Anesthesiology
83:169–177, 1995

Lippincott–Raven Publishers

Does Early Posttreatment with Lidocaine Attenuate Endotoxin-induced Acute Lung Injury in Rabbits?

Kahoru Nishina, M.D., Katsuya Mikawa, M.D.,† Nobuhiro Maekawa, M.D.,‡ Yumiko Takao, M.D.,§ Hidefumi Obara, M.D.‖*

Background: **It is well known that endotoxin causes acute lung injury, resulting in adult respiratory distress syndrome. Lidocaine pretreatment has recently been shown to attenuate endotoxin-induced lung injury in rabbits. The aim of the current study was to determine whether early postinjury treatment with intravenous lidocaine could attenuate acute lung injury induced by endotoxin in rabbits.**

Methods: **Thirty-two male anesthetized rabbits were randomly assigned to receive one of four treatments (n = 8 for each group): infusion of saline (group S-S), infusion of saline with lidocaine treatment (group S-L), infusion of *Escherichia coli* endotoxin (100 $\mu g \cdot kg^{-1}$ over a 60-min period) without lidocaine treatment (group E-S), or infusion of endotoxin with lidocaine treatment (group E-L). Ten minutes after the end of infusion of endotoxin (groups E-L and E-S) or saline (groups S-S and S-L), the animals received a bolus injection followed by continuous infusion of lidocaine (2 $mg \cdot kg^{-1}$ + 2 $mg \cdot kg^{-1} \cdot h^{-1}$ in groups S-L and E-L) or saline (groups S-S and E-S). The rabbits' lungs were ventilated with 40% O_2. Hemodynamics, peripheral leukocyte and platelet counts, and arterial O_2 tension (Pa_{O_2}) were recorded during the ventilation period (6 h). After the observation, lung mechanics; the cell fraction of bronchoalveolar lavage fluid (BALF); and concentrations of activated complement components C3a and C5a, cytokines, and arachidonic acid metabolites in BALF were measured and analyzed. The ratio of lung wet weight to dry weight (W/D weight ratio) and albumin concentrations in BALF were analyzed as indexes of pulmonary edema. The *Cypridina* luciferin analogue–dependent chemiluminescence (representing O_2^- production) by neutrophils isolated from the pulmonary artery and light-microscopic findings of the lung were compared among the four groups.**

Results: **Endotoxin caused decreases in peripheral leukocyte and platelet counts, lung compliance, and Pa_{O_2}. It caused increases in lung W/D weight ratio; polymorphonuclear cell counts in BALF; and albumin, C3a, C5a, tumor necrosis factor-α, interleukin (IL)–1β, IL-6, IL-8, and thromboxane B_2 concentrations in BALF. Lidocaine attenuated the changes in W/D weight ratio and morphologic lung damage. The change in compliance, decrease in Pa_{O_2}, and albumin concentrations in BALF were slightly but significantly less in rabbits receiving lidocaine after injury. The *Cypridina* luciferin analogue–dependent chemiluminescence by neutrophils was greater in rabbits receiving endotoxin without lidocaine than in those receiving endotoxin with lidocaine.**

Conclusions: **These results indicate that early treatment with lidocaine attenuates endotoxin-induced lung edema in rabbits without affecting chemical mediators in BALF. However, the improvement is slight and likely to be of little clinical significance. (Key words: Anesthetics, local: lidocaine. Immune response: neutrophils; superoxide anions. Lung(s): edema; lavage; respiratory distress syndrome.)**

INTRAVENOUS infusion of *Escherichia coli* endotoxin causes acute lung injury and alterations in pulmonary physiologic processes similar to those that occur in septicemia in humans.[1,2] There have been a variety of experimental approaches to the prevention of acute lung injury.[3–5] We have shown that pretreatment with lidocaine significantly attenuated endotoxin-induced acute lung injury in rabbits.[6] Lidocaine effectively treats endotoxin-induced lung injury by attenuating O_2^- production by neutrophils, which is the final step of lung injury, without affecting the secretion of cytokines. In the current study, we investigated whether lidocaine would be effective when administered shortly after the onset of a gram-negative septic insult (postinjury treatment). We hypothesized that lidocaine administered soon after injury also may be effective in reducing endotoxin-induced lung injury by attenuation of neutrophil activation. In our previous study, we demonstrated severe peripheral neutropenia and pulmonary hypertension in rabbits within 60 min after the start of infusion of endotoxin.[6] Thus, we chose 10 min after administering endotoxin (over a 60-min period) as an

* Research Fellow in Anesthesiology.

† Assistant Professor in Anesthesiology.

‡ Associate Professor of Anesthesiology.

§ Instructor in Anesthesiology.

‖ Professor and Chair of Anesthesiology.

Received from the Department of Anesthesiology, Kobe University School of Medicine, Kobe, Japan. Submitted for publication July 14, 1994. Accepted for publication March 29, 1995.

Address reprint requests to Dr. Nishina: Department of Anesthesiology, Kobe University School of Medicine, Kusunoki-cho 7, Chuo-ku, Kobe 650, Japan.

appropriate time both after septic insult and before deterioration of oxygenation to test the efficacy of lidocaine.

Materials and Methods

Animal Preparation and Protocol

This study was conducted according to the guidelines of the animal care review board of Kobe University School of Medicine. Thirty-two male Japanese white rabbits weighing 2.0–2.4 kg were used in this study and randomly divided into four groups (n = 8 for each group) in a blinded manner as follows: rabbits in group S-S received saline alone without lidocaine posttreatment; group S-L received saline with lidocaine posttreatment; group E-S received endotoxin from *E. coli* (055:B5 from the same lot, Difco, Detroit, MI), without lidocaine posttreatment; and group E-L received endotoxin followed by lidocaine (fig. 1).

After the rabbits were sedated with 4 $mg \cdot kg^{-1}$ ketamine, tracheostomy was performed aseptically, and a 3.5-mm noncuffed endotracheal tube was inserted and tied in place. Anesthesia was maintained with continuous infusion of ketamine at a rate of 4 $mg \cdot kg^{-1} \cdot h^{-1}$. The lungs of the rabbits were ventilated with an infant ventilator (IV100B, Sechrist, Anaheim, CA) at an inspired O_2 concentration of 40%. Tidal volume was set to 10 $ml \cdot kg^{-1}$ measured by pneumotachograph. Respiratory rate was adjusted to produce an initial arterial CO_2 tension (Pa_{CO_2}) of 35–42 mmHg; Pa_{CO_2} was maintained at less than 50 mmHg throughout the study period.

Via femoral cutdown, a catheter was placed in the distal aorta to monitor arterial pressure and to take samples for blood gas analysis. Pulmonary arterial pressure was continuously monitored with a pulmonary artery catheter (3-French, Baxter, Chicago, IL) inserted through the right internal jugular vein. Central venous pressure was also monitored with a catheter inserted through the femoral vein. The animals were placed on a heating pad under a radiant heat lamp so that the body temperature could be kept at 37.7–40.3°C. Lactated Ringer's solution was intravenously administered at a rate of 8 $ml \cdot kg^{-1} \cdot h^{-1}$.

Immediately after the baseline measurement of lung mechanics, hemodynamics, peripheral leukocyte and platelet counts, and arterial blood gas analysis, groups S-S and S-L received infusion of saline for 60 min. Rabbits in groups E-L and E-S received endotoxin 100

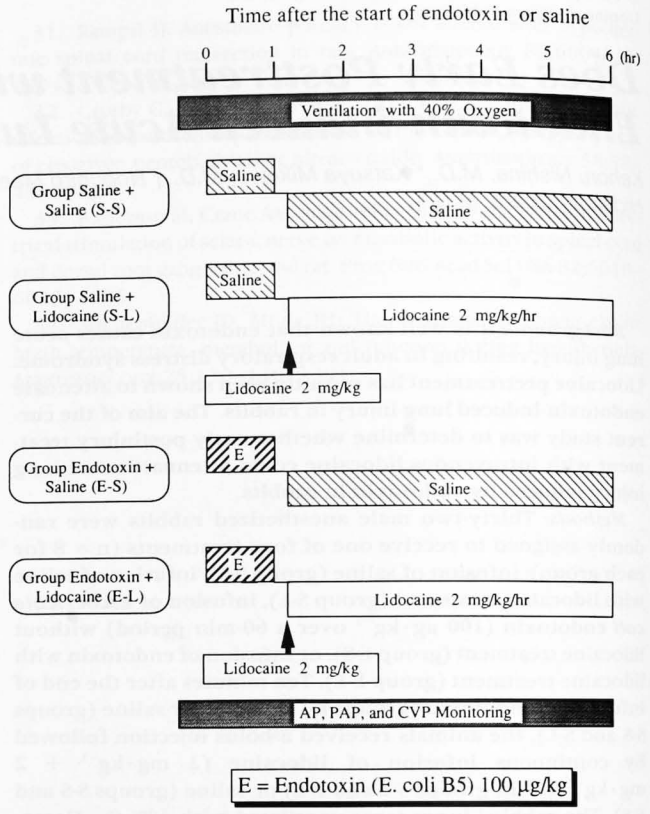

Fig. 1. Study protocol. AP = arterial blood pressure; PAP = pulmonary arterial pressure; CVP = central venous pressure.

$\mu g \cdot kg^{-1}$ over a 60-min period, with or without lidocaine treatment. Groups E-L and S-L received a bolus of lidocaine 2 $mg \cdot kg^{-1}$ (Fujisawa, Osaka, Japan), 10 min after the end of administration of endotoxin (E-L) or saline (S-L), followed by continuous infusion of lidocaine at a rate of 2 $mg \cdot kg^{-1} \cdot h^{-1}$ until the rabbits were killed. This infusion rate of lidocaine was also used in our previous study.[6]

All rabbits were killed 6 h after the start of endotoxin treatment by injection of thiamylal. In groups S-L and E-L, arterial blood samples were obtained at 0, 0.5, 1, 2, 3, and 4 h after the start of administration of lidocaine and at the time of killing to measure plasma concentrations of the drug using fluorescence polarization immunoassay (TDX system, Abbott, North Chicago, IL).

Estimation of Acute Lung Injury

Measurement of Lung Mechanics. During each experimental period, we obtained arterial blood specimens for analyzing arterial O_2 tension (Pa_{O_2}), Pa_{CO_2},

and *p*H using an automatic blood gas and electrolyte analyzer (ABL2, Radiometer, Copenhagen, Denmark) and for counting the number of peripheral leukocytes and platelets (counter, Coulter Electronics, Harkenden, United Kingdom). Immediately after the start of mechanical ventilation (before infusion of saline or endotoxin), at the end of administration of endotoxin, and immediately before the rabbits were killed (after the period of observation), lung mechanics were measured by the passive expiratory flow–volume technique as described by LeSouef *et al.*[7] The air flow was measured with a Fleish 00 pneumotachograph and a differential pressure transducer (MP-45, Validyne Engineering, Northridge, CA). Airway pressure was measured at the proximal end of the pneumotachometer with a semiconductor pressure transducer (P-300 501G, Copal Electronics, Tokyo, Japan). The volume was measured for each breath by digital integration of air flow using a respiration monitor (Aivision, Tokyo, Japan) and a personal computer (PC9801 VM11, NEC, Tokyo, Japan). The lungs were inflated and the air flow was interrupted at 20 cmH_2O. The occlusion was rapidly released after airway pressure reached a plateau. Compliance and resistance of the total respiratory system were then calculated by means of the personal computer.

At the end of the experiment, after the thorax was opened, blood (15 ml) was drawn into a heparinized syringe (20 $U \cdot ml^{-1}$) from the pulmonary artery for chemiluminescence assay (see below). The rabbits were killed by administration of thiamylal after sampling of blood. The heart and lungs were then removed *en bloc* by observers blinded to the nature of experiment.

Ratio of Lung Wet Weight to Dry Weight. The left upper lobe was weighed and then dried to constant weight at 60°C for 24 h in an oven. The ratio of wet weight to dry weight (W/D weight ratio) was calculated to assess tissue edema.

Preparation of Bronchoalveolar Lavage Fluid and Measurements. Through the right mainstem bronchus 40 ml saline with ethylenediamine tetraacetic acid–2Na at 4°C was slowly infused and withdrawn. This procedure was repeated five times. Indomethacin was added to the bronchoalveolar lavage fluid (BALF) to inhibit further metabolism of arachidonic acid to prostaglandins during analysis. The BALF was analyzed for cell count and cell differentiation. A cytocentrifuged preparation (Cytospin 2, Shandon Southern Products, United Kingdom) of the BALF was stained with Wright-Giemsa for cell differentiation. The cells present in the fluid were counted with the Coulter counter and the Bürker-Türk method.[8]

The fluid was centrifuged at 250*g* at 4°C for 10 min to remove the cells. The cell-free supernatant was divided into several aliquots and stored at −70°C until assayed. The following substances, metabolites, and mediators in the BALF were then measured. (1) The activated complement components C3a and C5a were quantified by radioimmunoassay (Amersham, Bucks, UK). (2) Albumin concentrations were measured by nephelometry with immunoglobulin G fraction of goat antirabbit albumin (Cappel, PA). (3) Concentrations of tumor necrosis factor α (TNFα), interleukin (IL)–1β, IL-6, and IL-8 were measured by enzyme immunoassay (Amersham, Bucks, United Kingdom). (4) Concentrations of thromboxane A_2 (TxA_2) and prostacyclin were quantified by radioimmunoassay (Amersham) as thromboxane B_2 (TxB_2) and 6-keto-prostaglandin $F_1\alpha$, the stable metabolites of TxA_2 and prostacyclin, respectively.

Chemiluminescence Assay. ***Reagents.*** *Cypridina* luciferin analogue (CLA) (2-methyl-6-phenyl-3,7-dihydroimidazo [1,2-a]-pyrazine-3-one), dimethyl sulfoxide, Hank's balanced salt solution (HBSS), Histopaque-1119, Histopaque-1077, N-formyl-L-methionyl-L-luecyl-L-phenylalanine (FMLP), and zymosan A were obtained from Sigma Chemical (St. Louis, MO).

The CLA was dissolved to 56 $\mu g \cdot ml^{-1}$ in distilled water. The solution was stored in 1-ml aliquots at −80°C. The CLA concentrations were based on E410 nm = 8900 $M^{-1} \cdot cm^{-1}$.[9] FMLP 5 mg was dissolved in 1.14 ml DSMO. The solution was stored at −80°C until the time of the assay. Just before use, the stored solution was diluted with 50% dimethyl sulfoxide–50% HBSS to 100 μM. Zymosan A was opsonized by the method of Nishida *et al.*[10] with modification. Zymosan A was suspended in HBSS at a concentration of 2 $mg \cdot ml^{-1}$ and heated in a boiling water bath for 100 min, washed twice with HBSS, and opsonized with pooled serum in a shaking water bath for 30 min at 37°C. The opsonized zymosan (OZ) was then washed twice, resuspended in HBSS to a concentration of 20 $mg \cdot ml^{-1}$, and stored at −80°C until use.

Isolation of Neutrophils. Histopaque-1119, Histopaque-1077, and whole blood were layered in a test tube and centrifuged at 700*g* for 30 min at room temperature. The layer containing granulocytes (at the interphase between Histopaque-1077 and Histopaque-1119) was transferred to another tube. The cells were washed in HBSS and centrifuged twice at 200*g* for 10

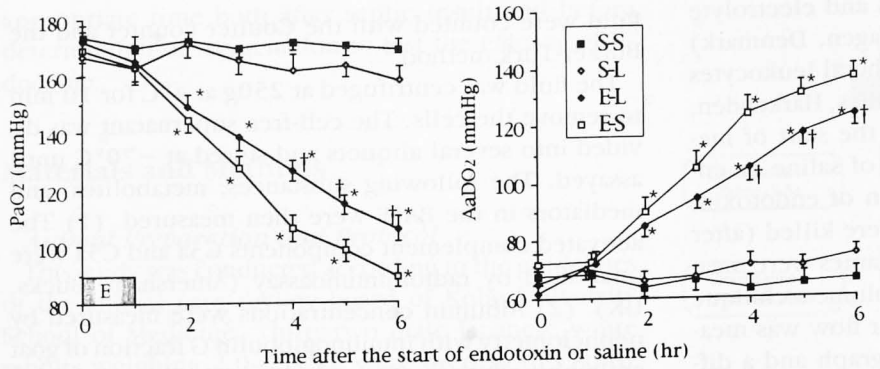

Fig. 2. Changes in arterial O_2 tension (Pa_{O_2}) and alveolar − arterial difference in O_2 tension (AaD_{O_2}) (mean ± SEM) in the three groups. E = intravenous infusion of *Escherichia coli* endotoxin (100 μg/kg) or saline; S-S = group receiving saline; S-L = group receiving saline and lidocaine; E-S = group receiving endotoxin and no lidocaine; E-L = group receiving endotoxin and lidocaine. *$P < 0.05$ *versus* group S-S; †$P < 0.05$, group E-L *versus* group E-S.

min. The resultant leukocytes were suspended to 1×10^7 cells · ml^{-1} in HBSS and were kept at 0°C for no longer than 3 h before use. The cell analysis showed that more than 96% of the cells were neutrophils, and the trypan blue dye exclusion test confirmed that more than 95% of the cells were viable.

Measurement of Chemiluminescence. Measurement of chemiluminescence was made by the method of Sugioka *et al.*[11] The incubation mixture contained 4×10^5 white blood cells (WBC), 20 μl FMLP or 80 μl OZ, 50 μl 40 μM CLA, and HBSS to a total volume of 2 ml. Cells and HBSS were preincubated for 3 min and the reaction initiated by the simultaneous addition of the other two components. CLA-dependent luminescence, which is thought to reflect primarily O_2^- production, was monitored with a luminescence reader (Lumicounter-1000, Nichion, Chiba, Japan). During luminescence measurement, the incubation mixture was agitated at 37°C in the luminescence reader. Ketamine used as an anesthetic in the current study has been shown to have no effect on O_2^- production by neutrophils at doses used in the clinical setting.[12]

Histopathologic Examination

Shortly after the rabbits were killed (<5 min), the left lower lobe was fixed by instillation of 10% formaldehyde solution through the left lower bronchus at 20 cmH_2O. The specimens were embedded in paraffin wax, and stained with hematoxylin and eosin and examined under a light microscope. Lung injury was scored as 0 (minimal damage) to 4+ (maximal damage) according to combined assessments of alveolar congestion; hemorrhage and edema; infiltration or aggregation of neutrophils in air space or vessel wall; thickness of alveolar wall, and hyaline membrane formation by two observers unaware of the group assignment of the animal.

Statistical Analysis

Data except lung injury score are expressed as means ± SEM; data on lung injury score are given as medians and range. The degree of attenuation of lung injury by lidocaine was calculated from the following formula: percentage attenuation = 100 × (b − c)/(b − a), where a = value in S-S group; b = value in E-S group; and c = value in E-L group. This value indicates the degree of efficacy of lidocaine treatment in each subject: 0% indicates that the mean scores for groups E-S and E-L were equal (no attenuation of lung injury by lidocaine), and 100% indicates that the mean scores for group E-L and S-S were equal (maximum attenuation). Statistical analysis was performed by repeated-measures analysis of variance for continuous variables, except for lung injury score, for which the Kruskall-Wallis rank test was used. $P < 0.05$ was deemed significant. When analysis of variance indicated a significant difference, Bonferroni's multiple-comparison test was used to determine which groups were significantly different from each other.

Results

Changes in Arterial O_2 Tension, Hemodynamics, and Peripheral Leukocyte and Platelet Counts

No rabbits died of endotoxemia. In groups S-L and E-L, plasma lidocaine concentrations were maintained between 1.2–2.3 μg · ml^{-1}. As shown in figure 2, Pa_{CO_2} in groups S-S and S-L remained at a level exceeding 150 mmHg, whereas Pa_{O_2} in group E-S gradually decreased to 89 mmHg during the experiment. In group E-L, however, the decrease in this parameter was attenuated maximally by 29% ($P < 0.05$). The values of Pa_{CO_2} in four groups were similar (38 to 40 mmHg) and gradually increased to 50 ± 1.2 and 52 ± 1.3 in

Table 1. Changes in Hemodynamics and Peripheral Leukocyte and Platelet Counts in the Four Groups

	Time after the Start of Endotoxin or Saline							
	0 h	0.5 h	1 h	2 h	3 h	4 h	5 h	6 h
MPAP (mmHg)								
S-S	19 ± 1	20 ± 1	19 ± 1	17 ± 1	19 ± 1	17 ± 1	20 ± 1	18 ± 1
S-L	18 ± 1	20 ± 1	18 ± 1	19 ± 1	18 ± 1	20 ± 1	18 ± 1	17 ± 1
E-S	17 ± 1	30 ± 1*	32 ± 1*	29 ± 1*	25 ± 1*	24 ± 1*	22 ± 2*	22 ± 1*
E-L	19 ± 1	32 ± 1*	33 ± 1*	28 ± 1*	23 ± 1*	22 ± 1*	20 ± 1*	19 ± 1*
Leukocytes ($\times 10^2$ cells/mm^3)								
S-S	40 ± 4	38 ± 4	39 ± 5	40 ± 4	43 ± 3	37 ± 4	35 ± 3	39 ± 4
S-L	38 ± 4	37 ± 4	36 ± 6	40 ± 4	43 ± 3	37 ± 4	35 ± 3	39 ± 4
E-S	41 ± 4	16 ± 1*	9 ± 0.7*	5 ± 0.2*	5 ± 0.3*	6 ± 0.3*	8 ± 0.3*	9 ± 0.7*
E-L	42 ± 4	15 ± 1*	9 ± 0.7*	5 ± 0.2*	6 ± 0.3*	6 ± 0.3*	9 ± 0.3*	10 ± 0.7*
Platelets ($\times 10^4$ cells/mm^3)								
S-S	29 ± 3	32 ± 3	30 ± 3	33 ± 3	29 ± 3	27 ± 3	29 ± 3	26 ± 3
S-L	31 ± 3	29 ± 3	28 ± 3	30 ± 3	29 ± 3	29 ± 3	28 ± 3	27 ± 3
E-S	30 ± 3	29 ± 3	26 ± 3	22 ± 3*	17 ± 3*	15 ± 3*	16 ± 3*	14 ± 3*
E-L	33 ± 3	31 ± 3	28 ± 3	23 ± 3*	19 ± 3*	20 ± 3*	19 ± 3*	20 ± 3*

Values are mean ± SEM.

MAP = mean arterial pressure; HR = heart rate; CVP = central venous pressure; MPAP = mean pulmonary arterial pressure.

* $P < 0.05$ *versus* group S-S, $P > 0.05$ for group E-L *versus* group E-S.

groups E-L and E-S, respectively. The alveolar − arterial difference in O_2 tension was increased in groups E-L and E-S as Pa_{O_2} decreased. Lidocaine attenuated the increase in the alveolar − arterial difference in O_2 tension maximally by 31% ($P < 0.05$). The heart rate, arterial blood pressure, or central venous pressure did not differ among the four groups at any point (data not shown). Infusion of endotoxin rapidly increased pulmonary arterial pressure, with a peak reached at the end of endotoxin infusion. Lidocaine posttreatment failed to attenuate the pulmonary hypertension (table 1). Peripheral blood leukocyte counts decreased with infusion of endotoxin, reached their nadir 1–2 h after the end of endotoxin infusion, and remained low during the experiment. Lidocaine posttreatment also failed to attenuate the decrease in leukocyte. Peripheral blood platelet counts decreased gradually in the endotoxin-treated group (E-S and E-L). No effect of lidocaine on platelet counts was observed.

Lung Mechanics

Neither compliance nor resistance immediately after the start of mechanical ventilation and at the end of endotoxin treatment was different among the four groups (table 2). Compliance 6 h after the start of treatment with endotoxin was greater in group E-L than in group E-S (attenuation 29%; $P < 0.05$). In contrast, resistance in group E-L 6 h after endotoxin was similar to that in group E-S (attenuation 27%).

Ratio of Lung Wet Weight to Dry Weight

The lung W/D weight ratio was calculated as a parameter of lung edema. The ratio increased in rabbits receiving endotoxin (E-S and E-L) compared with those

Table 2. Lung Mechanics before and after Endotoxin or Saline

	Time after the Start of Endotoxin or Saline		
	0 h	1 h	6 h
Compliance (ml/cmH_2O)			
Group S-S	2.92 ± 0.09	2.88 ± 0.10	2.81 ± 0.14
Group S-L	2.88 ± 0.08	2.93 ± 0.11	2.78 ± 0.13
Group E-S	2.94 ± 0.08	2.62 ± 0.11	1.75 ± 0.12*
Group E-L	2.87 ± 0.08	2.64 ± 0.09	2.06 ± 0.12*†[.29]
Resistance ($cmH_2O \cdot L^{-2} \cdot s^{-1}$)			
Group S-S	97 ± 4	101 ± 5	102 ± 6
Group S-L	100 ± 4	98 ± 5	104 ± 7
Group E-S	95 ± 4	106 ± 6	125 ± 6*
Group E-L	101 ± 4	105 ± 5	119 ± 6*[.27]

Values are mean ± SEM. The percent attenuation of the variables by lidocaine is shown in parentheses.

% attenuation = (group E-S − group E-L) × 100/(group E-S − group S-S).

* $P < 0.05$ *versus* group S-S.

† $P < 0.05$ for group E-L *versus* group E-S.

receiving saline (S-S and S-L) (table 3). Lidocaine posttreatment attenuated the increase in W/D weight ratio (attenuation 43%; $P < 0.05$).

Analysis of Bronchoalveolar Lavage Fluid

Recovery in BALF in the three groups was 83–89%, indicating no difference between the four groups. Table 3 shows that the total number of leukocytes recovered in BALF was significantly higher in groups E-S and E-L compared with that in group S-S. Leukocyte counts in BALF were not significantly different in groups E-L and E-S (attenuation 15%). Differential counts revealed that BALF leukocytes in group S-S were mostly macrophages. Polymorphonuclear cells accounted for 1% of the WBC in BALF obtained from group S-S. In contrast, the ratio of polymorphonuclear cells to total WBC increased to 12% in group E-S and 8% in group E-L ($P > 0.05$).

Albumin concentrations in the supernatant of BALF were higher in endotoxin-treated rabbits (E-S and E-L) than in the saline-treated rabbits (S-S and S-L). Lidocaine posttreatment significantly decreased the albumin concentrations in endotoxin-treated rabbits (E-L) (attenuation 37%). Table 3 indicates that the concentrations in BALF of C3a and C5a, which are known to be chemotactic factors, were similar in groups E-L and E-S. The BALF concentrations of cytokines significantly increased in endotoxin-treated rabbits (E-S and E-L). The TxB_2 concentration in BALF increased in group E-S compared with that in group S-S. Posttreatment with lidocaine failed to attenuate the increase. In contrast, there were no differences in BALF concentrations of 6-keto-prostaglandin $F_1\alpha$ among the four groups.

Chemiluminescence

The CLA-dependent chemiluminescence (representing O_2^- production) by neutrophils isolated from the pulmonary artery blood in group E-S was significantly higher compared with that in group S-S when stimulated by OZ or FMLP (table 4). Posttreatment with lidocaine attenuated the increase in chemiluminescence (attenuation 75% for OZ and 64% for FMLP).

Histopathologic Findings

The results of the grading of lung damage are summarized in table 5. The score for E-L group was statistically less than that for group E-S. Light-microscopic findings in group E-S included hemorrhage and edema, thickened alveolar septa, and the presence of inflammatory cells in alveolar spaces; these changes were slightly less pronounced in group E-L.

Discussion

In the current study, we have shown that early posttreatment with lidocaine slightly attenuated deterio-

Table 3. Wet/Dry Weight Ratio and Analysis of Bronchoalveolar Lavage Fluid (BALF)

Variable	Group				% Attenuation
	S-S	S-L	E-S	E-L	
Total white blood cells (cells/mm^3)	196 ± 22	215 ± 24	513 ± 38*	455 ± 31*	18
% PMN	1 ± 0.1	1 ± 0.1	12 ± 3*	8 ± 2*	36
Wet/dry weight ratio	4.8 ± 0.1	4.9 ± 0.1	5.5 ± 0.1*	5.2 ± 0.1†	43
Concentrations in BALF					
Albumin (mg/dl)	1.3 ± 0.3	1.2 ± 0.2	9.2 ± 1.0*	6.3 ± 0.7*†	37
C3a (ng/dl)	25 ± 4	23 ± 4	52 ± 9*	43 ± 6*	33
C5a (ng/dl)	6 ± 1	5 ± 1	19 ± 4*	17 ± 5*	15
TNFα (fmol/ml)	1 ± 1	1 ± 1	21 ± 5*	18 ± 3*	15
IL-1β (fmol/ml)	1 ± 1	1 ± 1	19 ± 3*	15 ± 3*	22
IL-6 (fmol/ml)	3 ± 1	3 ± 1	76 ± 10*	57 ± 6*	26
IL-8 (fmol/ml)	3 ± 1	2 ± 1	48 ± 7*	31 ± 6*	38
T × B_2 (pg/ml)	115 ± 28	107 ± 25	214 ± 34*	196 ± 31*	18
6-keto-$PGF_{1\alpha}$ (pg/ml)	202 ± 38	228 ± 50	221 ± 42	205 ± 41	—

Values are mean ± SEM.

—: because 6-keto-$PGF_{1\alpha}$ is thought to be a beneficial mediator for attenuation of lung injury, percent attenuation is not calculated.

PMN = polymorphonuclear cell; IL = interleukin; T × B_2 = thromboxane B_2; $PGF_{1\alpha}$ = prostaglandin $F_{1\alpha}$; % attenuation = (group E-S − group E-L) × 100/(group E-S − group S-S).

* $P < 0.05$ *versus* group S-S.

† $P < 0.05$ for group E-L *versus* group E-S.

Table 4. *Cypridina* Luciferin Anal
Chemiluminescence (Peak) by Ne
Pulmonary Artery Blood

Group	Opsonized Zymosan -stimulated (× 10^6 cp
S-S	2.1 ± 0.2
S-L	1.3 ± 0.1*
E-S	4.1 ± 0.4*
E-L	2.6 ± 0.3† (75)

Values are mean ± SEM. The percent att
by lidocaine shown in parentheses was c
formula: % attenuation = (group E-S − gr
S-S).
* $P < 0.05$ *versus* group S-S.
† $P < 0.05$ for group E-L *versus* group E-

ration of oxygenation in e
(E-S and E-L). Lidocaine post
endotoxin-induced pulmonar
D weight ratio. The drug was
(assessed by lung injury sco
sessed by compliance for t
contrast, lidocaine posttreatm
release of chemotaxins (C3
IL-8 in BALF) in rabbits re
There were no differences be
E-S in the percentage of alveo
in BALF and leukocyte cou
These observations suggest th
could not inhibit the release
sulting in failure to reduce ac
in the lung.

The pulmonary arterial pre
receiving endotoxin (E-S and
of endotoxin treatment. The
continued until 3 h after th
ment. Lidocaine had no effe
monary arterial pressure. No
centrations in BALF was obse
groups with or without lidoc
ure of lidocaine to decrease
be responsible for failure of
pulmonary artery pressure.
lessened the endotoxin-indu

≠ Dwenger A, Regel G, Ellendorff
brock H, Sturm JA, Tscherne H. Alv
minescence response of blood neutr
in sheep after endotoxin infection.
163-168, 1990.

Table 4. *Cypridina* Luciferin Analogue-dependent Chemiluminescence (Peak) by Neutrophils Isolated from the Pulmonary Artery Blood

Group	Opsonized Zymosan -stimulated ($\times 10^6$ cpm)	FMLP-stimulated ($\times 10^6$ cpm)
S-S	2.1 ± 0.2	2.5 ± 0.2
S-L	1.3 ± 0.1*	1.7 ± 0.1*
E-S	4.1 ± 0.4*	6.7 ± 0.5*
E-L	2.6 ± 0.3† (75)	4.0 ± 0.4*† (64)

Values are mean ± SEM. The percent attenuation of the chemiluminescence by lidocaine shown in parentheses was calculated according to the following formula: % attenuation = (group E-S − group E-L) × 100/(group E-S − group S-S).

* $P < 0.05$ *versus* group S-S.

† $P < 0.05$ for group E-L *versus* group E-S.

ration of oxygenation in endotoxin-treated rabbits (E-S and E-L). Lidocaine posttreatment also attenuated endotoxin-induced pulmonary edema as assessed by W/D weight ratio. The drug was effective morphologically (assessed by lung injury score) and functionally (assessed by compliance) for the acute lung injury. In contrast, lidocaine posttreatment did not decrease the release of chemotaxins (C3a, C5a, TNFα, IL-1β, and IL-8 in BALF) in rabbits receiving endotoxin (E-L). There were no differences between the groups E-L and E-S in the percentage of alveolar neutrophils recovered in BALF and leukocyte counts in peripheral blood. These observations suggest that lidocaine posttreatment could not inhibit the release of the chemotaxins, resulting in failure to reduce accumulation of leukocytes in the lung.

The pulmonary arterial pressure increased in rabbits receiving endotoxin (E-S and E-L), peaking at the end of endotoxin treatment. The pulmonary hypertension continued until 3 h after the end of endotoxin treatment. Lidocaine had no effect on this increased pulmonary arterial pressure. No difference in TxB_2 concentrations in BALF was observed in endotoxin-treated groups with or without lidocaine (E-S or E-L). This failure of lidocaine to decrease TxB_2 concentrations may be responsible for failure of lidocaine to decrease of pulmonary artery pressure. Lidocaine posttreatment lessened the endotoxin-induced increase in BALF concentrations of albumin, which is an index of endothelial hyperpermeability leading to pulmonary edema. The beneficial effect of lidocaine on pulmonary edema, which is assessed by W/D weight ratio and pathologic changes, may be due to attenuation of vascular hyperpermeability. Successful use of lidocaine to reduce lung extravascular protein accumulation, as an index of endothelial hyperpermeability, in thiourea-induced lung injury has been reported.[13]

We began the lidocaine treatment 10 min after the end of endotoxin infusion. We chose this timing because significant pathophysiologic events (pulmonary hypertension and profound leukopenia) have already occurred at this time.[6] It is well known that pulmonary hypertension and neutropenia are initial events in endotoxin-induced lung injury.[14–16]

We have hypothesized that posttreatment with lidocaine attenuates lung injury by the suppression of activation of neutrophils, as does pretreatment. Thus we evaluated the effect of lidocaine on acute lung injury until approximately 5 h after starting treatment with lidocaine, focusing our attention on the period of activating neutrophils and macrophages. As in the control group of the current study, endotoxin causes increases in production and release of cytokines, O_2^-, thromboxanes, and complement components, resulting in accumulation of neutrophils in lung, deterioration of oxygenation, and increase in extravascular lung water within 6 h. Lung vascular permeability to proteins increased between 2 and 6 h after endotoxin injection.[17,18] Chemiluminescence response reveals that neutrophils in blood are activated early after endotoxin, followed by metabolic exhaustion with a minimal chemiluminescence response after 2 h. The response is augmented thereafter by new granulocytes liberated from the bone marrow.# Accumulation of neutrophils in affected tissue probably became peaked by 6 h after

Table 5. Mean Lung Injury Score (median (range))

Group	n	Lung Injury Score
S-S	8	0 (0)
S-L	8	0 (0)
E-S	8	3 (2–4)*
E-L	8	2 (1–4)*†

Values are medians (with range in percentages).

Scores 0 = minimal (little) damage; 1+ = mild damage; 2+ = moderate damage; 3+ = severe damage; 4+ = maximal damage.

* $P < 0.05$ *versus* group S-S.

† $P < 0.05$ for group E-L *versus* group E-S.

Dwenger A, Regel G, Ellendorff B, Schweitzer G, Funck M, Limbrock H, Sturm JA, Tscherne H. Alveolar cell pattern and chemiluminescence response of blood neutrophils and alveolar macrophages in sheep after endotoxin infection. J Clin Chem Clin Biochem 28: 163–168, 1990.

endotoxin administration, because the WBC counts in peripheral blood have started increasing.

We previously reported that lidocaine administered before endotoxin reduced endotoxin-induced lung injury.[6] Lidocaine pretreatment attenuates the deterioration of pulmonary hypertension, peripheral leukopenia, oxygenation, lung compliance, W/D ratio, and WBC counts, percentage of polymorphonuclear cells in total WBC, albumin in BALF, and O_2^- production by neutrophils with stimulation. However, lidocaine posttreatment was effective only in oxygenation, lung edema (assessed by albumin concentration in BALF, W/D ratio, and morphologic examination), and lung compliance. Lidocaine attenuated chemiluminescence of neutrophils to the same degree regardless of the timing of administration (attenuation 65% and 83% [pretreatment] and 64% and 75% [posttreatment], FMLP- and OZ-stimulated chemiluminescence, respectively). This attenuation of O_2^- production, which is known to be a major factor involved in lung injury as a final step, is a putative mechanism for reducing lung edema by lidocaine in this study.

Lidocaine after endotoxin failed to suppress chemical mediators despite improved pulmonary hyperpermeability. Some mediators that concern lung injury induced by endotoxin were not measured in the current study (*e.g.,* protease derived from neutrophils, phospholipase A_2, platelet-activating factor [PAF], and endothelin). Protease from activated neutrophils and macrophages directly attacks lung matrix, causing destruction of lung structure. Lidocaine stabilizes the cell membrane to decrease the release of proteases from neutrophils or macrophages.[19] Phospholipase A_2 activated by endotoxin causes degradation of phospholipid to arachidonic acids, as substrates of eicosanoids. TxA_2, produced by endothelium, platelets, and pulmonary macrophages, causes early phase pulmonary hypertension lasting about 90 min after starting endotoxin infusion.[20,21] It was not affected by lidocaine posttreatment probably due to its prompt release. As the results of this study showed that the concentration of TxA_2 and 6-keto-prostaglandin $F_1\alpha$ concentration in BALF were similar in groups E and E-L, lidocaine posttreatment would not prevent activation of phospholipase A_2. PAF directly and indirectly increases pulmonary vascular resistance and permeability. In the rat model, within 20 min of intraperitoneal injection of endotoxin, blood and lung PAF concentrations increased for more than 120 min.[22] TNFα stimulates peritoneal macrophages, polymorphonuclear neutrophils, and vascular endothelial cells to synthesize and release PAF 1–4 h after TNFα administration *in vivo*.[23] The PAF and proteases remain as yet to be determined to clarify the mechanisms through which lidocaine posttreatment attenuated endotoxin-induced lung injury.

Cytokines, such as IL-1, TNF-α, and IL-6, might have been induced and released at the early phase of post endotoxin administration, namely before starting lidocaine treatment. These cytokines injure lung not directly but through activating many cell types, including neutrophils and macrophages. Lidocaine might be effective in the control of the next phase related to cytokines, because even pretreatment with lidocaine possessed partial effect on attenuation of cytokine secretion. Fletcher and Ramwell reported that posttreatment with the drug only for 3 h successfully decreased mortality (as assessed 72 h after endotoxin) due to endotoxin shock in baboons and dogs.[24] Our observations were inconsistent with their study. Lidocaine suppressed O_2^- production from neutrophils but did not cut off the cytokine cascade that may be responsible for the late phase of lung injury.

In conclusion, lidocaine administered after endotoxin slightly attenuated deterioration of oxygenation probably due to reduction of edema. A comparison of these findings with our previous findings[6] suggests that posttreatment with lidocaine may have only limited clinical application in patients with adult respiratory distress syndrome.

References

1. Brigham KL, Begley C, Bernard GR, Hutchinson AA, Loyd JE, Lucht WD, Meyric B, Newman JH, Niedermeyer ME, Ogletree ML, Sheller JR, Snapper JR: Septicemia and lung injury. Clin Lab Med 3: 717–744, 1983

2. Snapper JR, Hutchinson AA, Ogletree ML, Brigham KL: Effects of cyclooxygenase inhibitors on the alterations in lung mechanics caused by endotoxemia in unanesthetized sheep. J Clin Invest 72: 63–76, 1983

3. Ishizaka A, Wu Z, Stephens KE, Harada H, Hogue RS, O'Hanley PT, Raffin TA: Attenuation of acute lung injury in septic guinea pigs by pentoxifylline. Am Rev Respir Dis 138:376–382, 1988

4. Jenkins JK, Carey PD, Byrne K, Sugerman HJ, Fowler AA III: Sepsis-induced lung injury and the effects of ibuprofen pretreatment: Analysis of early alveolar events via repetitive bronchoalveolar lavage. Am Rev Respir Dis 143:155–161, 1991

5. Said SI, Fooda HD: Pharmacologic modulation of lung injury. Am Rev Respir Dis 139:1553–1564, 1989

6. Mikawa K, Nishina K, Maekawa N, Takao Y, Yaku H, Obara H: Effect of lidocaine pretreatment on endotoxin-induced lung injury in rabbits. ANESTHESIOLOGY 81:689–699, 1994

7. LeSouef PN, England SJ, Bryan AC: Total resistance of the respiratory system in preterm infants with and without an endotracheal tube. J Pediatr 104:108–111, 1984

8. Miwa S: Blood testing, Rinshoukensahou Teiyou (in Japanese). Edited by Kanai M. Tokyo, Kanehara, 1983, pp 226–319

9. Nakano M, Sugioka K, Ushijima Y, Goto T: Chemiluminescence probe with *Cypridina* luciferin analog, 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-a]- pyrazin-3-one, for estimating the ability of human granulocytes to generate O_2^-. Anal Biochem 159: 363–369, 1986

10. Nishida A, Kimura H, Nakano M, Goto T: A sensitive and specific chemiluminescence method for estimating the ability of human granulocytes and monocytes to generate O_2^-. Clin Chim Acta 179: 177–182, 1989

11. Sugioka K, Nakano M, Kurashige S, Akuzawa Y, Goto T: A chemiluminescent probe with a *Cypridina* luciferin analog, 2-methyl-6-phenyl-3,7-dihydroimidazo-[1,2-a]pyrazin-3-one, specific and sensitive for O_2^- production in phagocytizing macrophages. FEBS Lett 197:27–30, 1986

12. Nakagawara M, Hirokata Y, Yoshitake J: Effects of anesthetics on superoxide releasing activity of human polymorphonuclear leukocytes. Masui 34:754–759, 1985

13. Stelzner TJ, Welsh CH, Berger E, McCullough RG, Morris K, Repine JE, Weil JV: Antiarrhythmic agents diminish thiourea-induced pulmonary vascular protein leak in rats. J Appl Physiol 63:1877–1883, 1987

14. Koyama S, Toyofuku T, Hirai K, Yoshimura K, Sakai A, Kobayashi T: Dibutyryl cyclic AMP attenuates lung responses induced by endotoxin in conscious sheep. Am Rev Respir Dis 146:32–38, 1992

15. Chen T-Y, Zapol WM, Greene E, Robinson DR, Rubin RH: Protective effects of E5, an antiendotoxin monoclonal antibody, in the ovine pulmonary circulation. J Appl Physiol 75:233–239, 1993

16. Gross D, Dahan JB, Landau EH, Krausz MM: Effect of leukotriene inhibitor LY-171883 on the pulmonary response to *Escherichia coli* endotoxemia. Crit Care Med 18:190–197, 1990

17. Brigham KL, Meyrick B: Endotoxin and lung injury. Am Rev Respir Dis 133:913–927, 1986

18. Brighan KL, Bowers RE, Mckeen CR: Methylpredonisolone prevention of increased lung vascular permeability following endotoxemia in sheep. J Clin Invest 67:1103–1110, 1981

19. Wachtfagel YT, Kucich U, Greenplate J, Gluszko P, Abrams W, Weinbaum G, Wenger RK, Rucinski B, Niewiarowski S, Edmunds LH, Colman RW: Human neutrophil degranulation during extracorporeal circulation. Blood 69:324–330, 1987

20. Kubo K, Kobayashi: Effects of OKY-046, a selective thromboxane synthase inhibitor, on endotoxin-induced lung injury in unanesthetized sheep. Am Rev Res Dis 132:494–499, 1985

21. Harris RH, Zmudka M, Ramwell MPW, Fletcher JR: Relationships of TxB2 and 6-keto PGF1a to the hemodynamic changes during baboon endotoxic shock. Adv Prostaglandin Thromboxane Res 7: 843–849, 1980

22. Chang SW, Feddrsen CO, Henson PM, Voelkel NF: Platelet-activating factor mediates hemodynamic changes and lung injury in endotoxin-treated rats. J Clin Invest 79:1498–1509, 1987

23. Camussi G, Bussolino F, Salvidio G, Baglioni C: Tumor necrosis factor/cachectin stimulates peritoneal macrophages, polymorphonuclear neutrophils, and vascular endothelial cells to synthesize and release platelet activation factor. J Exp Med 166:1390–1404, 1987

24. Fletcher JR, Ramwell PW: Lidocaine treatment following baboon endotoxin shock improves survival. Advances in Shock Research 2:219–232, 1979