Delayed treatment with an \( \text{LTD}_4/\text{E}_4 \) antagonist limits pulmonary edema in endotoxic pigs

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FINK, MITCHELL P., KEITH L. KRUITHOFF, JAN B. ANTONSSON, HAILONG WANG, AND HEIDIE R. ROTHSCHILD. Delayed treatment with an \( \text{LTD}_4/\text{E}_4 \) antagonist limits pulmonary edema in endotoxic pigs. Am. J. Physiol. 260 (Regulatory Integrative Comp. Physiol. 29): R1007–R1013, 1991.—We used a selective leukotriene (LT) \( \text{D}_4/\text{E}_4 \) receptor antagonist, (LY 203647) to investigate the role of cysteinyl LTs as mediators of several important pathophysiological events in a porcine model of endotoxic shock. Pentobarbital-anesthetized pigs (11.8–17.5 kg) were mechanically ventilated with 100% \( \text{O}_2 \). Pigs in groups I (n = 10), IIA (n = 10), and IIB (n = 5) were infused with Escherichia coli lipopolysaccharide (LPS; 250 pg/kg) from time \( t \) = 0–240 min with Ringer lactate (0.8 ml·kg\(^{-1} \)·min\(^{-1} \)). Pigs in group I received no further treatment. At \( t \) = 30 min, groups IIA and IIB were injected with LY 203647 (30 mg/kg) and were started on an infusion of the compound at 10 (group IIA) or 30 mg·kg\(^{-1} \)·h\(^{-1} \) (group IIB). Delayed treatment with LY 203647 significantly (\( P < 0.05 \)) and persistently ameliorated LPS-induced pulmonary hypertension. The compound also abrogated LPS-induced pulmonary edema, as assessed by gravimetrically determined lung weight. Despite the beneficial effect on pulmonary edema, delayed treatment with LY 203647 did not improve arterial oxygenation. Delayed treatment with LY 203647 transiently improved mesenteric perfusion. These data suggest that cysteinyl LTs are important mediators in porcine endotoxicosis.

LEUKOTRIENES (LTs) are a group of potent lipid mediators derived from arachidonic acid via the action of the enzyme 5-lipoxygenase. LTC\(_4\), LTD\(_4\), and LTE\(_4\) are collectively referred to as cysteinyl or sulfidopeptide LTs and have been identified as the active components of the slow-reacting substance of anaphylaxis (28). The cysteinyl LTs increase microvascular permeability (40), constrict airway smooth muscle (14), and promote pulmonary, mesenteric, coronary, and renal arteriolar vasoconstriction (1, 2, 16, 33). This constellation of biological actions suggests that the cysteinyl LTs may be key mediators in a variety of shock states (27).

Data that have accumulated suggest that the cysteinyl LTs are pathophysiologically important in several animal models of endotoxic shock and the adult respiratory distress syndrome (ARDS). In unanesthetized sheep, LY 171883, an LTD\(_4\)/LTE\(_4\) receptor antagonist, has been shown to ameliorate lipopolysaccharide (LPS)-induced pulmonary hypertension and bronchoconstriction (21). In endotoxic rats, LY 171883 has been shown to prevent hemoconcentration, improve cardiac output, and preserve visceral perfusion (13, 17). SK&F 104353, a potent cysteinyl LT receptor antagonist that is chemically distinct from LY 171883, has been shown to improve 48-h survival in a rat model of endotoxic shock (36).

Recent studies from our laboratory further implicate the cysteinyl LTs as mediators of some of the physiological derangements occurring in experimental endotoxicosis. We showed that IY 171883 improves mesenteric perfusion and ameliorates ileal mucosal acidosis in an acute porcine endotoxicosis model (11). Subsequently, we showed that pretreatment with 1-[2-hydroxy-3-proplyl-4-(4-{2-[4-(1H-tetrazol-5-yl)butyl]-2H-tetrazol-5-yl}-butoxy)phenyl] (LY 203647, Ref. 29), another cysteinyl LT receptor antagonist, also improves mesenteric perfusion in this model, albeit only transiently (12). In addition, treatment of pigs with LY 203647 before challenge with endotoxin was shown to improve post-LPS arterial oxygen tension (\( \text{Pao}_2 \)) and diminish LPS-induced pulmonary edema (12).

Our previous studies of LY 171883 and LY 203647 in porcine endotoxicosis employed a pretreatment design. While useful for demonstrating biological activity, this experimental design fails to simulate adequately the usual clinical situation wherein pharmacological intervention is instituted therapeutically rather than prophylactically. Therefore, the present study was undertaken to determine whether beneficial effects are still demonstrable when IY 203647 is administered after an endotoxic challenge.

METHODS

Animal preparation. The studies described herein were approved by the institutional review board for experiments involving animal subjects at the University of Massachusetts Medical Center and met National Institutes of Health guidelines for animal use in research.

Male Yorkshire random-bred pigs (11.8–17.5 kg) without clinical evidence of infection were sedated with ketamine (17 mg/kg im) and anesthetized with pentobarbital sodium (17 mg/kg). Light general anesthesia was maintained throughout the experiment with intermittent bolus doses of pentobarbital (10–20 mg). After a tracheostomy was performed, the animals were mechanically ventilated with 100% \( \text{O}_2 \) using a Harvard ventilator (tidal ventilation is instituted therapeutically rather than prophylactically). Therefore, the present study was undertaken to determine whether beneficial effects are still demonstrable when IY 203647 is administered after an endotoxic challenge.

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volume = 10 ml/kg; fractional inspiratory time (t) = 0.33]. Rate was adjusted to maintain the partial pressure of CO₂ in arterial blood at 40 ± 5 Torr.

The animals were instrumented as previously described (19). Polyethylene (PE 160) catheters were positioned in the abdominal aorta and inferior vena cava via femoral cutdowns. A 3.0-Fr catheter for injecting the thermal indicator for cardiac output determinations and measuring central venous pressure (CVP) was placed in the right atrium via a cervical cutdown. A 2.5-Fr thermistor-tip catheter was positioned in the abdominal aorta, and a balloon-tip catheter was floated into the pulmonary artery. Via a midline celiotomy incision, a 6-mm ultrasonic flow probe (Transonic Systems, Ithaca, NY) was positioned around the superior mesenteric artery (SMA) close to its origin from the abdominal aorta. The probe was connected to a previously calibrated Transonic Systems model T101 blood flowmeter, and perfusion through the SMA (Q_SMA) was recorded from the digital readout. A PE-90 catheter was threaded into the superior mesenteric vein (SMV) via a distal tributary. A tonometric catheter (Tonometrics, Worcester, MA) was placed within the lumen of the distal ileum via an antimesenteric incision and was secured in place with a purse-string suture.

**Systemic hemodynamics.** Mean systemic arterial pressure (MAP) and mean pulmonary arterial pressure (MPAP) were determined using Transpac II transducers (Sorenson; Abbott Laboratories, North Chicago, IL) driving a Honeywell amplifier/monitor with digital readout. Cardiac output indexed to body weight (Q) was measured by thermodilution using an Edwards model 1302 blood gas analyzer. Because equilibration of CO₂ is only 70% complete at 20 min (18), the measured CO₂ tension in the tonometer fluid (Ptonco_z) was corrected to account for incomplete equilibration: Ptonco_z = Ptonco_ztrue/0.7, where Ptonco_ztrue is the corrected partial pressure of CO₂ in the saline contained within the tonometer balloon. A modified form of the Henderson-Hasselbalch equation (24) was used to calculate [H⁺]:

\[ [H^+] = 24 \times \left( \frac{[\text{HCO}_3^-]}{[\text{H}_2\text{CO}_3]} \right) \]

where [HCO₃⁻] is the simultaneously measured concentration of bicarbonate in arterial blood. We recently reported data validating this tonometric methodology for measuring ideal intramucosal pH in porcine endotoxicosis (4).

**Extravascular water.** Extravascular water (EVW) was determined gravimetrically as previously described (8). At the termination of each experiment, duplicate arterial blood samples (3 ml) were obtained for determination of hemoglobin (CO-oximeter). The blood samples were weighed wet and were then weighed again after drying (37°C for 48-72 h) to constant weight. The animals were killed using a commercially available euthanasia cocktail, and the entire right lung was removed. The major bronchi were removed from the lung, and the pulmonary parenchyma was homogenized with 100 g of added water in a Waring blender.

Wet and dry weights were recorded for duplicate 10-mI samples of the homogenates. Detergent (Tween 20; 1.0 ml) was added to the remaining lung tissue, which was subjected to additional homogenization in the blender. Duplicate 5-ml samples of the homogenate were centrifuged (3,000 g). The supernatants were aspirated and stored at -70°C until spectrophotometrically assayed for hemoglobin (cyanohemoglobin method) using commercially available reagents (Fisher Diagnostics). In the subsequent calculations for determining extravascular wet-to-dry weight ratios by successive applications of the law of conservation of mass, it was assumed that hemoglobin was the same for arterial and pulmonary parenchymal blood.

**Plasma prostanoid concentrations.** Arterial plasma samples were collected, stored, and assayed for immunoreactive thromboxane (Tx) B₂ (TX₂ metabolite) and 6-ketoprostaglandin (PG) F₁₅ (prostacyclin metabolite) as previously described (19). All samples for the experiment were run in single assay, obviating problems with interassay variability. Samples were run in duplicate and intra-assay variation was always <10%. The lower limit of sensitivity was 39 pg/ml for both prostanoids.

**Protocol.** A total of 28 pigs were studied in four groups. From t = 0-240 min, all pigs were infused with a large volume of Ringer lactate (0.8 ml·kg⁻¹·min⁻¹). From t = 0-20 min, pigs in groups I (n = 10), IIA (n = 10), and IIIB (n = 5), were infused with Escherichia coli LPS (0111:B4; DIFCO, Detroit, MI: 250 μg/kg). At t = 30 min, pigs in groups IIA and IIIB were injected intravenously over 2-3 min with LY 203647 (30 mg/kg). The vehicle for LY 203647 was 40 mg/ml mannitol-25 mg/ml
tris(hydroxymethyl)aminomethane buffer (pH = 8.0–8.5). The doses of this compound that were utilized in the present study were based on preliminary pharmacodynamic studies of the effect of LY 203647 on the pressor response to LTD4 in pithed rats that showed that the half-maximal effective dose is 7.5 mg/kg (29). From t = 30–240 min, pigs in groups IIA and IIB were infused with LY 203647 at 10 and 30 mg·kg⁻¹·h⁻¹, respectively. Pigs in group III were infused with Ringer lactate but received neither LPS nor LY 203647. All pigs were killed at t = 240 min.

**Analyses of data.** Data were analyzed using commercially available software (Boeingcalc, Boeing, Seattle, WA; CSS, Statsoft, Tulsa, OK; and Fig.P, Biosoft, Cambridge, UK) running on a Hewlett-Packard Vectra ES/12 computer. Results are expressed as means ± SE. The primary objective of the study was to determine whether delayed treatment with LY 203647 alters one or more of several physiological responses to LPS in pigs, i.e., the contrasts of greatest interest were those between LPS controls (group I) and the two LPS plus LY 203647 subgroups (groups IIA and IIB). Thus, in an effort to maximize the power to detect treatment effects attributable to LY 203647, results from group III were not included in the statistical analyses of any of the parameters except EVLW. Selected results from group III are presented here. Because of the small size of the sample, however, within-group analyses for group III were not performed.

Except for the EVLW and plasma prostanoid results, data were analyzed using two-way analysis of variance (ANOVA) for repeated measures (15). When group × time or time effects were present, comparisons were made using ANOVA, contrasts with the baseline value at individual time points after the initiation of treatment were assessed using two-tailed paired-sample t tests, using Holm’s sequentially rejective procedure to control for the increased risk of a type I error due to multiple tests (23). If significant group or group × time effects were present by ANOVA, between-group contrasts at each time point after the initiation of treatment (i.e., t = 40–240 min) were performed using analysis of covariance with the t = 20 min value serving as a covariate to control for animal-to-animal variability.

Individual contrasts between groups were performed using the Student-Newman-Keuls (SNK) multiple-range test. Between-group analyses at baseline and at t = 20 min were performed using one-way ANOVA and the SNK test. EVLW data were also analyzed using one-way ANOVA and the SNK test. Plasma prostanoid data were available for groups I and IIA and were analyzed using paired and unpaired t tests. The null hypothesis was rejected for P < 0.05.

**RESULTS**

The effects of time and crystalloid “resuscitation” on several variables in normal (group III) pigs are summarized in Table 1. Changes over time for most variables were <20% of the t = 0 min value. For PaO2, [H+], and MPAP, time-related changes were <30% of the baseline value.

At the two time points before infusion of LY 203647 or saline (i.e., t = 0 and 20 min), there were no significant differences among groups I, IIA, and IIB for all of the repetitively measured variables except MAP. At t = 20 min (i.e., after the infusion of LPS, but before treatment with vehicle or LY 203647), MAP was significantly higher in group I (121 ± 5 mmHg) than in groups IIA or IIB (108 ± 5 and 102 ± 6 Torr, respectively).

Between-group (i.e., group I vs. IIA and group I vs. IIB) effects were not significant for the following variables: MAP, Q, systemic DO2 and VO2, mesenteric DO2 and VO2, [H+], and Q/S/Q. These data are shown in Table 2. In all three of the groups infused with LPS, MAP increased at t = 20 min and then decreased to ~70–80% of the baseline value from t = 60–240 min. The decrease in MAP achieved statistical significance in group I but not in groups IIA or IIB. Irrespective of group, the effect of LPS on Q was triphasic, being characterized by an early transient decrease at t = 20 min, transient recovery to values near baseline at t = 60 min, and sustained deterioration thereafter. In all endotoxic groups, SVRI increased transiently at t = 20 min. In group I, SVRI subsequently decreased significantly to ~70% of the baseline value from t = 60–120 min. In contrast, SVRI was not significantly different from baseline from t = 40–240 min in the two LY 203647-treated groups. In all three endotoxic groups, systemic and mesenteric DO2 decreased significantly, whereas systemic VO2 and [H+] increased significantly. Mesenteric VO2 increased significantly only in group I.

The effects of LY 203647 on QSMa in endotoxic pigs are shown in Fig. 1. QSMa diminished to ~55% of baseline in all three endotoxic groups at t = 20 min. Infusion of LY 203647 in groups IIA and IIB improved mesenteric perfusion, but only for a short while; from t = 60–240 min, QSMa was similar in groups I and IIA, and it was significantly lower at t = 120 min in group IIB.

In all three endotoxic groups, MPAP increased to >250% of baseline at t = 20 min and remained significantly elevated for the duration of the experiment (Fig. 2A). In groups IIA and IIB, however, treatment with LY 203647 resulted in a significant decrease in MPAP relative to untreated endotoxic controls. By repeated-measures ANOVA, the group × time effect for MPAP was highly significant (P = 0.0001). Statistically significant between-group differences were evident at all posttreat-
TABLE 2. Effect of LPS on several physiological variables

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<th>Variable</th>
<th>Group</th>
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<th>60</th>
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<td>I</td>
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<td>102±6‡</td>
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<td>310±55</td>
<td>289±37</td>
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<td>354±98*</td>
<td>380±27*</td>
<td>439±15</td>
<td>398±14*</td>
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<td>340±13*</td>
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<td>9±1</td>
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<td>102±13*</td>
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<td>11.8±1.0</td>
<td>12.6±1.2*</td>
<td>18.7±1.5*</td>
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<td>18.7±1.1</td>
<td>18.7±0.8</td>
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<td>21.3±3.4</td>
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Values are means ± SE. SYRIV, systemic vascular resistance index; SYSDO, systemic oxygen delivery; SYSVO, systemic oxygen uptake; MESDO, mesenteric oxygen delivery; MESVO, mesenteric oxygen uptake; [H+], ileal intramural hydrogen ion concentration; Q_s/Q_t, intrapulmonary shunt. * P < 0.05 vs. baseline (t = 0 min) within group. † P < 0.05 vs. group I.

FIG. 1. Effect of delayed treatment with LY 203647 on perfusion through superior mesenteric artery (SMA) in endotoxic pigs. Pigs in group I (c) were endotoxic controls. Pigs in group IIA (b) were infused with lipopolysaccharide (LPS) and then treated with LY 203647 (30 mg/kg loading dose, 30 mg kg^(-1).h infusion). Pigs in group IIB (a) were infused with LPS and then treated with LY 203647 (30 mg/kg loading dose, 30 mg kg^(-1).h infusion). * P < 0.05 vs. t = 0 min (pre-LPS) value within same group. † P < 0.05 vs. time-matched value in group I.

ment time points except t = 180 and 240 min. At baseline and t = 20 min, Pa_o was significantly higher in group IIB than in group I; however, by repeated-measures ANOVA, neither group nor group x time effects for this variable were statistically significant (Fig. 2B). Q_s/Q_t increased significantly over time in group I (16.9 ± 1.0% at t = 0 min to 27.4 ± 3.3% at t = 240 min). Q_s/Q_t also increased in groups IIA and IIB, although the changes were not statistically significant. By repeated-measures ANOVA, the group x time effect for Q_s/Q_t was significant (P = 0.021), although significant between-group differences at individual time points were not demonstrated (Table 2).

Pulmonary wet to dry weight ratios (corrected for intravascular water) were significantly increased in endotoxic (group I) pigs as compared with normal (group III) pigs resuscitated with a similar volume of Ringer lactate (Fig. 3). Posttreatment of group IIA pigs with the lower dose of LY 203647 decreased EVLW relative to group I, although the effect was not statistically significant. Compared with group I, EVLW was significantly less in group IIB pigs that were posttreated with the higher dose of LY 203647.

Plasma levels of 6-keto-PGF_1α and TxB_2 increased after the infusion of LPS in groups I and IIA (Table 3). Posttreatment with LY 203647 did not affect the plasma prostaglandin response to endotoxin.

DISCUSSION

Most of the observations in the present study corroborate results obtained in a previous study from our laboratory of the effects of LY 203647 on several hemodynamic and pulmonary variables in anesthetized pigs challenged with endotoxin (12). In our earlier study of LY 203647, we administered the drug before infusion of LPS; in the present study, treatment with the drug was not initiated until 30 min after starting the infusion of LPS (i.e., 10 min after the entire dose of endotoxin was administered). Consistent with our previous findings...
using a pretreatment design, the present data showed that posttreatment with LY 203647 1) attenuated LPS-induced pulmonary hypertension, 2) ameliorated LPS-induced pulmonary edema, and 3) transiently improved post-LPS mesenteric perfusion.

Infusion of LPS leads to acute pulmonary hypertension in a variety of species, including sheep (26, 31), baboons (10), cats (5), and pigs (12, 19). The early phase of this phenomenon seems to be mediated largely by the release of TxA2, as evidenced by the following observations: 1) circulating concentrations of TxB2, which increase dramatically after the infusion of LPS, are temporally correlated with the early increase in MPAP in experimental models of acute endotoxicosis (5, 10, 26, 31); and 2) LPS-induced pulmonary hypertension is prevented when experimental animals are pretreated with cyclooxygenase inhibitors (19), TX synthase inhibitors (5, 10, 31), or TX receptor antagonists (26).

The data presented here suggest that the cysteinyl LTs are also important in the pathogenesis of LPS-induced pulmonary hypertension, since MPAP was significantly lower in endotoxic pigs treated with LY 203647 than in endotoxic controls (Fig. 2). These findings are consistent with previously reported data from our group, showing that pretreatment with LY 203647 (12) or LY 171883 (11), another cysteinyl LT receptor antagonist, diminishes pulmonary hypertension in porcine endotoxicosis.

Pretreatment with LY 171883 also ameliorates pulmonary hypertension in an unanesthetized ovine model of endotoxin-induced acute lung injury (21). Because treatment with LY 203647 did not affect plasma levels of TxB2 (Table 3), it is unlikely that the effect of this compound on MPAP was due to interference with LPS-induced TxA2 release. Conceivably, LY 203647 could have been functioning as a TxA2 receptor antagonist, but data are unavailable to confirm (or refute) this notion.

Delayed therapy with LY 203647 prevented the accumulation of EVLW in endotoxic pigs (Fig. 3). Similar results were obtained when this compound was used to pretreat pigs before the administration of LPS (12). In the present study, a larger dose of LY 203647 seemed to be more effective than a smaller dose in preventing the accumulation of EVLW. These data suggest that the cysteinyl LTs are pathophysiologically important in edema formation during acute lung injury induced by endotoxin. This notion is further supported by several observations obtained in both experimental and clinical studies, including the following: 1) in isolated perfused rat lungs, LTC4 increases the escape of albumin from the vascular space and promotes the accumulation of EVLW (34, 39); 2) infusing LTD4 increases EVLW in anesthetized dogs (35); 3) in rats, both treatment with LY 171883 (6) and induction of a state of essential fatty acid deficiency (7) attenuate oleic acid-induced leakage of albumin.

**TABLE 3. Plasma immunoreactive prostanoid concentrations in endotoxic pigs**

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Group</th>
<th>Time, min</th>
<th>0</th>
<th>20</th>
<th>80</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>TxB2</td>
<td>I</td>
<td>309±104</td>
<td>2,652±119*</td>
<td>2,601±149*</td>
<td>3,187±614*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IIA</td>
<td>283±87</td>
<td>2,908±84*</td>
<td>2,479±113*</td>
<td>2,159±180*</td>
<td></td>
</tr>
<tr>
<td>6-keto-PGF1α</td>
<td>I</td>
<td>474±78</td>
<td>407±71</td>
<td>1,693±253*</td>
<td>882±167*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IIA</td>
<td>697±137</td>
<td>594±116</td>
<td>1,559±310*</td>
<td>1,420±315</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE. TxB2, thromboxane B2; 6-keto-PGF1α, 6-keto prostaglandin F1α. * P < 0.05 vs. 0 min value within group.
min from the intravascular space into the pulmonary parenchyma; and 4) increased levels of immunoreactive cysteinyl LTs are demonstrable in bronchoalveolar lavage fluid obtained from animals with experimental acute lung injury (38) or patients with ARDS (3, 30, 37).

Although the data presented here and elsewhere provide strong support for the idea that the cysteinyl LTs are key mediators leading to the accumulation of EVLW in acute endotoxicosis, the mechanisms underlying this phenomenon are incompletely delineated. Some data suggest that the cysteinyl LTs increase microvascular permeability (40), and this phenomenon may account for the effect of these compounds on edema formation in the lung. Recently, however, Sakai et al. (34) have presented findings suggesting that pulmonary venoconstriction, rather than alterations in membrane integrity, underlies the edemagenic effect of LTC4.

The relative importance of the cysteinyl LTs in edema formation in various models of acute lung injury may be species specific. Recent in vivo studies suggest that the cysteinyl LTs are not important mediators of LPS-induced pulmonary edema in sheep (21). Similarly, data obtained using an isolated perfused lung preparation suggest that the cysteinyl LTs are unimportant in the pathogenesis of oleic acid-induced pulmonary edema in rabbits (25). However, in rats (6, 7), dogs (35), and pigs (present data and Ref. 12), it seems likely that the cysteinyl LTs are capable of promoting the accumulation of EVLW and are pathophysiologically important in the development of pulmonary edema associated with acute lung injury.

In a previous study, we showed that pretreatment with LY 203647 ameliorates LPS-induced arterial hypoxemia (12). Although this effect was statistically significant, the magnitude of the difference between treated and untreated animals was relatively small, particularly at later time points. In other studies, pretreatment with LY 171883 did not affect $P_{A_{o_2}}$ in sheep (21) or pigs (11) with acute lung injury due to endotoxin or rats with acute lung injury induced by oleic acid (7). In the present investigation, posttreatment with LY 203647 did not significantly alter the adverse effect of LPS on $P_{A_{o_2}}$, although there was evidence of a modest beneficial effect on intrapulmonary shunt fraction, since $Q_s/Q_{t}$ increased significantly over time in endotoxic controls but not in endotoxic animals posttreated with LY 203647. Thus, in the present study, the pathological accumulation of EVLW was prevented by LY 203647, but LPS-induced arterial hypoxemia was unaffected. Numerous other studies of acute lung injury have similarly documented dissociation between the degree of pulmonary edema (or the magnitude of the derangement in pulmonary microvascular permeability) and the extent of arterial hypoxemia (6, 8, 9, 12).

The cysteinyl LTs have been shown to be potent mesenteric arteriolar constrictors (16). We have shown that infusion of authentic LTC4 also leads to mesenteric hyperperfusion in pigs (unpublished observations). Because acute endotoxicosis in pigs is associated with mesenteric hyperperfusion and ileal mucosal acidosis (8, 11, 12, 19), we were interested in testing the hypothesis that this phenomenon is mediated by cysteinyl LTs. When LY 203647 is used to pretreat pigs before infusion of LPS, the early ($t = 20-40$ min) decrease in $Q_{sMA}$ is abrogated, although late-phase changes in mesenteric perfusion and the development of ileal mucosal acidosis are unaffected (12). Very similar results were obtained in the present study. In a previous study, L. Y. 171883 was shown to have a somewhat more durable beneficial effect on $Q_{sMA}$ in porcine endotoxicosis (11). Presently available data are insufficient to account for the differences in the results obtained with LY 171883 and LY 203647 with respect to mesenteric perfusion, although the former compound is known to have some activity as a phosphodiesterase inhibitor (20), and perhaps this explains its greater effect on $Q_{sMA}$ in porcine endotoxicosis. Alternatively, the compounds may be interacting with different subclasses of cysteinyl LT receptors. In any event, it seems probable that the early decrement in mesenteric perfusion in this porcine model of endotoxic shock is mediated, at least in part, by the release of cysteinyl LT.

We can only speculate as to why the beneficial effect of LY 203647 was only transient, although data obtained in rats suggest that LPS-induced release of cysteinyl LT is an early and transient event (22). Thus it is possible that the late-phase decrease in $Q_{sMA}$ in endotoxic pigs was due to mediators other than the cysteinyl LT.

In the present study, we did not include a group of animals treated with LY 203647 in the absence of LPS, since we have already shown that this compound does not affect important pulmonary or circulatory variables in normal pigs, at least over a 20-min period of observation (12). We did not attempt to measure cysteinyl LT concentrations in blood or bronchoalveolar lavage fluid. Hence our data are insufficient to establish beyond all shadow of doubt that cysteinyl LTs are involved in several important pathophysiological events in this porcine endotoxicosis model. Nevertheless, other investigators have documented that production of these compounds is increased in an experimental model of acute endotoxicosis (22). Thus our data, in combination with results obtained by other laboratories, strongly support the notion that the cysteinyl LTs are important mediators in acute endotoxicosis.

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20.  