

Neutrophil Elastase Inhibitor, ONO-5046, Modulates Acid-induced Lung and Systemic Injury in Rabbits

Kazuhiro Kaneko, M.D.,* Ichidai Kudoh, M.D., Ph.D.,† Satoshi Hattori, M.D., Ph.D.,* Hiroshi Yamada, M.D.,* Maria Ohara, M.D.,* Jeanine Wiener-Kronish, M.D.,‡ Fukuichiro Okumura, M.D., Ph.D.§

Background: Acid instillation leads to direct lung and to secondary systemic organ injury, probably *via* activated macrophages and neutrophils. This study investigated the effects of neutrophil elastase on organ injury after unilateral lung acid instillation by administering a specific neutrophil elastase inhibitor, ONO-5046, before acid instillation.

Methods: Three groups of anesthetized rabbits ($n = 12$ in each group) underwent tracheostomies, and instillations were made into their right lower lobe airspaces with either phosphate buffered saline (pH, 7.4; volume, 1.2 ml/kg; $n = 12$) or HCl (pH, 1.25; volume, 1.2 ml/kg; $n = 24$). In half of the acid-instilled rabbits, ONO-5046, 10 mg/kg, was given intravenously 15 min before the HCl instillation, and then $10 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$ of the drug was continuously infused throughout the experiment. The other groups of animals received the vehicle intravenously. Anesthesia and mechanical ventilation was continued for 8 h, whereas arterial blood gases were sampled intermittently. Eight hours after saline or acid instillation, the animals were killed, and their lungs, heart, kidneys, liver, and small intestines were harvested. Wet-to-dry weight ratios (W/D) and myeloperoxidase (MPO) assays of these organs were done, and elastase assays on the bronchoalveolar lavage fluids (BALF) obtained from each lung also were performed.

Results: Pretreatment with ONO-5046 attenuated the physiologic changes seen in the vehicle-treated animals. Significant decreases in W/D of the noninstilled lungs and of the small intestine and normalization of the oxygenation of the experimental animals occurred. The ONO-5046 pretreatment did not affect the neutrophil sequestration in the lungs or in the other

organs as determined by neutrophil counts in BALF and by the MPO assays.

Conclusions: A neutrophil elastase inhibitor, ONO-5046, administered immediately before acid instillation attenuated the physiologic changes seen in the vehicle-treated animals. The drug blocked neutrophil elastase but did not block neutrophil sequestration in the lungs, although the drug improved measurements of lung injury. (Key words: Lung: aspiration; pneumonia. Neutrophil: elastase; inhibitor.)

ASPIRATION of acid into the lungs of patients is the second-most frequent clinical disorder associated with the development of acute lung injury.^{1,2} The effects of the instillation of acid into the lungs of experimental animals has been extensively studied.³⁻⁸ The instillation of acid into one lung of an experimental animal causes an increase in the permeability of the lung's endothelium to protein in the instilled lung, but it also increases this permeability in the contralateral lung and in remote organs.^{6,7} Activated neutrophils are believed to be an important component in causing the increase in the protein permeability of the endothelia.⁵

ONO-5046 {[N-[2-[4-(2,2-dimethylpropionyloxy)phenylsulphonyl-amino] benzoyl] aminoacetic acid} is a new, specific, and reversible inhibitor of human neutrophil elastase (NE).⁹ In sheep with endotoxin-induced lung injury, the administration of ONO-5046 blocked the pulmonary hypertension, decreased the protein permeability of the lung endothelium, and attenuated the accumulation of neutrophils in the endotoxin-exposed lungs.¹⁰

In the present study, we investigated the effects of ONO-5046 on the lung injury and the remote organ injury caused by the unilateral instillation of acid in anesthetized rabbits. Unilateral instillation of acid is used to determine the direct, injurious effects of the acid and the indirect, injurious effects caused by neutrophil activation and other inflammatory processes. The effects of pretreatment with ONO-5046 on the direct and indirect acid-induced injuries could be measured.

* Staff, Department of Anesthesiology, Yokohama City University School of Medicine.

† Associate Professor, Department of Anesthesiology, Yokohama City University School of Medicine.

‡ Professor, Department of Anesthesiology and Medicine, University of California San Francisco.

§ Professor and Chairman, Department of Anesthesiology, Yokohama City University School of Medicine.

Received from Yokohama City University School of Medicine, Yokohama, Japan, and from the University of California San Francisco, San Francisco, California. Submitted for publication September 17, 1996. Accepted for publication May 19, 1997.

Address reprint requests to Dr. Kudoh: Department of Anesthesiology, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama, Japan 236. Address electronic mail to: ikudoh@med.yokohama-cu.ac.jp

Methods

Surgical Preparation and Ventilation

Specific pathogen-free Japanese White rabbits (range of weight, 3–4 kg) were anesthetized with pentobarbital (25 mg/kg), and anesthesia was maintained with 0.5% halothane. A 20-gauge intravenous catheter was inserted into an ear vein for administering fluid and drugs. A catheter was inserted into the carotid artery to monitor blood pressure and for blood sampling. An endotracheal tube was inserted through the tracheotomy. The rabbits were in the supine position, and their lungs were ventilated with a constant-volume pump (Harvard, Millis, MA) with an inspired oxygen fraction of 1.0 and a positive end-expiratory pressure of 3 cmH₂O. The tidal volume was adjusted to maintain an arterial P_{CO₂} between 35 and 45 mmHg. Two ml · kg⁻¹ · h⁻¹ of lactated Ringer's solution with pancuronium (2 mg/h) was administered intravenously throughout the experiment.

These animal experiments were done in conformity with the "Guiding Principles for Research Involving Animals" of the Yokohama City University School of Medicine, and the protocols were approved by the animal care committee.

Experimental Protocol

All the rabbits were placed in the right decubitus position to facilitate liquid deposition into the right lung.

Control Group (n = 12). An intravenous infusion of normal saline (1 ml/kg) was started; 15 min after this event, 1.2 ml/kg of phosphate buffered saline (PBS; pH, 7.4) mixed with Evan's blue dye was delivered into the right lung (primarily the right lower lobe) using a 10-ml syringe and a pediatric feeding tube. The intravenous infusion of saline was continued throughout the experiment.

HCl Group (n = 12). Fifteen minutes after the initiation of an infusion of normal saline (1 ml/kg), the acid instillate, HCl (pH, 1.25; volume, 1.2 ml/kg) mixed with Evan's blue dye, was delivered into the right lung. HCl (0.1 N) was diluted to a pH of 1.25 with normal saline. This quantity of acid was instilled because it had been shown to cause significant lung damage but did not lead to the death of the animals.^{6,11} The placement of the instillate in the lung was confirmed by the visualization of the Evan's blue dye at post-mortem.

ONO Group (n = 12). ONO-5046 (kindly provided by ONO Pharmaceutical, Osaka, Japan) was dissolved

in sterile normal saline to a concentration of 10 mg/ml and filtered through a 0.45-mm filter (Millipore, MA) before injection. Fifteen minutes before HCl instillation, ONO-5046 (10 mg/kg) was administered intravenously and then continuously infused (10 mg · kg⁻¹ · h⁻¹, dissolved in the saline) throughout the experiment. This dose of ONO-5046 was chosen based on an investigation done in endotoxemic rabbits.¹² Each animal received the same volume of instillate and the same quantity of intravenous fluid.

Measurements

Blood samples to measure arterial blood gases and for leukocyte counts were obtained before the infusion of normal saline for the control and HCl groups or before the infusion of ONO-5046 for the ONO group, before the acid instillation, and 30 min, 1, 2, 4, and 8 h after the instillation of fluid into the right lungs. The animals remained anesthetized, ventilated, and monitored for the experimental interval. Eight hours after the instillation of acid or PBS, the rabbits were deeply anesthetized, their abdomens and chests were opened, and the rabbits were exsanguinated.

Six animals in each group were used solely for the determination of the W/Ds. In the other six animals in each group, a 1-g sample of the kidney, liver, small intestine, and heart were harvested for the measurements of myeloperoxidase (MPO) activity. One-half of the lower lobes of the right and left lungs were clamped and removed for MPO measurement. The remainder of the lungs were used for bronchoalveolar lavage (BAL). BAL of the left and then the right lungs was performed separately with 5 ml of normal saline per lung (three times) using a 3.5-mm (inner diameter) tracheostomy tube. Aliquots of the BAL fluid (BALF) were used for leukocyte counts, and the remainder of the BALF was centrifuged at 1,500g for 20 min at 4°C, and these supernatants were frozen at -70°C. These supernatants were subsequently used for measurements of protein concentration, using the Lowry method,¹³ and for elastase assay (to be described). Leukocytes in the BALF and blood were quantified using Gentian Violet and Wright-Giemsa stains and a hemocytometer.

Myeloperoxidase Assay

Activity of this neutrophil enzyme was used to quantify polymorphonuclear neutrophil (PMN) sequestration in tissue; this assay has been found to be a more sensitive method for the detection of entrapped neutrophils than quantitative histology.¹⁴ One gram of tissue

ASPIRATION AND NEUTROPHIL ELASTASE

blotted dry was homogenized in 10 ml of 0.01 M potassium phosphate buffer (PPB; pH, 7.4) containing 1.0 mM ethylene diamine tetraacetic acid (EDTA). Two milliliters of homogenate and 5 ml of 0.01 M PPB containing 1.0 mM EDTA were mixed gently and then centrifuged at 10,000g for 20 min at 4°C. The pellet was rehomogenized in 5 ml of 0.05 M PPB (pH, 6.0) containing 0.5% hexadecyltrimethylammonium bromide. This suspension was freeze-thawed and sonicated with a Branson cell disrupter at 65 W for 1 min. A 0.1-ml aliquot was mixed with 0.79 ml of 0.08 M PPB (pH, 5.4) and 0.1 ml of 16 mM tetramethylbenzidine dissolved in N,N-dimethylformamide at 37°C. After 2 min, 0.01 ml of 30 mM H₂O₂ was added. After incubating 3 min at 37°C, 0.05 ml of catalase solution (300 mg/ml) was added. The mixture was diluted with 4 ml of 0.2 M sodium acetate (pH, 3.0) and then centrifuged at 12,000g for 10 min at 4°C. The supernatant was analyzed by a spectrophotometer (UV-2200, Shimadzu, Kyoto, Japan). One unit of MPO activity was defined arbitrarily as the amount of enzyme necessary to catalyze an increase in absorbance of 1.0/min at 655 nm at 37°C.

Wet-to-Dry Weight Ratios

Freshly harvested parts of the hearts, livers, kidneys, and small intestines were weighed, dried at 60°C for 72 h, and weighed again. The W/Ds were calculated as wet weights/dry weights. The W/Ds of the lungs also were measured using the method of Selinger *et al.*¹⁵ to calculate the extravascular lung waters.

Elastase Assay

The quantification of neutrophilic elastase activity in BALF was determined using the method of Barnett *et al.*,¹⁶ with a specific substrate, N-succinyl-(L-alanine)3-p-nitroanilide (Sigma, St. Louis, MO), for elastase. Twenty-five microliter aliquots of BALF were incubated in a 96 well plate at room temperature for 3 days with 1 mM substrate, 0.1 M Hepes, 0.5 M NaCl, pH of 7.5 in a total volume of 150 ml. Absorbance was measured at 405 nm using a microplatereader (Model 450, BIORAD, Hercules, CA). Elastase activity in the sample was determined from a calibration curve obtained using purified human neutrophilic elastase (Wako, Osaka, Japan).

Statistics

All data are presented as mean \pm SD. The data between the groups were analyzed by ANOVA and Scheffe's test. The within-group data were analyzed by Stu-

dent's paired *t* test. We accepted a *P* < 0.05 as statistically significant.

Results

Hemodynamics and Blood Gases

There were no significant differences in the mean arterial pressures, heart rates, or in the arterial pH, or PaCO₂ values measured in the three experimental groups (table 1). Eight hours after the instillation of HCl, the arterial PaO₂ had decreased significantly from 547 \pm 30 mmHg to 338 \pm 138 mmHg in the HCl group; in contrast, the arterial PaO₂ did not change in the control group or in the ONO pretreatment group.

Number of Neutrophils in Blood

The peripheral blood neutrophil counts over the 8-h experimental interval are shown in figure 1. The neutrophil counts in the animals that received acid instillations transiently decreased from their baseline counts; the decrease occurred 30 min after the instillation of the acid. Pretreatment with ONO-5046 inhibited this decrease in the number of circulating neutrophils.

The transient decrease of neutrophils was followed by a subsequent increase in the number of circulating neutrophils; the circulating neutrophil counts became elevated 2–8 h after the instillation of the PBS in the control group animals; the circulating neutrophil counts were elevated 4–8 h after the acid instillation in the animals in the ONO group, and the circulating neutrophils were significantly elevated only by the eighth hour after the instillation of acid in the animals in the HCl group. The increase in the circulating neutrophil counts in the control group may have been a result of the instillation of PBS or of the 8 h of anesthesia.

Myeloperoxidase Activities

The instillation of acid in the HCl and ONO groups was associated with an increase in the MPO activities in both of the lungs and in the small intestines (fig. 2). The MPO activities in the hearts, kidneys, and livers in these animals were not different from the MPO activities found in these organs in the control animals. The ONO-5046 pretreatment did not affect the myeloperoxidase activities measured in any of the organs.

Wet-to-Dry Weight Ratios

The instillation of acid increased the W/D ratios of both lungs in the HCl group (fig. 3). Pretreatment with

Table 1. Changes in Heart Rate, Blood Pressure, and Arterial Blood Gas (n = 6)

Condition	Time (h)	pH	P _{CO₂} (mmHg)	P _{O₂} (mmHg)	Heart Rate (beats/min)	Mean Arterial Pressure (mmHg)
Control	0	7.49 ± 0.05	36 ± 5	544 ± 42	323 ± 10	87 ± 8
	8	7.44 ± 0.06	37 ± 5	570 ± 13	294 ± 22	73 ± 5
HCl	0	7.48 ± 0.09	37 ± 7	547 ± 30	320 ± 22	95 ± 11
	8	7.41 ± 0.07	37 ± 5	338 ± 138*	307 ± 30	81 ± 17
HCl + ONO	0	7.47 ± 0.07	38 ± 5	575 ± 48	326 ± 3	98 ± 6
	8	7.46 ± 0.04	36 ± 4	569 ± 36†	299 ± 13	84 ± 3

Values are mean ± SD.

* $P < 0.05$ versus control group.

† $P < 0.05$ versus HCl group.

ONO-5046 prevented the increase in the W/D ratios in the noninstilled lungs of the ONO group. The W/D ratios of the hearts, livers, and kidneys in the three experimental groups did not differ; the W/D ratios of the small intestines in the HCl group were significantly greater than those measured in the control and ONO groups.

Protein Concentration in BALF

The protein concentrations of the BALF obtained from the acid-instilled lungs from the animals of the HCl and the ONO groups were significantly increased compared

with the protein concentration of the BALF obtained from the PBS-instilled lungs from the control animals (fig. 4). The protein concentrations of the BALF obtained from the noninstilled lungs from the three groups of experimental animals were not different. The ONO-5046 pretreatment did not significantly decrease the protein concentration of the BALF obtained from the HCl-instilled lungs.

Neutrophil Counts in BALF

The neutrophil counts in the BALF are shown in the table 2. The BALF from the acid-instilled lungs had neutrophil counts of $85 \pm 15 \times 10^6/\text{ml}$ compared with the counts from the BALF of the control group ($5 \pm 0.6 \times 10^6/\text{ml}$). ONO-5046 pretreatment did not decrease the neutrophil counts in the BALF ($69 \pm 23 \times 10^6/\text{ml}$). In the BALF from the noninstilled lungs, there were $26 \pm 8 \times 10^6/\text{ml}$ neutrophils in the BALF from the animals in the HCl group, and the neutrophil counts were similar in the BALF from the animals in the ONO group ($19 \pm 12 \times 10^6/\text{ml}$).

Elastase Activity in BALF

There was no elastase activity in the BALF from the lungs of the animals in the control group. The elastase activity in the BALF from the acid-instilled lungs in the HCl animals was $3.5 \pm 0.2 \text{ U/ml}$ and $1.8 \pm 0.3 \text{ U/ml}$ in the BALF from the contralateral lung. There was no elastase activity detected in the BALF from the acid-instilled or from the noninstilled lungs from the animals in the ONO-5046 group.

Discussion

In this study, we showed that a specific neutrophil elastase inhibitor, ONO-5046, attenuated some of the

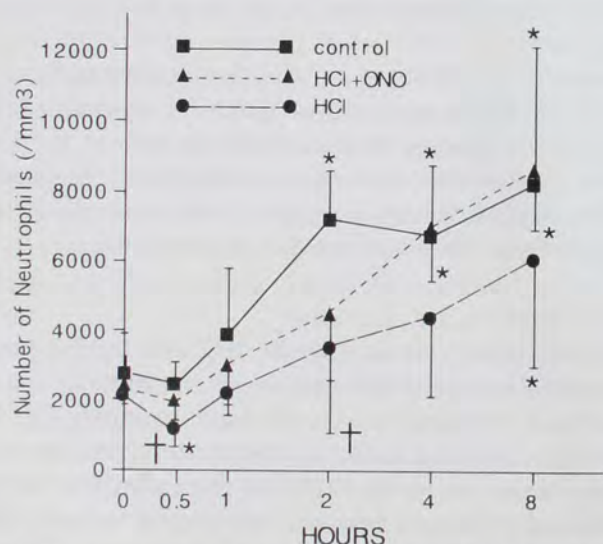


Fig. 1. The time course of neutrophil counts in the blood in the three experimental groups. In the HCl group, the neutrophils decreased significantly at 0.5 h; the comparison is to the neutrophil counts measured in the HCl group of animals at baseline. The administration of ONO-5046 prevented this early neutropenia. * $P < 0.05$ compared with 0 h; † $P < 0.05$ compared with the control group.

ASPIRATION AND NEUTROPHIL ELASTASE

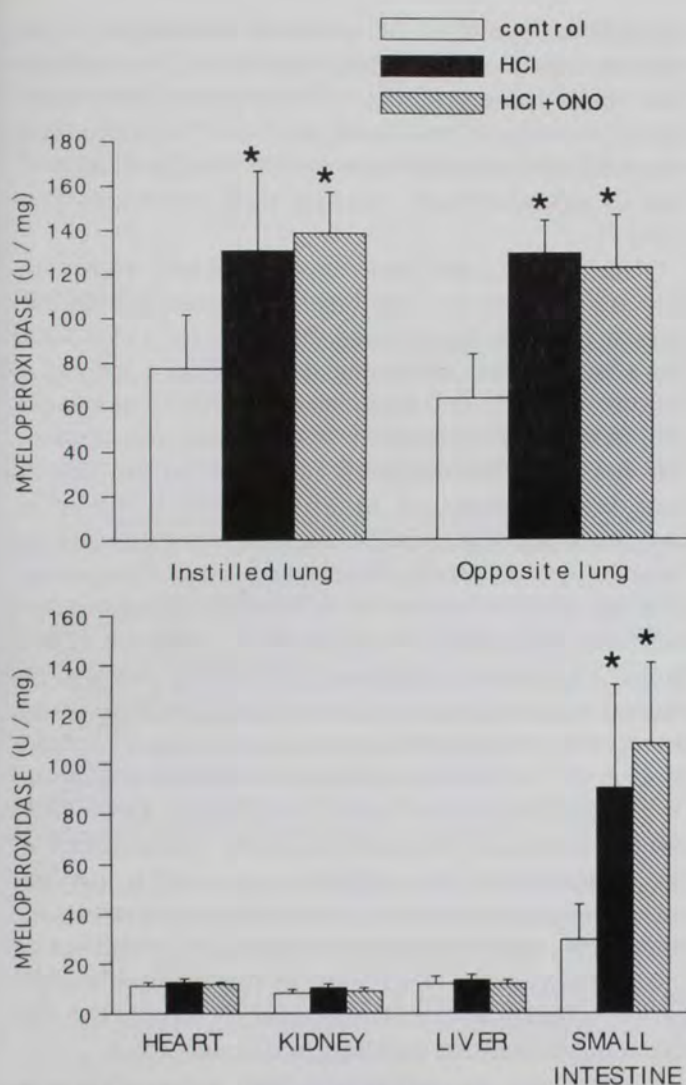


Fig. 2. Myeloperoxidase activities (a tracer that quantitates neutrophil sequestration) in the lungs and other organs. The instillation of acid increased myeloperoxidase activities in the instilled and in the noninstilled lungs and in the small intestines; the administration of ONO-5046 did not attenuate these increases. * $P < 0.05$ compared with the control group.

pathophysiologic effects of acid-induced lung and small intestine injury. The ONO-5046 pretreatment prevented the increases in the W/Ds of the noninstilled lungs and of the small intestines and normalized the Pa_{O_2} in rabbits who had been exposed to the unilateral acid instillations. Lung histologic sections confirmed that the lung edema was reduced in the ONO-5046 pretreated animals. Notably, these effects occurred despite the fact that the ONO-5046 pretreatment did not affect the neutrophil sequestration in the lungs or small intestines, nor did the pretreatment affect myeloperoxidase activi-

ties in the lungs or the necrosis seen on the histologic sections.

Activation of neutrophil and endothelial adhesion molecules are thought to contribute to the increased endothelial permeability in the noninstilled lungs^{3,4} in acid-induced unilateral lung injury experiments. There are CD18-dependent and CD18-independent pathways that activate neutrophil adherence. Doerschuk *et al.*¹⁷ showed that the anti-CD18 monoclonal antibody, 60.3, did not inhibit neutrophil emigration into acid-instilled lungs, suggesting that CD18-independent pathways

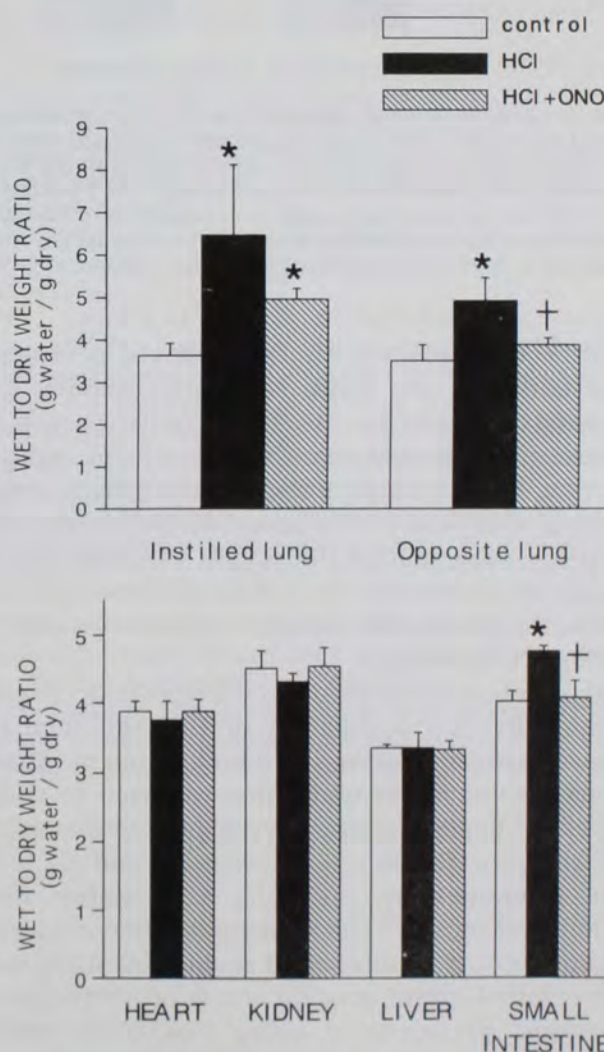


Fig. 3. Wet-to-dry weight ratios (W/D) of the lungs and other organs. The instillation of acid increased the W/D in the instilled and in the noninstilled lungs and in the small intestines; the administration of ONO-5046 prevented the increase in the W/D in the noninstilled lungs and in the small intestines. * $P < 0.05$ compared with the control group; † $P < 0.05$ compared with the HCl group.

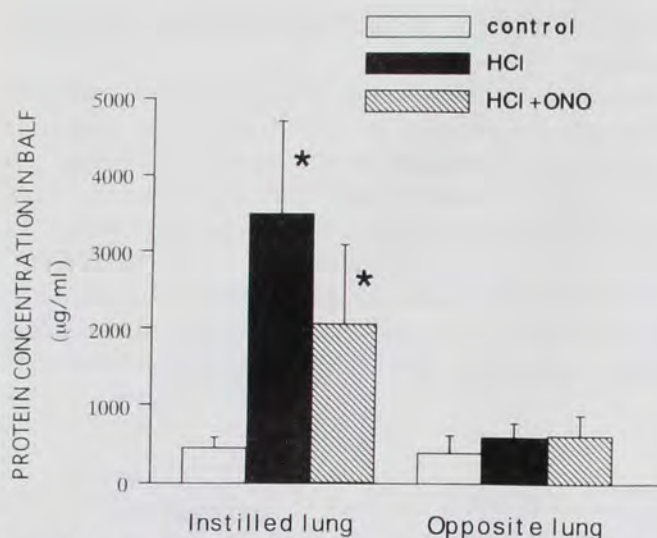


Fig. 4. The protein concentrations measured in the bronchoalveolar lavage fluids (BALF). In the instilled lungs, the instillation of acid increased the protein concentrations in the BALF in the HCl group and in the ONO-5046 group. The instillation of acid did not increase the protein concentrations of the BALF obtained from the noninstilled lungs in either the HCl or ONO groups. * $P < 0.05$ compared with the control group.

might be more important in acid-induced neutrophil sequestration in the lungs. Neutrophil stiffness and ICAM and other selectins have been found to contribute to neutrophil sequestration in acid-injured lungs.¹⁸ However, this investigation suggests that lung endothelial permeability can be normalized despite the fact that neutrophil sequestration still occurs; blockade of neutrophil elastase function was sufficient to normalize the endothelial permeability and the oxygenation defects in acid-exposed animals.

The number of circulating neutrophils decreased 30 min after the instillation of HCl and then increased gradually during the 8-h experiment. These results are consistent with those reported by Goldman *et al.*³ Recent reports have suggested that adhesion receptor families (*i.e.*, selectins and β_2 integrins) promote early neutrophil sequestration after the onset of sepsis.¹⁹ The neutropenia that occurred 30 min after the instillation of acid in this study may have resulted from L-selectin and E- or P-selectin interactions; pretreatment using ONO-5046 modulated this early neutropenia, suggesting perhaps that the blockade of neutrophil elastase may affect adhesion molecules. This is speculative; there may be other mechanisms for the neutropenia, and this investigation did not specifically address this issue.

The neutral protease, elastase, is released from the

azurophilic granules of activated neutrophils. The elastase has broad substrate specificity that includes basal membrane proteins, cell receptors, fibronectin, elastin, collagens, and proteoglycans.²⁰ This elastase also has been found to cause endothelial cell injury²¹ and to proteolytically destroy lung surfactant proteins.²²

ONO-5046 is a synthetic compound and is a reversible and competitive inhibitor of human, rabbit, rat, hamster, and mouse neutrophil elastase.⁹ ONO-5046 has a much lower molecular weight than α_1 -protease inhibitor (α_1 -PI) and may exert its effects in the microenvironment between neutrophils and tissues, whereas α_1 -PI incompletely blocks neutrophil-mediated tissue destruction. Inhibition of neutrophil elastase has been shown to be useful in other models of lung injury. Recently, Yoshimura *et al.*²³ reported that the administration of ONO-5046 inhibited leukotriene B₄-induced lung injury in isolated rabbit lungs. The administration of ONO-5046 was also reported to be effective in modulating endotoxin-induced lung injury in sheep and guinea pigs.^{10,12} Sakamaki *et al.*¹² demonstrated that in a guinea pig model of endotoxin-induced acute lung injury, ONO-5046 did not attenuate the increase in the permeability of the endothelium, but rather it appeared to protect the alveolar epithelium. A possible explanation for the discrepancy between the lack of protection of the epithelium by ONO-5046 in the present investigation is that acid instillation directly injures the epithelium, unlike the instillation of endotoxin.

In summary, we and others have shown that several agents have effects when used as pretreatments in animal models of acid-induced lung injury. The present experiments demonstrate that the administration of ONO-5046, a neutrophil elastase inhibitor, significantly improved oxygenation and improved lung edema induced by unilateral acid instillation. The utilization of this drug or others that decrease

Table 2. Neutrophil Counts in BALF ($\times 10^6/\text{ml}$)

	Control Group	HCl Group	ONO Group
Acid-instilled lung	5 \pm 0.6	85 \pm 15*	69 \pm 23*
Contralateral lung	4 \pm 0.4	26 \pm 8*	19 \pm 12*

Values are mean \pm SD.

* $P < 0.05$ versus control group.

BALF = bronchoalveolar lavage fluid.

ASPIRATION AND NEUTROPHIL ELASTASE

the inflammatory response in the clinical setting will depend on whether the drugs cause significant side effects or problems in host defense. Future experiments need to focus on whether this agent is useful when it is administered after the acid injury has occurred.

References

1. Olsson GL, Hallen B, Hambræus-Jonzon K: Aspiration during anaesthesia: a computer-aided study of 185,358 anaesthetics. *Acta Anaesthesiol Scand* 1986; 30:84-92
2. Doyle RL, Szaflarski N, Modin GW, Wiener-Kronish JP, Matthay MA: Identification of patients with acute lung injury. *Am J Respir Crit Care Med* 1995; 152:1818-24
3. Goldman G, Welbourn R, Kobzid I, Valeri CR, Shepro D, Hechtman HB: Tumor necrosis factor- α mediates acid aspiration-induced systemic organ injury. *Ann Surg* 1990; 212:513-20
4. Goldman G, Welbourn R, Kobzid I, Valeri CR, Shepro D, Hechtman HB: Synergism between leukotriene B₄ and thromboxane A₂ in mediating acid-aspiration injury. *Surgery* 1992; 111:55-61
5. John RCS, Mizer LA, Kindt GC, Weisbrode SE, Moore SA, Dorinsky PM: Acid aspiration-induced acute lung injury causes leukocyte-dependent systemic organ injury. *J Appl Physiol* 1993; 74:1994-2003
6. Kudoh I, Ohtake M, Nishizawa H, Kurahashi K, Hattori S, Okumura F, Pittet JF, Wiener-Kronish J: The effect of pentoxifylline on acid-induced alveolar epithelial injury. *ANESTHESIOLOGY* 1995; 82:531-41
7. Nishizawa H, Yamada H, Miyazaki H, Ohara M, Kaneko K, Yamakawa T, Wiener-Kronish J, Kudoh I: Soluble complement receptor type 1 inhibited the systemic organ injury caused by acid-instillation into a lung. *ANESTHESIOLOGY* 1996; 85:1120-8
8. Kudoh I, Nishizawa H, Miyashita T, Okumura F, Wiener-Kronish J: Effects of pentoxifylline administered after HCl-instillation into lung on TNF- α production and systemic injury in rabbits (Abstract). *Am J Respir Crit Care Med* 1995; 151:A761
9. Kawabata K, Suzuki M, Sugitani M, Imaki K, Toda M, Miyamoto T: ONO-5046, a novel inhibitor of human neutrophil elastase. *Biochem Biophys Res Commun* 1991; 177:814-20
10. Kubo K, Kobayashi T, Hayano T, Koizumi T, Honda T, Sekiguchi M, Sakai A: Effects of ONO-5046, a specific neutrophil elastase inhibitor, on endotoxin-induced lung injury in sheep. *J Appl Physiol* 1994; 77:1333-40
11. Knight PR, Druskovich G, Tait AR, Johnson KJ: The role of neutrophils, oxidants, and proteases in the pathogenesis of acid pulmonary injury. *ANESTHESIOLOGY* 1992; 77:772-8
12. Sakamaki F, Ishizaka A, Urano T, Sayama K, Nakamura H, Terasima T, Waki Y, Tasaka S, Hasegawa N, Sato K, Nakagawa N, Obata T, Kanazawa M: Effect of a specific neutrophil elastase inhibitor, ONO-5046, on endotoxin-induced acute lung injury. *Am J Respir Crit Care Med* 1996; 153:391-7
13. Lowly OH, Rosenbough NJ, Far AL, Randall J: Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 193:265-75
14. Goldblum SE, Wu KM, Jay M: Lung myeloperoxidase as a measure of pulmonary leukostasis in rabbits. *J Appl Physiol* 1985; 59:1978-85
15. Selinger SL, Bland RD, Demling RH, Staub NC: Distribution volumes of ¹³¹I albumin, ¹⁴C sucrose and ³⁶Cl in sheep. *J Appl Physiol* 1975; 39:773-9
16. Barnett CC, Moore EE, Moore FA, Carl VS, and Biffl WL: Soluble ICAM-1 provokes PMN elastase release. *J Surg Res* 1996; 63:6-10
17. Doerschuk CM, Winn RK, Coxson HO, Harlan JM: CD18-dependent and -independent mechanisms of neutrophil emigration in the pulmonary and systemic microcirculation of rabbits. *J Immunol* 1990; 144:2327-33
18. Williams TJ, Hellewell PG: Adhesion molecules involved in the microvascular inflammatory response. *Am Rev Respir Dis* 1992; 146:S45-46
19. Adrian UH, Chambers DJ, McEvoy LM, Bargatze F, Afros KE, Butcher EC: Two step model of leukocyte-endothelial cell interaction in inflammation: Distinct roles of LECAM-1 and leukocyte β 2 integrins in vivo. *Proc Natl Acad Sci U S A* 1991; 88:7538-42
20. Jochum M, Gippner-Steppert C, Machleidt W, Fritz H: The role of phagocyte proteinases and proteinase inhibitors in multiple organ failure. *Am J Respir Crit Care Med* 1994; 150:S123-30
21. Inauen W, Granger DN, Meininger CJ, Schelling ME, Granger HJ, Kvietys PR: Anoxia-reoxygenation-induced, neutrophil-mediated endothelial cell injury: Role of elastase. *Am J Physiol* 1990; 259:H925-31
22. Pison U, Tam EK, Caughey GH, Hawgood S: Proteolytic inactivation of dog lung surfactant-associated proteins by neutrophil elastase. *Biochim Biophys Acta* 1989; 992:251-7
23. Yoshimura K, Nakagawa S, Koyama S, Kobayashi T, Honma T: Roles of neutrophil elastase and superoxide anion in leukotriene B₄-induced lung injury in rabbit. *J Appl Physiol* 1994; 76:91-6