 44%, respectively. Due to a large scatter of data within each group, the inhibition by ALP was, however, not truly significant (p-value=0.06). The relatively poor inhibition of HNE-induced haemorrhage, based on actual ALP/HNE ratios in the airway lumen of 0.86 and 1.74 might be explained by the reversible character of the inhibitor.

It is known that HNE is capable of inducing SCM only when the enzyme is in its active form [21]. Based on this fact, we expected to observe relevant inhibition of SCM at a molar ALP/HNE ratio in the airway lumen of 1.74. However, in contrast to the effect on the induction of emphysema and haemorrhage, instillation of ALP only resulted in a minimal diminishing effect of HNE-induced SCM (fig. 6). Likewise, Lucey and co-workers [42] observed a significant effect of recombinant ALP on HNE-induced SCM only at very high inhibitor concentrations (molar ALP/HNE ratio of instilled dose 30). The characteristic of ALP, being a reversible inhibitor, may partly explain its low potency to inhibit SCM. However, the irreversible elastase inhibitor succinyl-(alanyl)-prolyl-valine-chloromethyl ketone, as investigated by Lucey and co-workers [39], also showed no significant inhibition of HNE-induced SCM, whereas the development of emphysema was clearly inhibited. Eglin-c, another potent irreversible HNE inhibitor, also produced minimal protection against the development of SCM [43].

In one out of two control experiments in which ALP was instilled followed by PBS, minimal haemorrhage and SCM was induced (see figs 5 and 6). The reason for this is unknown but can possibly be ascribed to some unknown contamination in this purified ALP preparation which was certainly not present in the preparation used in the second experiment (see figs 5 and 6).

Although the animal model as used in this study cannot be extrapolated to the human situation [19], we showed that ALP may act as a potent inhibitor of HNE in vivo. Starting from the hypothesis that emphysema is an HNE-mediated disease [1], our results add additional support to a role of ALP, as a modulating factor in the pathogenesis of emphysema. In addition, the present results might have clinical implications. Emphysema in *α*,*PI* deficient individuals is thought to be due to an unrestrained action of neutrophil elastase. Therefore, strategies to prevent deficiency-associated emphysema have focused on augmenting the elastase inhibitor activity in the lower respiratory tract [44]. ALP is a potent inhibitor of neutrophil elastase and of human origin. Moreover, as shown in this study, ALP, purified from mucoid sputum, is found to be effective in inhibiting experimental emphysema.

Therefore, ALP may be of therapeutic significance to restore the elastase/elastase-inhibitor balance in the lungs of *α*,*PI* deficient individuals. Very recently, ALP and its carboxy-terminal domain, have been successfully expressed in both *Saccharomyces cerevisae* and *Escherichia coli* [45, 46]. The encoded proteins show strong anti-elastolytic activity and may prove to be very useful in therapeutic applications.

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References


IN VIVO EFFECT OF ANTIHEUCOPROTEASE


Note added in proof

After acceptance of this manuscript, a publication appeared of Lucey et al. (J Lab Clin Med, 1990, 115, 224-232) who reported amelioration by recombinant human secretory leukocyte-protease inhibitor of elastase-induced emphysema and secretory cell metaplasia in hamsters.