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Effects of Pretreatment with SDZ MRL 953, a Novel Immunostimulatory Lipid A Analog, on Endotoxin-Induced Acute Lung Injury in Guinea Pigs

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SDZ MRL 953 (SDZ), a novel immunostimulatory lipid A analog, has been reported to have immunopharmacological activities similar to those of lipopolysaccharide (LPS) but to have little of the toxicity of LPS. We investigated the effects of pretreatment with SDZ on *Escherichia coli* endotoxin-induced acute lung injury in guinea pigs. Four experimental groups consisted of saline control ($n = 16$), SDZ (−12 h) plus LPS (2 mg/kg of SDZ per kg of body weight injected intravenously 12 h before intravenous injection of 2 mg of LPS per kg; $n = 15$), SDZ (−10 min) plus LPS (SDZ injected 10 min before LPS injection; $n = 10$), and LPS alone ($n = 16$). The animals were sacrificed, and lung tissue was sampled 4 h after LPS or saline infusion. Lung injury was assessed by measuring the wet weight-to-dry weight ratio and the level of ^{125}I-labeled albumin accumulation in bronchoalveolar lavage fluid relative to that in plasma. In the SDZ (−12 h) plus LPS group, these two parameters of acute lung injury were decreased compared with those in the LPS alone group. However, they were not decreased in the SDZ (−10 min) plus LPS group. We conclude that SDZ attenuates endotoxin-induced acute lung injury when it is administered 12 h before LPS injection. The attenuating effects of SDZ are speculated to be due to down regulation of the response to endotoxin rather than to receptor blocking.

Lipid A is the most toxic moiety contributing to the biological activities of endotoxin (9, 26). Certain analogs and partial structures of lipid A may be capable of acting as receptor antagonists of lipopolysaccharide (LPS) (3, 4, 15). Lipid X, a prototype reducing sugar moiety of lipid A, has been reported to block LPS-induced priming in a manner consistent with a competitive mode of inhibition (3, 15). Lipid X protected mice and sheep from lethal LPS challenge (7, 22). However, some early preparations of lipid X were contaminated with immunostimulatory factors (1, 7), while chemically pure lipid X was found to exhibit little immunostimulatory activity (15). Furthermore, no beneficial effects could be demonstrated in a therapeutic trial of synthetic lipid X by using a canine model of septic shock (2). Therefore, it has not yet been elucidated whether receptor blocking by a lipid A analog protects a host with sepsis, which may be exacerbated by subsequent LPS challenge. In contrast, synthetic lipid A subunits of nonreducing sugar moieties, such as GLA-27 and GLA-60, have been reported to induce the release of mediators including gamma interferon and tumor necrosis factor (TNF) at nontoxic doses (9). These analogs have also been found to be active in enhancing host resistance to microbial and viral infections in healthy and myelosuppressed mice (11, 12). These observations suggest that lipid A analogs may enhance the host defense against infections by inducing various cytokines.

SDZ MRL 953, 2-deoxy-3,4-bis-*O*-[(*R*)-3-hydroxytetradecanoyl]-2-[(*R*)-3-hydroxy-tetradecanoylamido]-1-*O*-phosphono-a-D-gluco-pyranose (SDZ), is a new synthetic monosaccharidic lipid A which has been shown to have immunopharmacological activities similar to those of LPS but to have little of the toxicity of LPS (16). SDZ has also been demonstrated to increase the survival rate in a murine sepsis model when it is administered before bacterial inoculation (17). Although acute lung injury has been considered one of the primary causes of death in patients with sepsis (10), lung injury was not evaluated in the murine model. Therefore, in the present study, we investigated the effects of pretreatment with SDZ on acute lung injury induced by a bolus injection of LPS in guinea pigs. Previous reports suggest that there may be two mechanisms which, if they exist, are responsible for the protective effects of lipid A analogs in sepsis. One is receptor blocking (7, 22) and the other is immunopharmacological modulation of the host defense (17). Therefore, in the present study, we designed two pretreatment groups, SDZ administration 10 min before LPS injection, which reflects receptor blocking, and 12 h before LPS injection, which reflects enhanced host defenses.

MATERIALS AND METHODS

Experimental design. Experiments were designed to evaluate the effects of pretreatment with SDZ on endotoxin-induced lung injury by comparing four experimental groups: a saline control group, SDZ (−12 h) plus LPS group (SDZ was injected 12 h before LPS injection), SDZ (−10 min) plus LPS group (SDZ was injected 10 min before LPS injection), and LPS alone group (Fig. 1). To investigate the effects of SDZ itself on lung injury, we also studied SDZ alone groups (0.02, 0.2, and 2 mg/kg of body weight). SDZ was synthesized at Sandoz Forschungsinstitut, Vienna, Austria, and was purified by using a Sephadex LH 20 column to >99% purity, as assessed by reverse-phase high-performance liquid chromatography.

Animal preparation. Specific-pathogen-free female guinea pigs weighing 800 to 1,100 g were used (Sankyo Labo Service, Shizuoka, Japan). While the guinea pigs were under general anesthesia with intramuscularly administered ketamine (100 mg/kg) and xylazine (5 mg/kg), catheters were placed in the right carotid artery and the jugular vein of each animal. All animals were allowed to recover from the cannulation procedure for 18 h before time zero. The preparation of the animals has previously been described in detail (14).

Experimental protocol. The experimental protocol is provided in Fig. 1.

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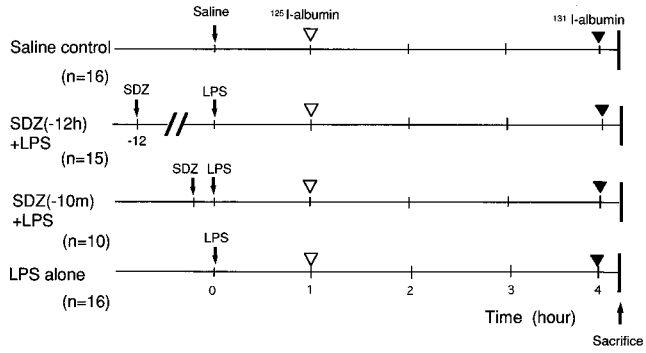

FIG. 1. Experimental protocol. ^{125}I-albumin was injected at 1 h to evaluate transvascular permeability. ^{131}I-albumin was injected 10 min before sacrifice to correct for blood contamination.

Twelve hours before time zero, 2 mg of SDZ per kg was injected intravenously in the SDZ (−12 h) plus LPS group. In the SDZ (−10 min) plus LPS group, 2 mg of SDZ per kg was injected intravenously 10 min before time zero. At time zero, the animals were injected intravenously with either 2 ml of saline or 2 mg of *Escherichia coli* endotoxin (serotype O127:B8; Sigma Chemical, St. Louis, Mo.) per kg. Endotoxin was suspended in 2 ml of saline, while SDZ was dissolved in 2 ml of purified water. Mean arterial pressure was measured via the carotid catheter at 0, 15, 60, and 240 min (VC-621; Nihon Koden, Tokyo, Japan). Blood samples were also collected to examine whole blood cell counts and leukocyte (WBC) differentials at 0, 15, 60, and 240 min (Sysmex K-1000; Toa Iyou Denshi, Kobe, Japan). Normal saline (25 ml/kg) was infused intravenously hourly to offset hypotension and dehydration. At time 1 h, 2 μCi of ^{125}I-labeled guinea pig albumin (Sigma) per 2 ml was injected slowly via the venous catheter. At time 4 h, 1.5 μCi of ^{131}I-labeled human albumin per 2 ml was injected to correct for blood contamination in the samples (14). At the same time, 1,000 IU of heparin was injected via the venous catheter. Ten minutes later, the animals were killed with an intravenous infusion of 4 meq of KCl. The standard chloramine T procedure was used to label albumin with ^{125}I and ^{131}I, as described in a previous report (20). To examine the effects of SDZ alone on lung injury, we injected SDZ at either 0.02 mg/kg (n = 3), 0.2 mg/kg (n = 3), or 2 mg/kg (n = 4) at time zero. The following procedures were the same as those described above.

BAL. The sequestration of neutrophils in pulmonary airspaces was assessed by bronchoalveolar lavage (BAL) in each animal. BAL was performed on the left cranial lobe after securing catheters within the left main bronchus. Each lung was lavaged with 10 ml of saline. Fluid recovery was always greater than 90%, and there were no significant differences in fluid recovery among groups. One-gram samples of BAL fluid and blood were placed in a well counter, and the counts of ^{125}I and ^{131}I were estimated, with appropriate corrections for crossover between ^{125}I and ^{131}I. The BAL fluid was centrifuged at 400 × g and 4°C for 10 min, and the supernatant was stored at −20°C. The cell pellet was resuspended in 1 ml of saline, and a cell count was done by a modified hemacytometer method (Unopet Microcollection System; Becton Dickinson, Rutherford, N.J.). For differential counting of WBCs in BAL fluid, cell monolayers were prepared from BAL fluid by cytocentrifugation. Differential counts were performed on 200 cells from smears stained with a modified Wright's stain (Diff-Quik; American Scientific Products, McGraw Park, Ill.).

Lung water measurement. The lungs were excised by opening the chest and were drained of free blood by gently blotting the hilus on paper towels. The gamma counts of tissue samples for ^{125}I and ^{131}I were determined in a gamma counter (ARC-300; Aloka, Tokyo, Japan), with appropriate corrections for cross-over. The tissues were then dried in a vacuum drying oven (DP22; Yamato Scientific, Tokyo, Japan) at 90°C and −200 mm Hg for 48 h, which removed any gravimetrically detectable water. Then, the weight of the dried lung tissue was measured. Approximately 1 ml of each blood sample was weighed, and gamma rays were counted. The blood wet weight/blood dry weight ratio was obtained after drying the specimen in a similar manner. Blood contamination in each sample was estimated from ^{131}I counts of the tissue sample, and the extravascular lung tissue wet weight/lung tissue dry weight ratio (W/D) was then calculated by correcting for the contamination (14).

Parameters for albumin leakage. The ratio of the concentration of ^{125}I-labeled albumin in BAL fluid to that in plasma (B/P) was used as an index for transalveolar septal flux of ^{125}I-labeled albumin, which is considered to reflect alveolar septal damage. Blood contamination in the BAL fluid was corrected by using ^{131}I counts, and the effects of the contamination were subtracted (14).

Histopathological examination. The right cranial lobe was fixed for histopathological examination by inflating it with formalin equal to 0.11 of the total lung capacity of the animal, which was calculated by using the following formula: milliliters of formalin = $(0.11) \times (BW)^{0.69}$, where BW is the body weight of the guinea pig (in grams) (5). The lung was fixed for at least 48 h, and then 2- to 5-mm sagittal sections were embedded in paraffin and 5-mm sections were cut and stained with hematoxylin and eosin. The entire lobe was examined at ×40 and ×100 magnifications for the presence of remarkable peribronchovascular cuff, alveolar edema, and hemorrhage. The numbers of polymorphonuclear leukocytes were counted in 20 randomly selected fields at ×400 magnification by an observer blinded to the animals' group assignments. Fields containing large vessels and bronchi were excluded. The numbers of cells were divided by the numbers of alveoli in order to compensate for variable lung inflation.

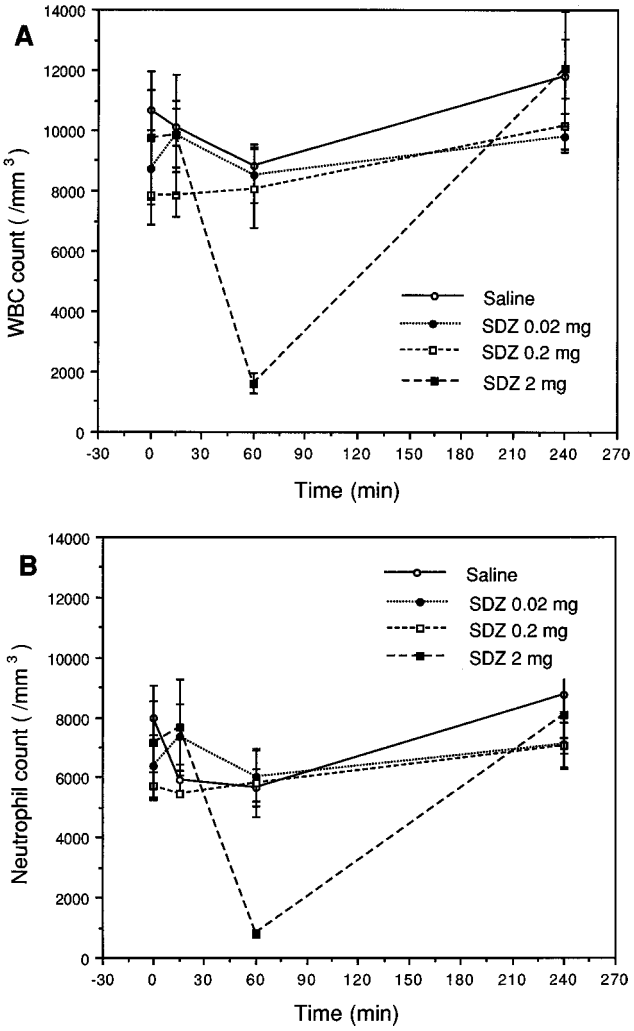

FIG. 2. (A) Peripheral WBC counts in the saline and SDZ alone groups. At 60 min, WBC counts were decreased in the group receiving SDZ at 2 mg/kg compared with the counts in the saline group ($P < 0.0001$). (B) Peripheral neutrophil counts. The time courses of the neutrophil counts in the four groups were similar to those of the WBC counts. At 60 min, neutrophil counts were decreased in the group receiving SDZ at 2 mg/kg compared with those in the saline group ($P < 0.001$).

TNF bioassay. A bioassay for TNF was performed with serum and BAL fluid samples obtained from the SDZ (−12 h) plus LPS, LPS alone, and SDZ alone groups. The bioassay was performed as described by Ruff and Gifford (23). Briefly, L-929 cells, which are sensitive to TNF, were seeded onto 96-well-type microtest plates (6×10^4 cells per well), and then serially diluted samples and 1 μg of actinomycin D per ml were added to the wells. The level of cell death was determined after 18 h of culture, as follows. The cells were fixed and stained in 0.2% crystal violet–2% methanol for 15 min. The stained plates were washed extensively with running water and dried. Viable cells retained the dye. The dye was solubilized with 0.5% sodium dodecyl sulfate, and the A_{550} of each well was read with a dual-wavelength microplate photometer (MTP-12; Corona Electric, Ibaraki, Japan).

Statistical analysis. All data are presented as the mean ± standard error of the mean. One-way analysis of variance and Fisher's least-significant-difference test were used to detect statistically significant differences between groups. Chi-

TABLE 1. Effects of SDZ alone on lung injury

Group	No. of animals	W/D ratio[a]	^{125}I-albumin B/P ratio[a]
Saline	16	4.14 ± 0.10	0.0028 ± 0.0007
SDZ alone			
0.02 mg/kg	3	4.15 ± 0.30	0.0035 ± 0.0004
0.2 mg/kg	3	4.10 ± 0.22	0.0035 ± 0.0013
2 mg/kg	4	4.05 ± 0.13	0.0032 ± 0.0013

[a] Values are means ± standard errors of the means.

square contingency analysis was used to compare the observed frequencies of histopathologic findings. A P value of <0.05 was considered significant.

RESULTS

Hemodynamic data and survival rate. No significant difference in mean arterial pressure was observed among the four experimental groups and the SDZ alone groups at the beginning of this experiment or during the observation period. Mean arterial pressure did not decrease during the experimental course in any of the groups. All animals survived until the completion of the experiment except those which died because of excess bleeding during the cannulation procedure.

Effects of SDZ alone on neutrophil accumulation, the parameters of lung injury, and TNF release. The peripheral WBC counts observed at each time point are given in Fig. 2A. There were no significant differences in WBC counts among the saline and the SDZ alone groups at times zero and 15 min and 240 min after SDZ administration. WBC counts decreased at 60 min in the group receiving 2 mg of SDZ per kg compared with the counts in the saline group ($P < 0.0001$). The time courses of the neutrophil counts were quite similar to those of the WBC counts (Fig. 2B). There were no significant differences in the total cell counts in BAL fluid among the saline and SDZ alone groups [saline, $(0.48 \pm 0.42) \times 10^6$/ml; SDZ at 0.02 mg/kg, $(1.24 \pm 0.49) \times 10^6$/ml; SDZ at 0.2 mg/kg, $(2.47 \pm 0.42) \times 10^6$/ml; SDZ at 2 mg/kg, $(2.94 \pm 1.77) \times 10^6$/ml], nor did the neutrophil counts in BAL fluid differ among the groups [$(0.20 \pm 0.29) \times 10^6$/ml, $(0.50 \pm 0.24) \times 10^6$/ml, $(1.13 \pm 0.42) \times 10^6$/ml, and $(1.10 \pm 0.61) \times 10^6$/ml, respectively]. The results of the parameters of lung injury are presented in Table 1. There were no significant differences in the two indices among the saline and SDZ alone groups. TNF levels in plasma and BAL fluid in the saline and SDZ alone groups are presented in Table 2. TNF levels in plasma were decreased in all of the SDZ alone groups compared with those in the LPS group at 60 and 240 min. There were no differences in TNF levels in BAL fluid among the saline and SDZ alone groups.

Peripheral blood cell counts. The peripheral WBC counts observed at each time point are given in Fig. 3A. There were no significant differences in WBC counts among the saline, SDZ (−12 h) plus LPS, SDZ (−10 min) plus LPS, and LPS groups at time zero. At 15 min after LPS administration, WBC counts decreased markedly in the SDZ (−12 h) plus LPS, the SDZ (−10 min) plus LPS, and LPS alone groups. In these three groups, WBC counts were subsequently maintained at approximately 1,000/mm^3 for as long as 1 h after LPS challenge. The WBC counts in the three groups then recovered, reaching approximately 2,500/mm^3 by 4 h after LPS injection. Peripheral neutrophil counts are also given in Fig. 3B. The time courses of the neutrophil counts in the four groups were quite similar to those of the WBC counts.

Cell findings in BAL fluid. The total cell and neutrophil counts in BAL fluid recovered at 4 h are given in Fig. 4. The total cell counts in the SDZ (−12 h) plus LPS, the SDZ (−10 min) plus LPS, and the LPS alone groups were significantly increased compared with those in the saline control group. The neutrophil counts in the three groups were also increased compared with those in the saline alone group.

Lung water. The lung W/D ratios are given in Fig. 5. The W/D ratios for the saline and the SDZ (−12 h) plus LPS groups were significantly decreased compared with those for the LPS alone group. The values for the SDZ (−10 min) plus LPS group were not significantly different from those for the LPS alone group.

Albumin leakage. The B/P ratios for the saline and the SDZ (−12 h) plus LPS groups were significantly decreased compared with that for the LPS alone group, although the value for the SDZ (−10 min) plus LPS group was not decreased compared with that for the LPS alone group (Fig. 6).

Histopathological findings. The histopathological findings for the right cranial lobe are presented in Table 3. The polymorphonuclear leukocyte/alveolus ratio did not differ significantly between the SDZ (−12 h) plus LPS and LPS alone groups. In contrast, peribronchovascular cuff and alveolar hemorrhage were more frequently observed in the LPS alone group than in the SDZ (−12 h) plus LPS group. Alveolar edema was seen only in one animal, which was in the LPS alone group.

TNF levels in plasma and BAL fluid. The TNF levels in plasma obtained in the SDZ (−12 h) plus LPS and the LPS alone groups at times zero, and 15, 60, and 240 min are given in Fig. 7. There were no significant differences in plasma TNF levels between the SDZ (−12 h) plus LPS group and the LPS alone group during the experimental course, nor did the TNF levels in BAL fluid in the SDZ (−12 h) plus LPS group differ from those in the LPS group (46.51 ± 11.44 versus 24.25 ± 15.59 U/ml).

TABLE 2. Effects of SDZ on TNF

Groups	No. of animals	TNF level in plasma (U/ml)[a]				TNF level in BAL fluid (U/ml)[a]
		0 min	15 min	60 min	240 min	
SDZ alone						
0.02 mg/kg	3	4.2 ± 4.2	0.1 ± 0.1	0.0 ± 0.0[b]	8.1 ± 4.2[c]	3.2 ± 1.1
0.2 mg/kg	3	0.0 ± 0.0	216.2 ± 216.2	217.2 ± 217.2[b]	17.3 ± 17.3[c]	2.8 ± 0.5
2 mg/kg	4	0.0 ± 0.0	3.2 ± 3.2	17.5 ± 12.6[d]	11.5 ± 11.5[b]	3.3 ± 0.8
LPS, 2 mg/kg	5	6.7 ± 2.1	39.1 ± 16.5	6,757.2 ± 1,625.8	507.5 ± 168.9	24.3 ± 15.6

[a] Values are means ± standard errors of the means.
[b] $P < 0.01$ compared with the LPS group.
[c] $P < 0.05$ compared with the LPS group.
[d] $P < 0.001$ compared with the LPS group.

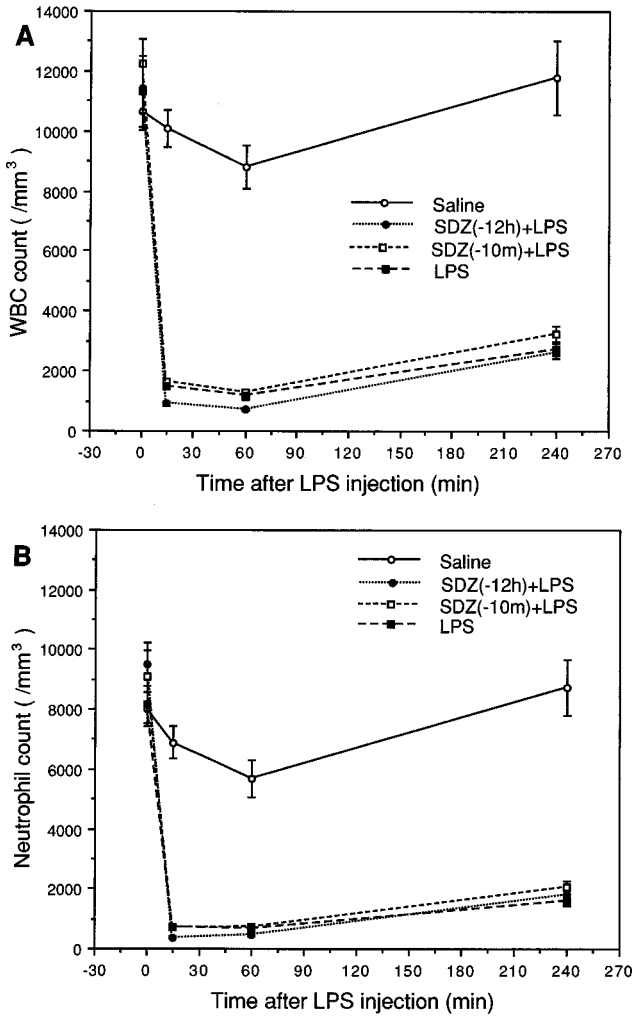

FIG. 3. (A) Peripheral WBC counts. At 15, 60, and 240 min after LPS administration, WBC counts were decreased in the SDZ (−12 h) plus LPS, SDZ (−10 min) plus LPS, and LPS alone groups compared with those in the saline group ($P < 0.00001$). (B) Peripheral neutrophil counts. The time courses of the neutrophil counts in the five groups were similar to those of the WBC counts. At 15, 60, and 240 min after LPS administration, neutrophil counts were decreased in the SDZ (−12 h) plus LPS, SDZ (−10 min) plus LPS, and LPS alone groups compared with those in the saline group ($P < 0.00001$).

DISCUSSION

Therapeutic trials with several lipid A analogs have been performed in animal models of septic shock (2, 7, 17, 22), and some of the results have suggested increased survival rates (7, 17, 22). However, the effects of lipid A analogs on LPS-induced pulmonary edema have not yet been evaluated quantitatively. We therefore investigated the effects of pretreatment with SDZ on acute lung injury following intravenous LPS administration. Our results indicate that SDZ attenuated lung injury when it was administered 12 h before LPS challenge, although it was not effective when it was injected 10 min before challenge. We speculate that the attenuating effects of SDZ on lung injury may be mediated by immunopharmacological modulation, leading to down regulation of the response to subsequent LPS injection rather than receptor blocking. The pharmacokinetics of SDZ have not been demonstrated, although the elimination and tissue distribution of lipid X have been reported (6). The elimination of lipid X following administration of a bolus injection suggests that the LPS receptor of peripheral WBCs may be blocked by the administration of lipid A analogs 10 min before LPS challenge. However, receptor blockade could still be occurring at the tissue level 12 h after the administration of a lipid A analog on the basis of the results of the distribution of lipid X in tissue (6). Therefore, we speculate that although the mechanisms underlying the attenuating effects of SDZ on lung injury are still uncertain, immunopharmacological modulation leading to down regulation subsequent to LPS injection is more likely than receptor blockade. Survival was also enhanced in a murine model when SDZ was administered repeatedly before bacterial inoculation (16), which is consistent with this speculation.

A previous report suggested that, in mice, SDZ was less toxic than LPS by a factor of 1×10^4- to $>7 \times 10^5$-fold (16). In the present study, no differences in the W/D and B/P ratios between the saline control and SDZ alone groups were found, as indicated in Table 1. These results suggest that SDZ is not toxic when it is administered at doses of no more than 2 mg/kg. However, neutrophil counts at 60 min in the peripheral blood of the group receiving SDZ at 2 mg/kg were significantly decreased compared with those in the group receiving saline, as shown in Fig. 2B. The neutrophil counts in the SDZ alone groups at 15 min were not decreased. Neutrophil accumulation in lungs within 15 min after LPS challenge has been shown to be mediated by activated complement in animal models of acute lung injury (8), while neutrophil attachment to pulmonary capillaries 60 min after LPS injection has been shown to be mediated by adhesion molecule induction (21). Therefore, we speculated that 2 mg of SDZ per kg caused neutrophil accumulation in the lungs, without complement activation, probably because of adhesion molecule induction. Cytokine induction may also be associated with transient adhesion of neutrophils 60 min after LPS injection (21, 30). However, TNF levels were not increased after injection of SDZ alone, as indicated in Table 2. Therefore, these chemotactic effects on neutrophils seem to be mediated by other cytokines. Because SDZ was not toxic but did have the biological activities described above when it was administered at a dose of 2 mg/kg, the pretreatment SDZ dose was determined to be 2 mg/kg in the present study.

As stated above, pretreatment with SDZ 12 h before LPS injection attenuated lung injury in our guinea pig model. Although the precise mechanisms by which SDZ pretreatment prevented lung injury are uncertain, pretreatment with SDZ did not decrease the level of accumulation of neutrophils in the lungs, as demonstrated by the results of analysis of the cell counts in BAL fluid and histopathological examination. Therefore, the attenuating mechanisms may not stem from inhibitory effects on neutrophil accumulation. A previous report suggested that recruitment of neutrophils into alveolar spaces occurs without changes in protein permeation (19), which indicates that other cells or mediators may also contribute to the development of lung injury. Although pretreatment with SDZ did not inhibit neutrophil migration, it is still possible that SDZ attenuated neutrophil activation when LPS was subsequently injected.

TNF is among the many factors which are involved in the pathogenesis of acute lung injury, so we measured the TNF levels in plasma and BAL fluid. Because TNF has been reported to exacerbate acute lung injury (24), it was speculated that TNF levels would decrease in the groups receiving SDZ pretreatment compared with those in the LPS alone group. However, we found that SDZ pretreatment did not result in a decrease in the TNF levels in plasma following LPS stimulation (Fig. 7). The mechanisms by which lung injury was atten-

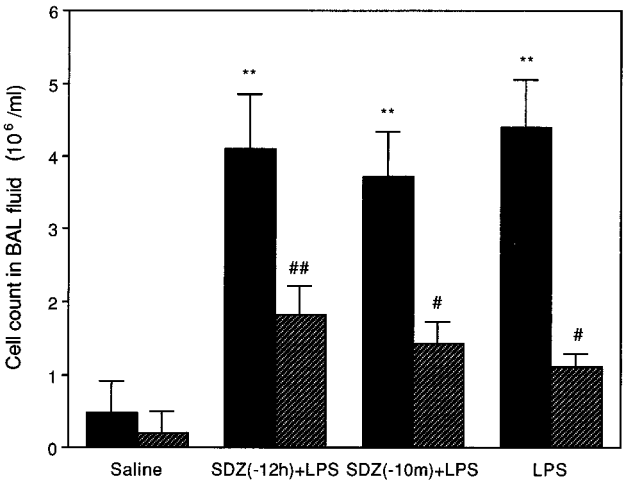

FIG. 4. The total cell (solid bars) and neutrophil (hatched bars) counts in BAL fluid recovered at 4 h. The total cell counts in the SDZ (−12 h) plus LPS, the SDZ (−10 min) plus LPS, and the LPS alone groups were significantly increased compared with those in the saline control group. The neutrophil counts in the SDZ (−12 h) plus LPS, the SDZ (−10 min) plus LPS, and the LPS alone groups were significantly increased compared with those in the saline group. Double asterisks indicate $P < 0.01$ versus the WBC count in the saline control group. A single number sign indicates $P < 0.05$ and double number signs indicate $P < 0.01$ versus the neutrophil count in the saline group. There was no significant difference in the neutrophil counts between the SDZ (−12 h) plus LPS group and the LPS alone group.

uated in the SDZ (−12 h) plus LPS group, despite the elevated TNF levels, are not known with certainty. However, these results suggest that there are many factors, in addition to TNF, contributing to the pathogenesis of acute lung injury after LPS administration, as reported previously (13, 25, 27, 29).

Pretreatment with interleukin-1 has been reported to induce tolerance to subsequent LPS challenge, although the levels of TNF in serum were elevated in mice (18). In the previous study (18), TNF levels decreased after pretreatment with sublethal doses of LPS; however, similar protective effects were observed following subsequent LPS injection (18). These results suggest that different mechanisms may be responsible for tolerance to

TABLE 3. Histopathological findings of lung injury

Group[a]	PMN[b]/alveolus[c]	No. (%) of animals		
		Peribroncho-vascular cuff	Alveolar edema	Alveolar hemorrhage
SDZ (−12 h) plus LPS	0.577 ± 0.033	1 (25)	0 (0)	1 (25)
LPS alone	0.459 ± 0.060	3 (75)[d]	1 (25)	3 (75)[d]

[a] There were four animals in each group.
[b] PMN, polymorphonuclear leukocytes.
[c] Values are means ± standard errors of the mean.
[d] $P < 0.05$ compared with the SDZ (−12 h) plus LPS group (chi-square contingency analysis).

subsequent LPS challenge. These observations appear to be in agreement with our speculation that acute lung injury may be attenuated, despite a lack of decrease in TNF levels in serum.

SDZ was found to stimulate macrophages to release cytokines such as interleukin-6, interleukin-8, TNF, and colony-stimulating activity in a previous in vitro study (16). The other lipid A analogs, such as GLA-27 and GLA-60, were also found to be active in enhancing host resistance to microbial and viral infections in mice when they were administered 1 to 3 days before inoculation (11, 12). These analogs were reported to activate B cells and macrophages and to induce the release of mediators including gamma interferon and TNF (9). These observations suggested that pretreatment with SDZ at 12 h before LPS challenge may have induced the release of a cocktail of beneficial cytokines that protect the lung from acute injury, although TNF levels did not increase after injection of SDZ alone.

Taken together, our results and these observations suggest that complicated mechanisms may exist in the regulation of the response to LPS challenge. Although neutrophils and TNF have been considered to exacerbate acute lung injury, both may have beneficial effects on the host defense against infections (11, 28). In the present study, SDZ may have induced certain mediators other than TNF that are responsible for attenuating the effects on lung injury. In addition, it was demonstrated that lung injury was attenuated in the setting of neutrophil accumulation in the lungs and increased plasma

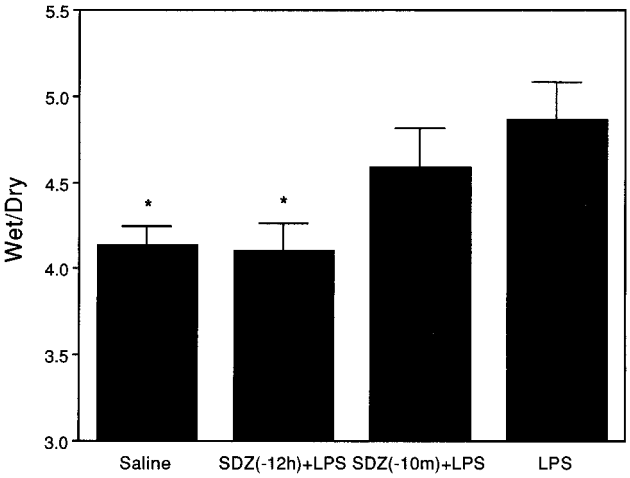

FIG. 5. Pulmonary extravascular water obtained from the W/D ratio corrected for blood contamination by using ^{131}I-albumin. Lung water accumulation caused by LPS was suppressed in the SDZ (−12 h) plus LPS group. Single asterisks indicate $P < 0.05$ compared with the LPS alone group.

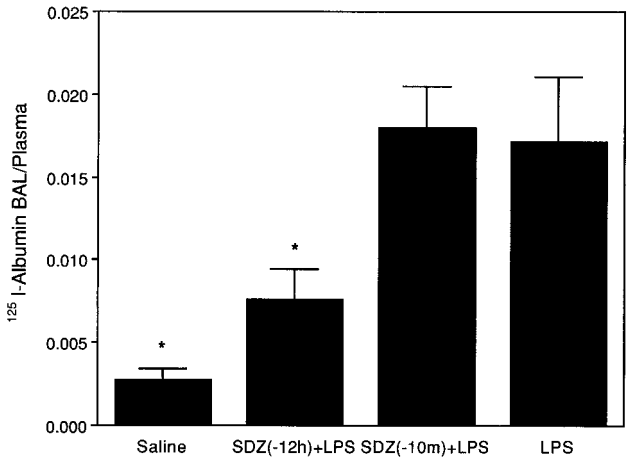

FIG. 6. Transalveolar septal albumin leakage as represented by the ^{125}I-albumin B/P ratio. The B/P ratio for the SDZ (−12 h) plus LPS group was decreased compared to that for the LPS alone group. Single asterisks indicate $P < 0.05$ compared with the LPS alone group.

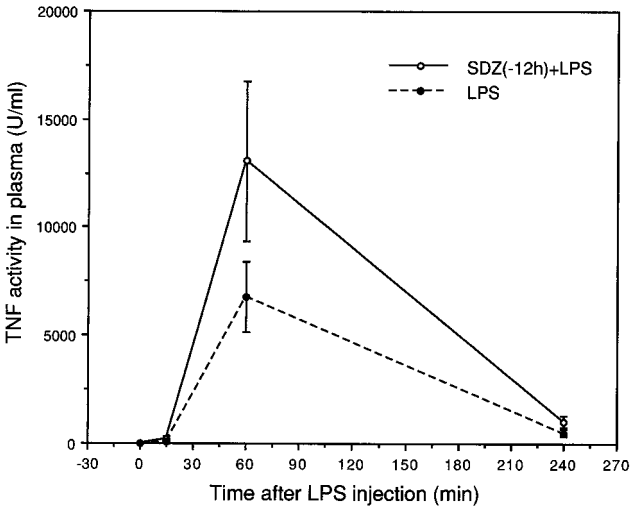

FIG. 7. TNF levels in plasma obtained from the SDZ (−12 h) plus LPS and the LPS alone groups at times zero and 15, 60, and 240 min. There were no significant differences in TNF levels between the SDZ (−12 h) plus LPS and the LPS alone groups during the course of the experiment.

TNF levels. Because various factors are involved in acute lung injury and its associated host defense mechanisms, it is difficult to elucidate these processes solely by measuring some of these factors.

In summary, SDZ, a lipid A analog, was considered to be effective in preventing LPS-induced acute lung injury when it was administered 12 h before LPS challenge, probably because of down regulation in response to LPS challenge. To our knowledge, there have been no reports in which the effects of lipid A analogs on lung injury were evaluated. Although the precise mechanisms underlying the attenuating effects of SDZ on acute lung injury were not elucidated, the results presented here demonstrate that SDZ may have therapeutic potential in preventing acute lung injury induced by LPS.

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