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Elastase inhibition reduced death associated with acid aspiration-induced lung injury in hamsters

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Abstract

This study examined whether the specific inhibition of neutrophil elastase by sivelestat sodium hydrate (sivelestat) reduced deaths associated with severe acute lung injury after hydrochloric acid (HCl) aspiration in hamsters. Animals that received a single intratracheal instillation of HCl (0.2 N, 200 μl) time-dependently died by occlusion of their trachea with inflammatory exudate. In a time course study, these animals developed severe lung injury, peaking 12 to 24 h after HCl instillation, as indicated by hemorrhage and a massive increase in the protein concentration of bronchoalveolar lavage fluid. These changes were closely correlated with neutrophil elastase activity in bronchoalveolar lavage fluid. Sivelestat (0.01, 0.1 and 1 mg/kg/h), when intravenously infused during the first 48 h post-HCl instillation, dose-dependently reduced death in HCl-instilled hamsters. In a separate experiment, analysis of bronchoalveolar lavage fluid parameters and partial pressure of arterial oxygen (PaO_2) 8 h post-HCl instillation showed that sivelestat at 1 mg/kg/h, i.v. significantly improved both bronchoalveolar lavage fluid parameters and PaO_2 levels with evidence of the inhibition of neutrophil elastase activity in bronchoalveolar lavage fluid. These results suggest that neutrophil elastase plays a significant role in this type of severe acute lung injury that leads to death by respiratory failure.

Keywords: Neutrophil elastase; Acid aspiration; Acute lung injury; Sivelestat

1. Introduction

Aspiration of gastric content can trigger the development of adult respiratory distress syndrome. The mortality for adult respiratory distress syndrome resulting from acid aspiration remains high, ranging from 40% to 50% (Pepe et al., 1982; Fowler et al., 1983). Earlier reports have shown that the development of this lung injury in animal models is associated with neutrophils (Goldman et al., 1993) rather than the acid itself (Awe et al., 1966). The published evidence shows that neutrophil depletion (Goldman et al., 1993), scavengers of reactive oxygen species such as superoxide dismutase and catalase, significantly reduce acid aspiration-induced acute lung injury in rats (Goldman et al., 1992), and suggests that neutrophil-derived reactive oxygen species play an important role in this type of acute lung injury. More recently, it has been demonstrated that neutrophil-derived proteases participate in the development of acid aspiration-induced lung injury (Goldman et al., 1992; Knight et al., 1992).

Neutrophil elastase is a protease, capable of degrading key structural elements of connective tissues such as elastin, collagen, and proteoglycan (Havemann and Gramse, 1984). Amongst many potentially injurious agents, neutrophil elastase has emerged as a key mediator of lung injury. 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 involved in lung edema. Increased neutrophil elastase enzyme activity has been demonstrated in patients with adult respiratory distress syndrome (Rocker et al., 1989). We have previously shown that neutrophil elastase plays an important role in the progression of endotoxin-induced acute lung injury in hamsters (Kawabata et al., 2000). However, the contribution of this enzyme to severe acute lung injury that leads to death by respiratory failure remains unknown. Studying a role of neutrophil elastase in such severe acute lung injury would be important to understand not only the role of neutrophil elastase but also the therapeutic potential of the inhibition of this enzyme in acute lung injury.

In an attempt to elucidate the role of neutrophil elastase in acid aspiration-induced lung injury, we have found that

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acid-aspirated hamsters develop severe acute lung injury that leads to death by respiratory failure. We used this model to investigate whether the specific inhibition of neutrophil elastase by sivelestat sodium hydrate (sivelestat) would reduce death associated with acute lung injury. Sivelestat (ONO-5046·Na/LY544349) has 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, matrix metalloproteases, cyclooxygenases, and lipoxygenases (Kawabata et al., 1991, 2002). Sivelestat also has no effect on the production of reactive oxygen species from neutrophils (Iwamura et al., 1993) or cytokines from inflammatory cells (Kawabata et al., 2002).

2. Materials and methods

2.1. Animals

Male Syrian golden hamsters (Keari, Osaka, Japan) weighing 90 to 110 g were housed in an air-conditioned room at 23 ± 2 °C and 55 ± 10% humidity with alternating 12-h light/dark cycles, and were given food and water ad libitum until use. All animal experiments were performed in accordance with our institutional animal care guidelines.

2.2. Surgical procedure and induction of lung injury

While under sodium pentobarbital (60 mg/kg, i.p.) anesthesia, a small diameter 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. Immediately after cannulation, each animal was incised (midline anterior neck) and intratracheally instilled with 200 μl of 0.2 N HCl via a 27-gauge syringe. The free end of the catheter in each animal was then connected to an infusion pump (model 55-1111, Harvard Apparatus, South Natick, MA) for intravenous infusion of sivelestat. Animals recovered from anesthesia within 1 h and, thereafter, were allowed free movement and access to water and food.

2.3. Experimental design

2.3.1. Hydrochloric acid (HCl) dose finding

HCl, at various concentrations (0.1 and 0.2 N) and volumes (100 and 200 μl) was intratracheally instilled into hamsters as described above. Their post-HCl instillation survival was then monitored every 24 for 144 h. In the control group, 200 μl of phosphate buffered saline (PBS) was intratracheally instilled.

2.3.2. Lung injury time course

Nineteen hamsters were intratracheally instilled with 200 μl of 0.2 N HCl as described above, and lung injury was evaluated in surviving animals at 12, 24, and 48 h. In the control group, 200 μl of PBS was intratracheally instilled.

2.3.3. Effect of sivelestat on survival

Groups of 12 hamsters were used. Animals were instilled either with HCl (0.2 N, 200 μl) or PBS, as described above, and their survival was monitored every 24 h for 6 days after instillation. In the sivelestat-treated groups, animals were intravenously infused with sivelestat (0.01, 0.1 and 1 mg/kg/h) for the first 48 h after HCl instillation. In the HCl control groups, animals underwent the same procedure except that saline was intravenously infused instead of sivelestat.

2.3.4. Effects of sivelestat on lung injury

In the sivelestat-treated group (surgical procedure same as above), animals were intravenously infused with sivelestat (1 mg/kg/h) for 8 h after HCl (0.2 N, 200 μl) instillation. In the HCl control group, saline was used instead of sivelestat. Immediately after the infusion (8 h after HCl instillation), blood was drawn via a catheter inserted into the carotid artery and the partial pressures of arterial oxygen (PaO_2) and carbon dioxide ($PaCO_2$) were determined using a portable blood oxygen gas analyzer (i-STAT, i-STAT, Princeton, NJ). After the PaO_2 and $PaCO_2$ determinations, animals were anesthetized and sacrificed by exsanguination to evaluate lung injury. Animals that developed gasping symptoms during the infusion period were considered to be moribund and were sampled for arterial blood gases, then anesthetized and sacrificed early. Lung injury was evaluated as described below.

2.4. Measurements of lung injury parameters

After the blood gas analysis, animals were anesthetized and sacrificed by exsanguination (transection of the abdominal aorta) and their whole lungs were lavaged with 2.8 ml of citrated saline using a tracheal cannula. Hemorrhage, white blood cell count, protein concentration, and neutrophil elastase activity in the bronchoalveolar lavage fluid were then determined by the methods described below.

2.4.1. Hemorrhage

Erythrocytes, in 0.1 ml bronchoalveolar lavage fluid samples, were lysed with 0.9 ml of distilled water. Lysates were then centrifuged (1700 × *g*, 10 min, room temperature), and the absorbance of the supernatants at 412 nm was measured spectrophotometrically and considered as an index of hemorrhage (Kawabata et al., 2000).

2.4.2. White blood cell count

The bronchoalveolar lavage fluid that was not used for the hemorrhage assay was centrifuged at 1700 × *g* for 10 min at 4 °C, and cell pellets were counted with an automatic blood cell counter (Sysmex E-2500; Sysmex,

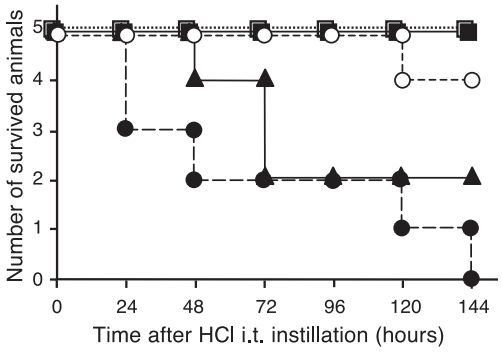

Fig. 1. HCl dose finding study. Different volumes (100 and 200 μl) and concentrations (0.1 and 0.2 N) of HCl were intratracheally instilled to five animals in each group, and animal survival was monitored. Legend: normal control group (■ closed squares: PBS 200 μl) and HCl control groups (□ open squares: 0.1 N 100 μl, ○ open circles: 0.1 N 200 μl, ▲ closed triangles: 0.2 N 100 μl, ● closed circles: 0.2 N 200 μl).

Kobe, Japan). The bronchoalveolar lavage fluid supernatants were used to assay protein concentration and neutrophil elastase activity.

2.4.3. Protein concentration

Total protein concentration in the bronchoalveolar lavage fluid supernatants was determined by the Lowry method using bovine serum albumin as the protein reference standard.

2.4.4. Neutrophil elastase activity

Neutrophil elastase activity was determined by a method using the highly neutrophil elastase specific synthetic substrate *N*-methoxysuccinyl-Ala-Ala-Pro-Val *p*-nitroanilide (Yoshimura et al., 1994). Briefly, samples were incubated in 0.1 M Tris–HCl buffer (pH 8.0) containing 0.5 M NaCl and 1 mM substrate for 24 h at 37 °C. After incubation, *p*-nitroaniline was measured spectrophotometrically at 405 nm and was considered to be a measure of neutrophil elastase activity.

2.4.5. Neutrophil sequestration in lung tissue

Myeloperoxidase activity in lavaged lungs was measured to determine neutrophil sequestration in lung tissues (Kawabata et al., 2000). Briefly, a whole lung, lavaged as described above, was blotted dry, homogenized in 3 ml of 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% cetyltrimethylammonium bromide, then centrifuged at 15,000 × *g* for 10 min at 4 °C. The supernatant was diluted five times in the same buffer, and the enzyme reaction was initiated by mixing 50 μl of the diluted supernatant with 1.4 ml of potassium phosphate buffer containing 0.00107% hydrogen peroxide solution and 50 μl of 3, 3-dimethoxybenzidine solution. The color change of the mixture was measured spectrophotometrically at 450 nm for 60 s and the developed absorbance was considered as myeloperoxidase activity in lung tissue.

2.5. Drugs and chemicals

Sivelestat sodium hydrate (sodium *N*-[2-[4-(2, 2-dimethylpropionyloxy)phenylsulfonyl amino]benzoyl]aminoacetate tetrahydrate was synthesized in our laboratory. We purchased hydrochloric acid (Wako, Osaka, Japan), and *N*-methoxysuccinyl-Ala-Ala-Pro-Val *p*-nitroanilide and 3, 3'-dimethoxybenzidine (Sigma, St. Louis, MO). Sivelestat was dissolved in saline with a small amount of Na_2CO_3 (2 μl of 0.5 M Na_2CO_3/mg of sivelestat). HCl was diluted with phosphate buffered saline (PBS). *N*-methoxysuccinyl-Ala-Ala-Pro-Val *p*-nitroanilide was dissolved in 1-methyl-2-

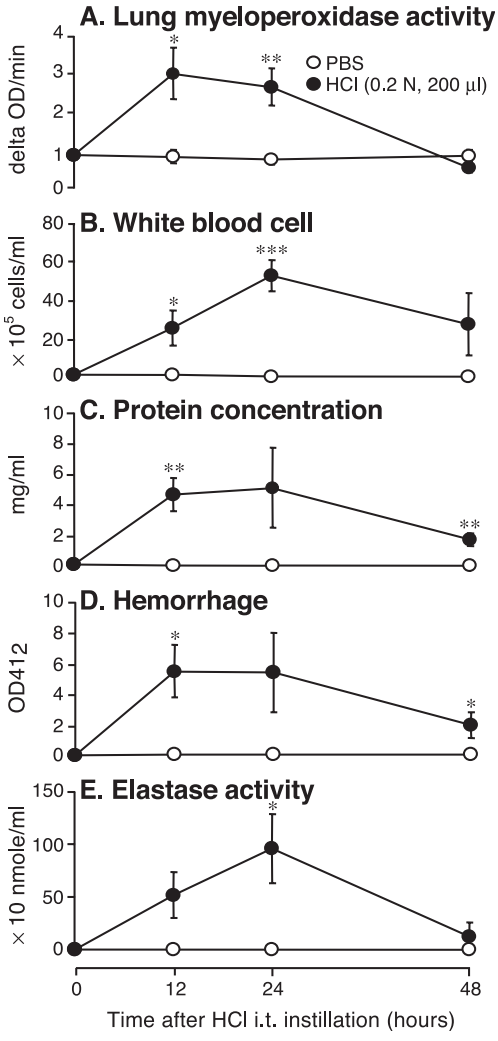

Fig. 2. Lung injury time course following HCl instillation. Initially, 19 animals were treated with 0.2 N HCl (200 μl) and five animals died within the first 24 h post-HCl instillation. Surviving HCl-instilled animals (● closed circles) and PBS treated-animals (○ open circles) were sacrificed at the specified time points, and bronchoalveolar lavage fluid parameters and myeloperoxidase activity in lavaged lungs were determined as described in the text. The myeloperoxidase activity in lavaged lungs was considered to represent the amount of neutrophil infiltration into lung tissue. Each point represents the mean ± S.E.M. of four to five animals. *$P<0.05$, **$P<0.01$ and ***$P<0.001$ vs. relevant PBS-treated group (Student's *t*-test).

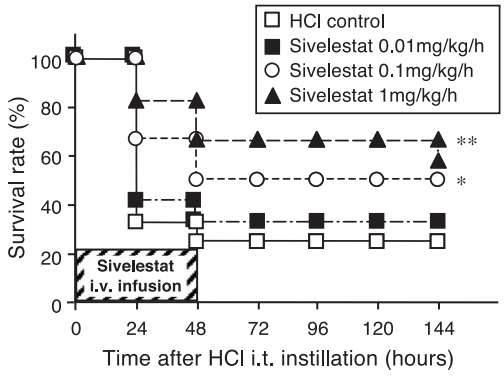

Fig. 3. Effects of sivelestat on the survival rate of hamsters treated with HCl. Animals were first intratracheally instilled with 200 μl of 0.2 N HCl and their survival was monitored. Sivelestat (0.01, 0.1 and 1 mg/kg/h) was intravenously infused for the first 48 h post-HCl instillation, and saline was intravenously infused into the controls. The results represent the survival rate in 12 animals in each group. The logrank test was used to determine the significance of differences in survival rate between HCl control- and sivelestat-treated groups. *$P<0.05$ and **$P<0.01$ vs. HCl control group.

pyrrolidone, and 3, 3'-dimethoxybenzidine was dissolved in distilled water.

2.6. Statistical analysis

Values are expressed as mean ± S.E.M. Statistical significance was determined either by Student's two-tailed *t*-test or one-way analysis of variance followed by Dunnett's *t*-test. The logrank test was used to determine the significance of difference in survival rate between HCl control and sivelestat-treated groups. *P* values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. HCl dose finding

Intratracheal instillation of HCl at various concentrations (0.1 and 0.2 N) and volumes (100 and 200 μl) decreased survival in hamsters in a concentration- and volume-related manner (Fig. 1). In the group of animals instilled with 200 μl 0.2 N HCl, all animals died within 144 h after HCl instillation with inflammatory exudate in the trachea.

3.2. Lung injury time course

Five animals died during the first 24 h following intratracheal HCl (0.2 N, 200 μl) instillation. In hamsters that survived to scheduled necropsy, this instillation increased the white blood cell count in bronchoalveolar lavage fluid (Fig. 2B) following an increase in myeloperoxidase activity in lavaged lungs (Fig. 2A), and caused acute lung injury as indicated by an increase in protein concentration (Fig. 2C) and hemorrhage (Fig. 2D) in bronchoalveolar lavage fluid. The increase in myeloperoxidase activity, white blood cell count, protein concentration, and hemorrhage caused by HCl instillation was maximal between 12 and 24 h post-HCl instillation and declined thereafter. Changes in these parameters were also associated with an increase in neutrophil elastase activity in bronchoalveolar lavage fluid (Fig. 2E). A microscopic examination of bronchoalveolar lavage fluid revealed that most of the white blood cells recovered were neutrophils.

3.3. Effects of sivelestat on survival

As the lung injury time course indicated that neutrophil elastase activity in bronchoalveolar lavage fluid returned to baseline 48 h after HCl (0.2 N, 200 μl) instillation (Fig. 2E), hamsters were intravenously infused with sivelestat for the

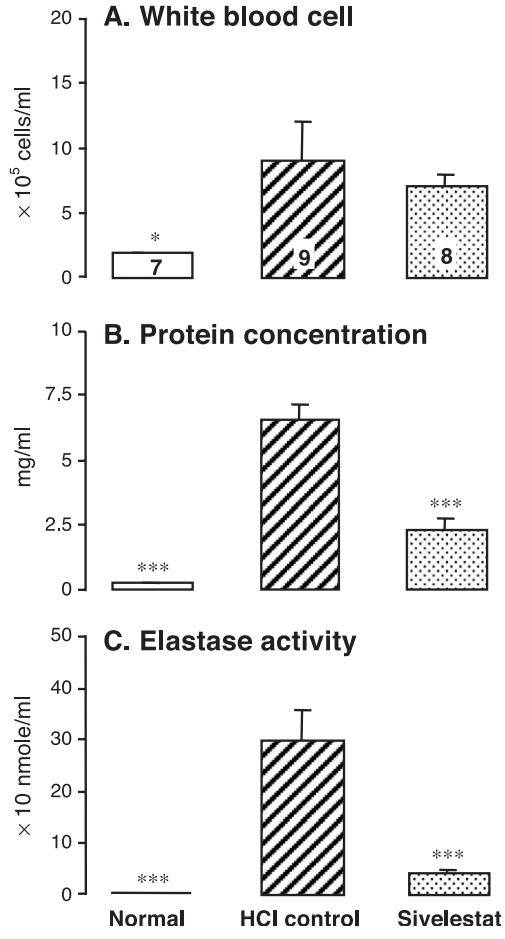

Fig. 4. Effect of sivelestat on lung injury parameters. Sivelestat (1 mg/kg/h) was intravenously infused for the first 8 h post-HCl instillation. HCl control and normal control groups were intravenously infused with saline. Eight hours after 0.2 N HCl (200 μl) instillation, arterial blood samples were collected from the carotid artery of each conscious animal to measure partial pressure of arterial oxygen (PaO_2). Animals were then sacrificed (exsanguinated) and the lungs were lavaged. Five out of nine animals in the HCl control group became moribund within 8 h after HCl instillation. The results represent mean ± S.E.M of seven to nine animals in each group. *$P<0.05$ and ***$P<0.001$ vs. HCl control group (Dunnett's *t*-test).

first 48 h following HCl instillation. In the HCl control group, the survival rate was as low as 33% for the first 24 h after HCl instillation, with a final survival rate of 25% (9 out of 12 animals died) at 144 h (Fig. 3). Sivelestat, at doses of 0.01, 0.1 and 1 mg/kg/h, dose-dependently improved the survival rate with a final survival rate of 33% (4/12), 50% (6/12) and 58% (7/12), respectively. The survival rate was significant at doses of 0.1 and 1 mg/kg/h as compared with the HCl control group (Fig. 3).

3.4. Effect of sivelestat on bronchoalveolar lavage fluid parameters

Five out of nine animals in the HCl control group became moribund within 8 h after HCl (0.2 N, 200 μl) instillation (Fig. 4). Moribund (gasping symptom) animals had arterial blood gases analyzed early, and were then sacrificed to evaluate lung injury. In the normal control and sivelestat-treated groups, no animals were observed gasping during this 8-h period. In the HCl control group (including both surviving and moribund animals), white blood cell counts (Fig. 4A), and protein concentrations (Fig. 4B), as well as neutrophil elastase activity in bronchoalveolar lavage fluid (Fig. 4C) were significantly increased 8 h after HCl instillation. When control animals were divided into two subgroups (i.e., those who survived to scheduled sacrifice, and moribund animals sacrificed early), bronchoalveolar lavage fluid white blood cell counts in the moribund animals were only 27% of that in the surviving animals (4 ± 2 vs. $15 \pm 4 \times 10^5$ cells/ml, $P < 0.05$), whereas bronchoalveolar lavage fluid protein concentrations in the moribund animals were about 160% of that in the surviving animals (7.9 ± 0.5 vs. 4.8 ± 0.3 mg/ml, $P < 0.01$). Neutrophil elastase activity was not statistically different between the two subgroups. The infusion of sivelestat (1 mg/kg/h, i.v.) reduced the increase in protein concentration and neutrophil elastase activity in bronchoalveolar lavage fluid without affecting the increase in white blood cell count. Sivelestat infusion also markedly reduced hemorrhage in bronchoalveolar lavage fluid (value in Normal, HCl control and Sivelestat group was 0.3 ± 0.05^a, 7.29 ± 0.96 and 2.74 ± 1.00^b OD, respectively; $^aP < 0.001$ and $^bP < 0.01$ vs. HCl control group with Dunnett's *t*-test).

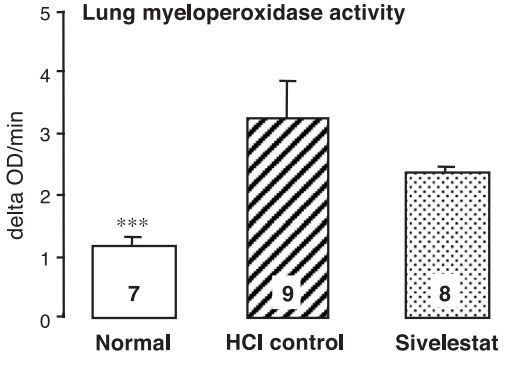

Fig. 5. Effect of sivelestat on lung neutrophil sequestration. Animals in each group were treated as described in Materials and methods. After lavage, the lungs of each animal were homogenized and the activity of myeloperoxidase in the supernatant of the homogenate was measured. The results represent mean ± S.E.M of seven to nine animals in each group. ***$P < 0.001$ vs. HCl control group (Dunnett's *t*-test).

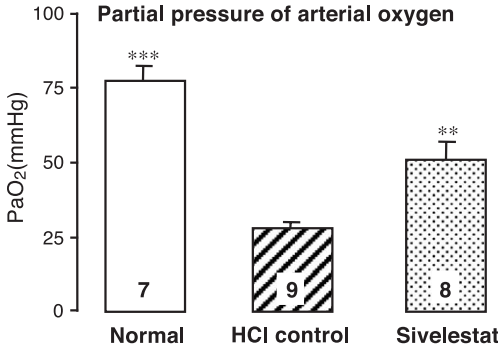

Fig. 6. Effect of sivelestat on lung dysfunction. Animals in each group were treated as described in Materials and methods. Eight hours after HCl instillation, arterial blood samples were collected from the carotid artery of each conscious animal and partial pressure of arterial oxygen (PaO_2) was determined. The PaO_2 value of moribund animals (gasping symptom within 8 h after HCl instillation) was determined early. The results represent mean ± S.E.M of seven to nine animals in each group. **$P < 0.01$ and ***$P < 0.001$ vs. HCl control group (Dunnett's *t*-test).

3.5. Effect of sivelestat on lung neutrophil sequestration

In the HCl control group (including surviving and moribund animals), myeloperoxidase activity in lavaged lungs was significantly increased 8 h after HCl instillation. Sivelestat, at 1 mg/kg/h i.v., did not affect this increase in myeloperoxidase activity (Fig. 5).

3.6. Effect of sivelestat on lung dysfunction

In the HCl control group (including the surviving and moribund animals), the partial pressure of arterial oxygen (PaO_2) was significantly decreased 8 h after HCl instillation. However, sivelestat at 1 mg/kg/h i.v., attenuated this decrease in PaO_2 (Fig. 6). There were no significant differences in the $PaCO_2$ values of each group: $PaCO_2$ 40.3 ± 1.4, 41 ± 1.5, and 37.9 ± 3.1 mm Hg in normal control, HCl control and sivelestat group, respectively.

4. Discussion

The main objective of this study was to examine the role of neutrophil elastase in deaths associated with severe acute lung injury after acid aspiration in hamsters. Our results indicate that the development of this acute lung injury paralleled an increase in neutrophil elastase activity in bronchoalveolar lavage fluid (Fig. 2), and that inhibition of the elevated neutrophil elastase activity by the neutrophil

elastase inhibitor, sivelestat, reduced not only acute lung injury (Fig. 4) but also death in hamsters (Fig. 3). The protective effect of sivelestat might be largely attributable to its specific inhibition of neutrophil elastase activity. As has been previously shown, sivelestat is a highly specific inhibitor of neutrophil elastase (Kawabata et al., 2002) and its continuous intravenous infusion (0.1 to 1 mg/kg/h) dose-dependently inhibits endogenous neutrophil elastase activity with total inhibition at 1 mg/kg/h in hamsters (Hagio et al., 2001). Sivelestat, at 1 mg/kg/h i.v., reduced the increase in neutrophil elastase activity in bronchoalveolar lavage fluid by 90% in this hamster model (Fig. 4).

Respiratory failure was the most frequent cause of death for hamsters in our study. The mortality was the highest during the first 24 h post-HCl instillation, a period characterized by severe acute lung injury as indicated by increased protein concentration and hemorrhage in bronchoalveolar lavage fluid (Figs. 2 and 3). Compared with other acute lung injury models, such as endotoxin inhalation-induced acute lung injury and cobra venom factor-induced acute lung injury, this model generates a larger increase in bronchoalveolar lavage fluid protein concentration and it is lethal. In fact, we noted that the peak increase in bronchoalveolar lavage fluid protein concentration in the endotoxin and cobra-venom models were, at most, around 1 mg/ml (Kawabata et al., 2000; Hagio et al., 2001), whereas in this model, concentrations were around 6 mg/ml at 8 h post-HCl instillation, and necropsies showed severe inflammatory exudates in the tracheas. Therefore, it is believed that sivelestat improves survival rate by significantly attenuating acute lung injury, but not other nonpulmonary organ failures, which might have been caused by acid aspiration (St John et al., 1993). This observation is supported by the findings that sivelestat-treated animals exhibited a significant reduction in protein concentration in bronchoalveolar lavage fluid and an increase in PaO_2. Our study does not, however, establish the therapeutic potential of neutrophil elastase inhibition in preventing death from nonpulmonary organ failure that is often one of the causes of death in patients with adult respiratory distress syndrome (Conner and Bernard, 2000).

Interestingly, subgroup analysis revealed that moribund animals in the HCl control group exhibited only minimal increases in bronchoalveolar lavage fluid-white blood cell counts in spite of the significant increase in bronchoalveolar lavage fluid-elastase activity. This finding suggests that the cellular source of elastase in these moribund animals is not white blood cells in alveolar space but those in lung tissue. Neutrophils in the lung interstitium are the most likely source of this enzyme, as all moribund animals had high myeloperoxidase activity that was comparable to that in the surviving HCl control animals. Sivelestat affected neither bronchoalveolar lavage fluid-white blood cell counts nor lung myeloperoxidase activity, suggesting that this compound specifically reduces the elastase burden on the lungs, but not other toxic mediators from neutrophils. It is also interesting to note that the cut-off bronchoalveolar lavage fluid-protein concentration that determines the survival of animals was between 5 and 8 mg/ml, as subgroup analysis indicated that the protein concentration in moribund animals was about 8 mg/ml, and the concentration in surviving animals was about 5 mg/ml.

An earlier report using rats has shown that treatment with the neutrophil elastase inhibitor, methoxysuccinyl-Ala-Ala-Pro-Val-chloromethylketone, attenuates the increase in bronchoalveolar lavage fluid protein concentration in nonacid-aspirated but not in acid-aspirated lungs (Goldman et al., 1992). Although, in our study, the effect of sivelestat on acute lung injury in nonacid aspirated lungs is not conclusive, our data clearly show that neutrophil elastase significantly contributes to acute lung injury after acid aspiration in hamsters. Treatment with sivelestat resulted in an over 70% reduction in bronchoalveolar lavage fluid protein concentration and a significant improvement in survival rate. Several reasons including the difference in experimental conditions can be addressed to explain the discrepancy between the findings. However, more importantly, species difference in neutrophil elastase regulation may be one of the main reasons for this discrepancy. It has been reported that endogenous protease inhibitors in some rodent species such as mouse, rat and guinea pig have an anti-neutrophil elastase activity several fold higher than that found in humans (Takahara et al., 1983). However, in hamsters, this anti-neutrophil elastase activity is relatively similar to that of humans (Takahara et al., 1983; Schulz et al., 1989), thus, the relative contribution of neutrophil elastase to lung injury in rats may be lower than that in humans and hamsters. The appropriate animal model must be selected when studying the role of neutrophil elastase in acute lung injury.

Acid aspiration-induced lung injury is primarily mediated by activated neutrophils. Recent studies have demonstrated that chemokines, especially interleukin-8, which is released after acid aspiration, is important in recruiting neutrophils into lung inflammation sites (Folkesson et al., 1995). It has also been shown that interleukin-8 can activate neutrophils that, in turn, release toxic products including neutrophil elastase (Walz et al., 1991). In fact, treatment with anti-interleukin-8 antibody markedly improves acid aspiration-induced lung injury in rabbits (Folkesson et al., 1995). Moreover, a significant correlation has been found between plasma levels of neutrophil elastase and interleukin-8 in patients with adult respiratory distress syndrome (Groeneveld et al., 1995). Neutrophil elastase, on the other hand, is known to release cytokines, particularly interleukin-8, from lung epithelial cells (Ruef et al., 1993). Thus, it can be speculated that following acid aspiration, neutrophil elastase and interleukin-8 mutually form a vicious cycle by increasing each others levels and subsequently aggravating the local neutrophil elastase burden on inflammation sites. Elevated local neutrophil elastase activity would then increase the permeability of

both lung endothelial (Suttorp et al., 1993) and epithelial cells (Peterson et al., 1995), leading finally to severe acute lung injury. Although not addressed in our study, it may be interesting to investigate whether neutrophil elastase inhibition reduces the level of interleukin-8 after acid aspiration. A recent study has shown that sivelestat reduced levels of a similar cytokine, cytokine-induced neutrophil chemoattractant-1, in lung after intestinal ischemia–reperfusion in rats (Takayama et al., 2001).

This study supports the recent evidence that neutrophil elastase can injure tissues, even in the presence of endogenous protease inhibitors such as α_1-protease inhibitor and α_2-macroglobulin (Weiss, 1989). Several mechanisms by which the local balance between neutrophil elastase and these protease inhibitors is disturbed have been proposed. First, these protease inhibitors are inactivated by neutrophil-derived reactive oxygen species at inflammatory sites (Weiss, 1989). Second, these endogenous protease inhibitors are large molecules and have limited access to the microenvironmental space between neutrophils and their substrate tissues (Campbell et al., 1982; Campbell and Campbell, 1988). Finally, these protease inhibitors are not fully effective in inhibiting tissue-bound neutrophil elastase (Morrison et al., 1990; Kawabata et al., 1996). In contrast to these endogenous protease inhibitors, sivelestat, a low molecular weight synthetic neutrophil elastase inhibitor (Kawabata et al., 1991), may be able to access such microenvironmental space, is structurally resistant to inactivation by reactive oxygen species, and is effective at inhibiting tissue-bound neutrophil elastase (Kawabata et al., 1996). Sivelestat appears to effectively inhibit neutrophil elastase activity at inflammatory sites, and thereby protects against the development of acute lung injury.

In conclusion, we suggest that neutrophil elastase plays an important role in acid aspiration-induced lung injury that leads to death by respiratory failure. Our results further support the importance of neutrophil elastase and the therapeutic potential of neutrophil elastase inhibitors in acute lung injury.

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