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Inhibition of neutrophil elastase activity attenuates complement-mediated lung injury in the hamster

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Abstract

The role of neutrophil elastase in complement-mediated lung injury was examined in hamsters using a specific neutrophil elastase inhibitor, sodium *N*-[2-[4-(2,2 dimethylpropionyloxy)phenylsulfonylamino]benzoyl]aminoacetate tetrahydrate (sivelestat). Intravenous injection with cobra venom factor (CVF) into hamsters transiently increased plasma neutrophil elastase activity by about 10-fold. This increase was followed by a sustained increase in lung vascular [^{125}I]bovine serum albumin permeability peaking 30 min after CVF injection. The increase in lung vascular permeability was associated with neutrophil accumulation in lung tissue and an increase in protein concentration in the bronchoalveolar lavage fluid. Inhibition of the elevated plasma neutrophil elastase activity (36.5%, 66.9% and 104.3%) by continuous i.v. infusion with sivelestat (0.1, 0.3 and 1 mg/kg/h), dose-dependently attenuated the increase in lung vascular permeability 30 min after CVF injection. Furthermore, sivelestat at 1 mg/kg/h almost totally prevented the increase in protein concentration in the bronchoalveolar lavage fluid without affecting lung neutrophil accumulation. These results suggest that neutrophil elastase is an important mediator in complement-mediated acute lung injury. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Neutrophil elastase; Cobra venom factor; Lung injury, acute; Sivelestat

1. Introduction

Complement activation is a critical event in many acute inflammatory responses that subsequently injure tissues. As a result of complement activation, the anaphylatoxin C5a is generated from a complement cascade causing an increase in vascular permeability, and the release of reactive oxygen species and proteases from neutrophils (Worthen and Henson, 1983). An increasing body of evidence has shown that systemic complement activation by bolus intravenous injection of cobra venom factor (CVF) into several animal species results in acute lung injury as indicated by an increase in lung vascular permeability (Till et al., 1982; Johnson et al., 1986; Doerschuk et al., 1996). Inhibition of cell adhesion, neutrophil depletion, and pretreatment with catalase, iron chelators, or hydroxy radical scavengers significantly attenuate CVF-induced lung injury in rats, suggesting that neutrophil-derived reactive oxygen species play an important role in this type of acute lung injury (Mulligan et al., 1994; Ward et al., 1985; Till and Ward, 1986). However, the contribution of other neutrophil-derived mediators such as proteases in complement-mediated acute lung injury remains unclear.

Among these proteases, neutrophil elastase may be of a particular interest. This protease is capable of degrading key structural elements of connective tissues such as elastin, collagen and proteoglycan (Havemann and Gramse, 1984), leading to lung vascular and trans-alveolar protein leakage and dysfunction. Indeed, neutrophil elastase has been implicated in the increase of permeability both in vascular endothelial (Suttorp et al., 1993) and alveolar epithelial cells (Peterson et al., 1995) that are considered to be deeply involved in lung edema. Furthermore, it has been assumed that neutrophil elastase and reactive oxygen species synergise to induce tissue damage by a mechanism in which reactive oxygen species inactivate α_1-protease inhibitor, a major endogenous elastase inhibitor in the body, thereby allowing neutrophil elastase to attack tissues (Weiss, 1989). In this study, we hypothesized that neutrophil elastase is an important mediator in complement-mediated acute lung injury. To verify this hypothesis, we examined the relationship between the development of acute lung injury and plasma neutrophil elastase activity,

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and the effect of a specific neutrophil elastase inhibitor, sodium *N*-[2-[4-(2,2-dimethylpropionyloxy)phenylsulfonylamino]benzoyl]aminoacetate tetrahydrate (sivelestat) on this injury in CVF-injected hamsters. Sivelestat, also known as ONO-5046 · Na, has previously been shown to inhibit neutrophil elastase derived from several animal species including hamsters and to have no effect on other proteases such as cathepsin G and plasmin (Kawabata et al., 1991) and on the production of reactive oxygen species from neutrophils (Iwamura et al., 1993).

2. Materials and methods

2.1. Animals

Male golden hamsters (Keari, Osaka, Japan) weighing 90–120 g were used in this study. The animals were housed in an air-conditioned room at 23 ± 2 °C and $55 \pm 10\%$ humidity with alternating 12-h light/dark cycle. Animals were given food and water ad libitum until use. Animal experiments were performed in accordance with the institutional animal care guidelines of Ono Pharmaceutical Company.

2.2. Animal model

A polyethylene catheter (outer diameter, 0.61 mm; inner diameter, 0.28 mm) was inserted into the left femoral vein, passed under the skin and exteriorized in the dorsal neck of each animal under ketamine hydrochloride (100–150 mg/kg, i.p.) anesthesia. The free end of the catheter was connected to an infusion pump (model 55-1111, Harvard Apparatus, South Natrick, MA) for drug administration. Animals usually recovered from anesthesia within 1 h and thereafter were allowed free movement. After the animals recovered from anesthesia, they were continuously infused with saline at a rate of 0.2 ml/h using the infusion pump. Ninety minutes after the start of saline infusion, CVF (10 U/2 ml/kg) followed by either [^{125}I]bovine serum albumin (0.5 μCi/0.2 ml/animal) or equal volume of saline were injected via the catheter. At specified time intervals after CVF injection, lung vascular permeability index, protein concentration in bronchoalveolar lavage fluid, neutrophil accumulation in lung tissue and plasma neutrophil elastase activity were measured by the methods described below.

2.3. Evaluation of lung injury

2.3.1. Lung vascular permeability index

Animals that had been injected [^{125}I]bovine serum albumin as described above were used to determine lung vascular permeability index according to the method of Till and Ward (1986). Under sodium pentobarbital (60 mg/kg, i.p.) anesthesia, 1 ml of citrated blood was taken from the abdominal aorta of each animal. Animals were then sacrificed by transection of the abdominal aorta and their lungs were perfused with 10–15 ml of saline via a catheter, which was inserted from the right cardiac ventricle to the pulmonary artery. Lungs were then isolated and radioactivity counts in the perfused lung tissue and aortic blood were determined with a gamma counter (ARC-370M, Aloka, Tokyo, Japan). Lung vascular permeability index was defined by the ratio of radioactivity in lung tissue to that in 1 ml of aortic blood.

2.3.2. Protein concentration in bronchoalveolar lavage fluid

Animals that had been injected saline instead of [^{125}I]bovine serum albumin were used. The procedures for aortic blood sampling and animals sacrifice were the same as those for the determination of lung vascular permeability index. After the animals were sacrificed, their whole lungs were lavaged five times with a single volume (2.8 ml) of citrated saline via a tracheal cannula attached to a syringe. The bronchoalveolar lavage fluid was then centrifuged ($1700 \times g$, 10 min, 4 °C) and the protein concentration in the supernatant determined by the Lowry's method.

2.3.3. Neutrophil accumulation in lung tissue

After bronchoalveolar lavage, whole lung tissues were excised and their myeloperoxidase activity was determined by a previously described method (Kawabata et al., 2000) as an index for neutrophil accumulation in lung tissue. Briefly, the lung was excised, added to 3 ml of 50 mM KPO_4 buffer (pH 6.0) containing 0.5% cetyltrimethylammonium bromide and homogenized with Physcotron (Bio-Mixer, model ABM-1, Nihonseiki kaisha, Tokyo, Japan). The tissue homogenate was centrifuged ($15{,}000 \times g$, 10 min, 4 °C), and the supernatant was diluted 5-fold with the KPO_4 buffer. Fifty microliters of the diluted supernatant was mixed with 1.4 ml of 0.00107% H_2O_2 (diluted with KPO_4 buffer). The mixture was then added to 50 μl of 30 mM *o*-dianisidine aq., and the change in absorbance was monitored at 450 nm for 1 min. An increase in absorbance was regarded as the tissue myeloperoxidase activity.

2.3.4. Measurement of plasma neutrophil elastase activity

Plasma was separated from citrated arterial blood which had been taken from the abdominal aorta of each animal and was used for the determination of neutrophil elastase activity. Plasma neutrophil elastase activity was determined spectrophotometrically using *N*-methoxysuccinyl-Ala-Ala-Pro-Val *p*-nitroanilide, a highly specific synthetic substrate for neutrophil elastase according to the method of Yoshimura et al. (1994). Briefly, plasma was incubated with 0.1 M Tris–HCl buffer (pH 8.0) containing 0.5 M NaCl and 1 mM substrate at 37 °C for 24 h and the amount of *p*-nitroanilide liberated was measured spectrophotometrically at 405 nm and was considered as neutrophil elastase activity.

2.4. Effect of sivelestat on CVF-induced complement activation in hamster

Based on the method described by Platts-Mills and Ishizaka (1974), the effect of sivelestat on CVF-induced complement activation in hamster was studied using CVF-induced hemolysis. Briefly, heparinized blood was taken from the abdominal aorta of normal hamsters under pentobarbital anesthesia (60 mg/kg, i.p.), and 350 μl of blood was added to 50 μl of gelatin–veronal buffer containing 0.1% Mg^{2+} and 30 mM ethylene glycol bis (β-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) (pH 7.3–7.4). After pre-incubation at 37 °C for 2 min, the mixture was added to 50 μl of CVF, dissolved in gelatin–veronal buffer (final concentration 30 mU/ml), and 50 μl of sivelestat (final concentration 100 μM) and was further incubated at 37 °C for 15 min. After incubation, 500 μl of gelatin–veronal buffer containing 10 mM of EDTA was added to the mixture to terminate the reaction. The mixture was then centrifuged (1700 × *g*, 10 min, 25 °C) and the supernatant was separated. The absorbance of the supernatant was measured at 412 nm and used as a measure of hemolysis.

2.5. Compounds administration and efficacy

Based on a previous ex vivo study, indicating that 90-min continuous infusion of sivelestat achieved steady state plasma neutrophil elastase inhibition in hamsters (Kawabata et al., 2000), we started continuous intravenous infusion of sivelestat 90 min before 30 min after CVF injection via a femoral catheter. A radical scavenging enzyme, catalase, was also infused in the same manner as sivelestat.

2.6. Drugs and chemicals

Sivelestat sodium hydrate, sodium *N*-[2-[4-(2,2-dimethylpropionyloxy)phenylsulfonylamino]benzoyl]aminoacetate tetrahydrate, (sivelestat; ONO-5046 · Na) was synthesized in our laboratory. Cobra venom factor (CVF, from Naja naja kaouthia), catalase, *N*-methoxysuccinyl-Ala-Ala-Pro-Val *p*-nitroanilide, *o*-dianisidine were purchased from Sigma (St. Louis, MO). [^{125}I]bovine serum albumin (specific activity 3.4–4.3 μCi/μg) was obtained from NEN Research Products (Boston, MA). Sivelestat was dissolved in saline with a small amount of Na_2CO_3 (2 μl of 0.5 M Na_2CO_3/mg sivelestat). *N*-methoxysuccinyl-Ala-Ala-Pro-Val *p*-nitroanilide was dissolved in 1-methyl-2-pyrrolidone. *o*-Dianisidine was dissolved in distilled water. Other agents were dissolved in saline.

2.7. Statistical analysis

All data were expressed as the mean ± S.E.M. Statistical significance was analyzed either by Student's two-tailed *t*-test or one-way analysis of variance followed by Dunnett's *t*-test. *P*-values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. Changes in plasma neutrophil elastase activity and lung vascular permeability index

A preliminary study indicated that CVF at 1, 3 and 10 U/kg increased both lung vascular permeability and plasma neutrophil elastase activity in a concentration-related manner. We, therefore, used 10 U/kg of CVF in all subsequent experiments. As shown in Fig. 1, intravenous

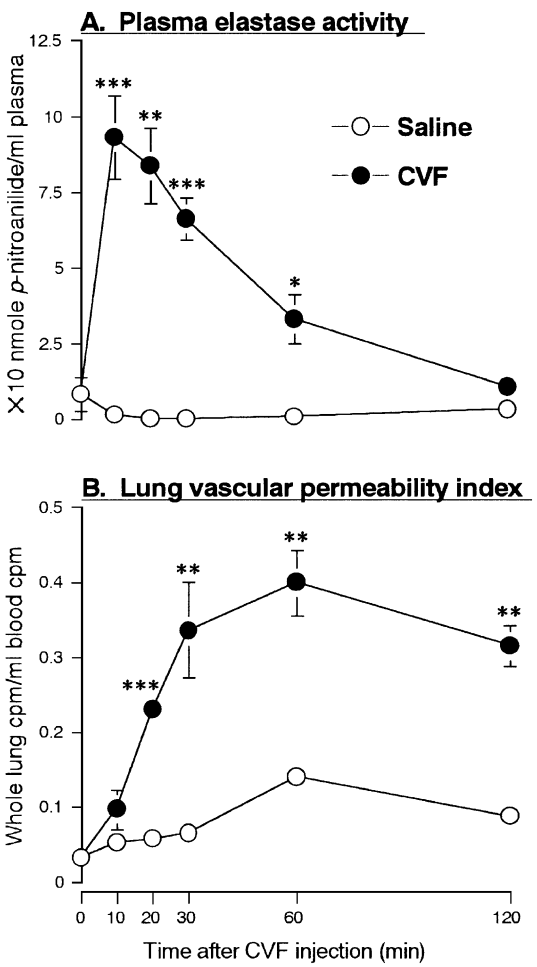

Fig. 1. Changes in plasma neutrophil elastase activity (A) and lung vascular permeability (B). [^{125}I]bovine serum albumin was intravenously injected immediately before the injection of CVF. At specified time points, 1 ml of citrated aortic blood was taken from each animal as described in the text and lung vascular permeability index as defined by the ratio of [^{125}I]bovine serum albumin radioactivity present in the lung tissue to that in 1 ml of aortic blood was measured. Plasma neutrophil elastase activity was determined spectrophotometrically with a synthetic substrate, *N*-methoxysuccinyl-Ala-Ala-Pro-Val *p*-nitroanilide. A group of three to four animals were used to study each point (saline-injected group: ○, CVF-injected group: ●). $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. relevant saline-injected group (Student's *t*-test).

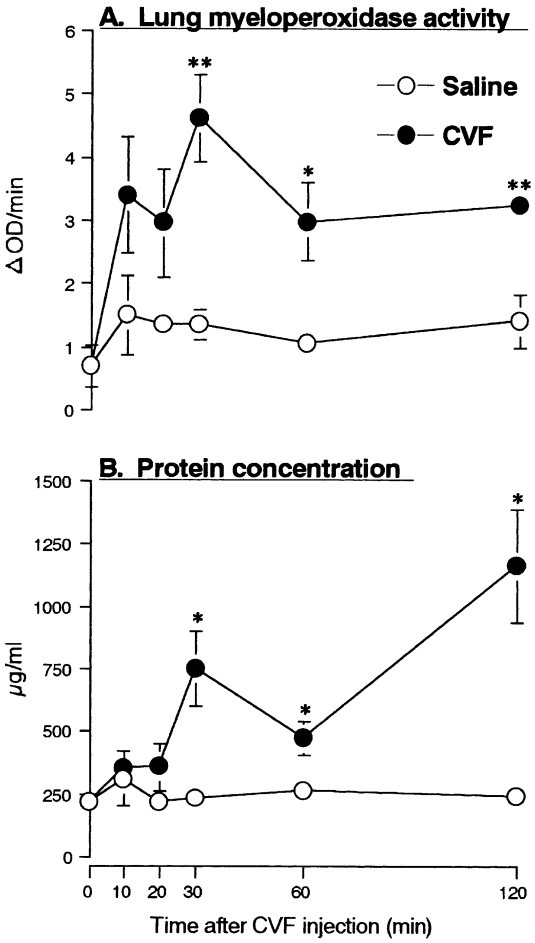

Fig. 2. Changes in lung myeloperoxidase activity (A) and protein concentration in bronchoalveolar lavage fluid (B). Animals were sacrificed at specified time points and protein concentration in bronchoalveolar lavage fluid and myeloperoxidase activity of lavage lungs were determined as described in the text. The myeloperoxidase activity in lavaged lungs was considered to be the number of neutrophils infiltrated in lung tissue. A group of four to six animals were used to study each point (saline-injected group: ○, CVF-injected group: ●). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. relevant saline-injected group (Student's *t*-test).

injection of CVF (10 U/kg) transiently elevated plasma neutrophil elastase activity by approximately 10-fold with the highest activity 10 min after CVF injection. The activity remained higher than that in the saline-injected animals for 60 min after CVF injection and thereafter returned to the basal level. Cobra venom factor injection also increased lung vascular permeability following the increase in plasma neutrophil elastase activity with a high level remaining at least until the end of the observation period (120 min after CVF injection). In the saline-injected animals, such changes were not observed throughout the observation period.

3.2. Changes in lung neutrophil accumulation and protein concentration in bronchoalveolar lavage fluid

Lung myeloperoxidase activity, an index for neutrophil accumulation in lung tissues, peaked 30 min after CVF injection (Fig. 2). The activity remained higher than that in the saline-injected group for observation period. Under our experimental conditions, inflammatory cell count and neutrophil elastase activity in bronchoalveolar lavage fluid were unchanged at least for 120-min post-CVF injection. Protein concentration in bronchoalveolar lavage fluid increased biphasically 30 and 120 min after CVF injection with the higher value 120 min after CVF injection.

3.3. Effects of sivelestat on the increase in plasma neutrophil elastase activity and lung vascular permeability

The efficacy of sivelestat was examined 30 min after CVF injection. In the CVF control, plasma neutrophil elastase activity and lung vascular permeability increased 4.5-fold and 4.9-fold as compared with the saline-injected

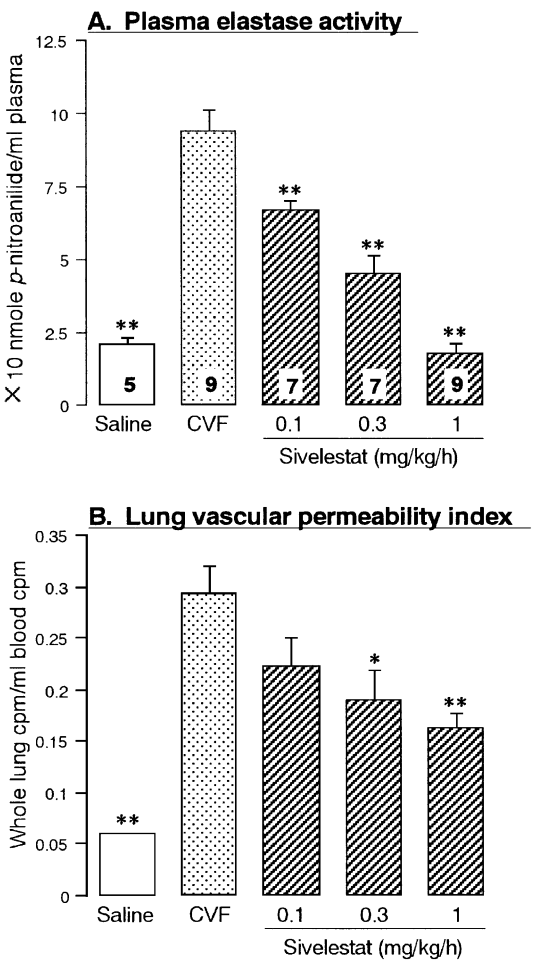

Fig. 3. Effect of sivelestat on the increase in plasma neutrophil elastase activity and lung vascular permeability. Thirty minutes after the injection of CVF and [^{125}I]bovine serum albumin, plasma neutrophil elastase activity (A) and lung vascular permeability (B) were determined as described in Fig. 1. In the saline control group, animals were intravenously injected saline instead of CVF. Sivelestat or saline was intravenously infused 90 min before to 30 min after CVF injection. Numbers attached to each column represent the number of total animals in each group. The results indicate mean ± S.E.M. of each group. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. CVF-injected group (Dunnett's *t*-test).

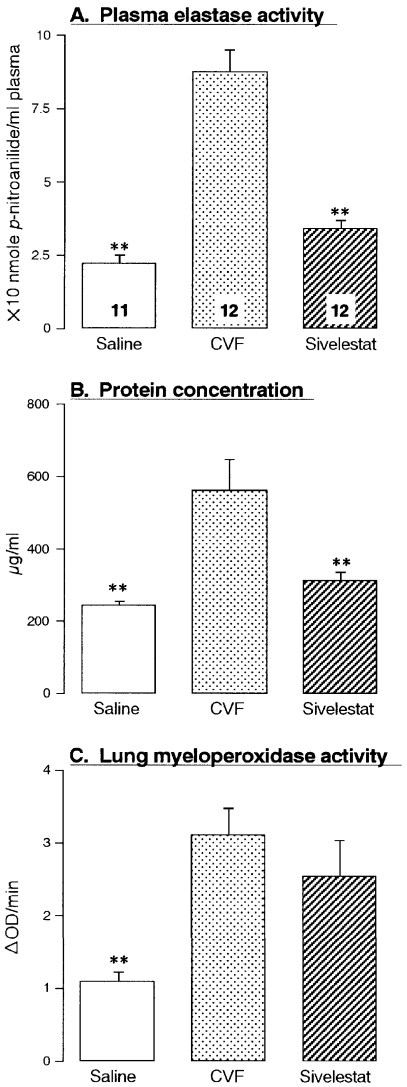

Fig. 4. Effects of sivelestat on the increase in plasma neutrophil elastase activity (A), protein concentration in bronchoalveolar lavage fluid (B) and lung myeloperoxidase activity (C). Thirty minutes after the injection of CVF, plasma neutrophil elastase activity, protein concentration in bronchoalveolar lavage fluid and myeloperoxidase activity in lavaged lungs were determined as described in the text. Sivelestat (1 mg/kg/h) or saline was intravenously infused 90 min before 30 min after CVF injection. In the saline control group, animals were intravenously injected saline instead of CVF. The number attached to each column represents the number of total animals in each group. The results indicate mean ± S.E.M. of each group. $^{**}P < 0.01$ vs. CVF-injected group (Dunnett's *t*-test).

group, respectively. Sivelestat at 0.1, 0.3 and 1 mg/kg/h dose-dependently reduced the increase in plasma neutrophil elastase activity by 36.5%, 66.9% and 104.3%, respectively, and that in lung vascular permeability by 30.0%, 44.6% and 56.2%, respectively (Fig. 3).

3.4. Effect of sivelestat on the increase in protein concentration in bronchoalveolar lavage fluid

Fig. 4 shows plasma neutrophil elastase activity, protein concentration in bronchoalveolar lavage fluid and myeloperoxidase activity in lung tissues 30 min after CVF injection in each group. In accordance with the results presented in Fig. 3, intravenous infusion of sivelestat at 1 mg/kg/h inhibited CVF-induced increase in plasma neutrophil elastase activity, and reduced that in protein concentration in bronchoalveolar lavage fluid. However, the treatment did not affect the increase in myeloperoxidase activity in lung tissues.

3.5. Effect of sivelestat on CVF-induced complement activation in hamsters

Addition of CVF into hamster blood resulted in hemolysis (absorbance was 0.115 ± 0.012 and 0.372 ± 0.064 nm in non-treated and treated blood, respectively ; $P < 0.001$ with Student's two-tailed *t*-test). Sivelestat had no effect on this hemolysis even at 100 μM (absorbance was 0.364 ± 0.062 nm).

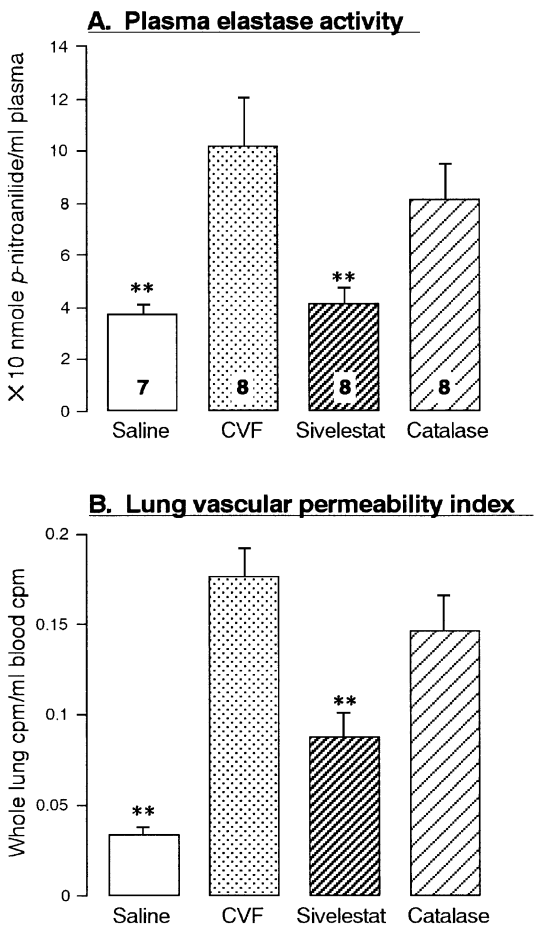

Fig. 5. Effects of catalase on the increase in plasma neutrophil elastase activity and lung vascular permeability. Plasma neutrophil elastase activity (A) and lung vascular permeability (B) were determined 30 min after CVF injection by the same method as described in Fig. 1. Sivelestat (1 mg/kg/h), catalase (2500 U/kg/h) or saline was intravenously infused 90 min before 30 min after CVF injection. In the saline-control and CVF-control group, animals were intravenously infused saline. The number attached to each column represents the number of total animals in each group. The results indicate mean ± S.E.M. of each group. $^{**}P < 0.01$ vs. CVF-injected group (Dunnett's *t*-test).

3.6. Effect of catalase on plasma neutrophil elastase activity and lung vascular permeability

The effect of catalase, a H_2O_2 scavenging enzyme, was also examined using the same animal model. The effect of sivelestat in this experiment was comparable to that shown in Fig. 3. Continuous intravenous infusion of catalase (2500 U/kg/h, 5000 U/kg in total over 2-h infusion) had no obvious effect on the CVF-induced increase either in the plasma neutrophil elastase activity or in lung vascular permeability (Fig. 5).

4. Discussion

In the present study, we have shown that i.v. injection with cobra venom factor (CVF) into conscious hamsters resulted in a marked elevation of plasma neutrophil elastase activity followed by a sustained increase in lung vascular permeability (Fig. 1) and protein concentration in bronchoalveolar lavage fluid (Fig. 2). Furthermore, we have found that inhibition of the elevated plasma neutrophil elastase activity by a specific neutrophil elastase inhibitor, sivelestat, attenuates the subsequent increase in both lung vascular permeability (Fig. 3) and the increase in protein concentration in bronchoalveolar lavage fluid (Fig. 4). These results are consistent with the current hypothesis that neutrophil elastase is an important mediator in complement-mediated acute lung injury.

Under physiological conditions, neutrophil elastase activity in the body is tightly regulated by endogenous protease inhibitors, such as α_1-protease inhibitor (Travis and Salvesen, 1983). However, at inflammatory sites, these protease inhibitors, typically α_1-protease inhibitor, are inactivated by neutrophil-derived reactive oxygen species (Weiss, 1989). Although neutrophil elastase-inhibitory activity and susceptibility of endogenous protease inhibitors to reactive oxygen species-inactivation are important regulatory factors for in vivo neutrophil elastase activity, these characteristics are known to vary widely among animal species. It has been reported that protease inhibitors in some rodent species such as mouse, rat and guinea pig have an anti-neutrophil elastase activity several folds higher than that in human (Schulz et al., 1989), whereas, those in sheep, rabbit and mini pig are much more susceptible to oxidative inactivation than those in human (Takahara et al., 1983). By contrast, anti-neutrophil elastase activity of endogenous protease inhibitors and their susceptibility to oxidative inactivation in hamsters are relatively similar to those in human (Schulz et al., 1989; Takahara et al., 1983). The present animal model, thus, may be useful in the study of neutrophil elastase in the pathogenesis of acute lung injury.

The protective effect of sivelestat might be largely attributable to the specific inhibition of neutrophil elastase activity. As reported previously, sivelestat inhibits hamster-neutrophil elastase activity with an IC_{50} value of 37 ± 4 nM but does not inhibit other neutrophil-derived proteases such as cathepsin G (Kawabata et al., 1991). In addition to its specificity among proteases, sivelestat neither affects the production of reactive oxygen species (Iwamura et al., 1993) nor inhibits the activity of lipoxygenase (5- and 15-lipoxygenase) and cyclooxygenase (cyclooxygenase I and II) (unpublished data) which have been implicated in the development of acute lung injury. Although, inhibition of CVF-induced complement activation (Till et al., 1987) or lung neutrophil accumulation (Mulligan et al., 1994) can attenuate CVF-induced lung injury, the present in vitro study indicated that sivelestat had no effect on CVF-induced complement activation in hamsters even at 100 μM. Furthermore, sivelestat (1 mg/kg/h) attenuated lung injury with the evidence of plasma neutrophil elastase inhibition but did not affect neutrophil accumulation (Fig. 4). Another factor that can modify CVF-induced lung injury is hemodynamics. Although it has been shown that CVF induces significant changes in hemodynamics and that agents that modify hemodynamics can affect lung injury via the alteration of lung vascular permeability. Sivelestat, however, does not affect systemic blood pressure and heart rate even at 30 mg/kg/h in normal hamsters (data not shown). These findings suggest that systemic complement activation increases neutrophil elastase activity that subsequently causes acute lung injury.

Sivelestat at 1 mg/kg/h, a dosage capable of abolishing plasma neutrophil elastase activity, almost totally blocked the increase in protein concentration in bronchoalveolar lavage fluid (Fig. 4) but partially reduced that in lung vascular permeability (Figs. 3 and 5). These results are consistent with previous findings that sivelestat prevents the increase in bronchoalveolar lavage fluid permeability index more evidently than that in lung vascular permeability in a rabbit model of neutrophil-dependent acute lung injury (Miyazaki et al., 1998). In the present model, neutrophil elastase appears to play a major role in trans-alveolar permeability, whereas, other factors also contribute to vascular permeability. It is considered that neutrophil elastase disturbs the functions of epithelial and alveolar endothelial cells and/or their tight junctions via its broad range of proteolytic activity. This possibility is supported by the finding that neutrophil elastase can increase the permeability of both endothelial and alveolar epithelial cells which are known to regulate vascular and trans-alveolar permeability. The target proteins by which neutrophil elastase increases permeability are unclear, however, the endothelial cell surface protein, thrombomodulin, and the cell junction protein, cadherin may be potential target proteins of neutrophil elastase as has been suggested recently (Carden et al., 1998).

It is not clear in the present study what factors other than neutrophil elastase contribute to the increase in lung vascular permeability. The anaphylatoxin C5a may be a potential mediator as it is generated in response to comple-

ment activation and can directly increase vascular permeability (Worthen and Henson, 1983). Furthermore, C5a is able to release histamine (Till et al., 1991) and leukotrienes (Worthen and Henson, 1983) that are capable of increasing vascular permeability. Indeed, in a rat model of CVF-induced acute lung injury, the increase in lung vascular permeability is partially prevented by anti-histamines (Till et al., 1991). Therefore, C5a may directly and/or indirectly contribute to the increase in lung vascular permeability in our model.

To study the involvement of neutrophil-derived reactive oxygen species in lung injury in the present model, we examined the effect of a hydrogen peroxide scavenging enzyme, catalase. It has been reported that bolus i.v. injection of catalase (1200 U/kg) attenuates CVF-induced lung injury in rats (Ward et al., 1985; Till and Ward, 1986). However, in our model, this enzyme failed to attenuate the increase in lung vascular permeability even by continuous i.v. infusion at 2500 U/kg/h (5000 U/kg over 2 h), a dose about 4-fold higher than that used in the rat study (Fig. 5). Although, the reason for this discrepancy is not clear, we speculate that hamsters are more resistant to oxidant injury than rats. As an earlier report has shown, endogenous lung catalase activity in hamsters, that has similar catalase activity in humans, is about four times higher than that in rats (Bryan and Jenkinson, 1987). In fact, catalase does not reduce neutrophil-mediated vascular permeability in hamster models (Rosengren et al., 1988). Thus, relative contribution of reactive oxygen species to lung injury in hamsters may be lower than that in rats. Further studies including broad range of catalase dose-setting, are needed to reach a firm conclusion.

Neutrophil elastase has been suggested to injure tissues at inflammatory sites due to local imbalance between neutrophil elastase and endogenous protease inhibitors. Three mechanisms by which local balance between neutrophil elastase and these protease inhibitors is disturbed have been proposed. First, these protease inhibitors have high molecular weight and cannot enter the microenvironment between neutrophils and their substrate tissues due to stereochemical limitations (Campbell et al., 1982; Campbell and Campbell, 1988). Second, a major endogenous inhibitor, α_1-protease inhibitor is inactivated via oxidation of its active center by neutrophil-derived reactive oxygen species (Weiss, 1989). Finally, these protease inhibitors are not fully effective to inhibit tissue bound neutrophil elastase (Kawabata et al., 1996). The present study adds to what has been proposed hypothesis that neutrophil elastase can injure tissues at inflammatory sites even in the presence of endogenous protease inhibitors. In contrast to these endogenous protease inhibitors, sivelestat, a low molecular weight neutrophil elastase inhibitor, can enter microenviroment between neutrophils and their substrate tissues, is not structurally inactivated by reactive oxygen species and effectively inhibits tissue bound neutrophil elastase. Therefore, sivelestat may effectively inhibit neutrophil elastase, resulting in a protection from acute lung injury associated with CVF-injection in hamsters.

In summary, we suggest that neutrophil elastase plays an important role in complement-mediated acute lung injury.

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