Adult respiratory distress syndrome (ARDS) is a form of acute lung injury characterized by diffuse pulmonary infiltrates in the absence of left heart failure. Severe hypoxemia and diminished compliance of the lungs are observed. ARDS is not infrequently a sequela of endotoxemia (31). There is much evidence indicating an etiologic role for lipid mediators, including platelet-activating factor (PAF), in endotoxin-induced lung dysfunction. In the experiments reported in this study, we utilize ABT-299, a new potent and highly specific PAF-receptor antagonist (38), in chronically instrumented sheep to more clearly define the role of PAF in endotoxin-induced lung dysfunction.

PAF is produced after endotoxemia, and increased levels of PAF have been observed in humans with septicemia (17, 22). The administration of exogenous PAF to a variety of species, including sheep, mimics many of the effects seen as a result of endotoxemia (16, 36, 40). The administration of PAF to sheep causes marked pulmonary hypertension, alterations in lung mechanics, hypoxemia, and leukopenia (12, 15, 36). These changes are similar to those observed after endotoxemia in sheep. Endotoxin also causes an initial marked pulmonary hypertension, alterations in lung mechanics, hypoxemia, and leukopenia (8, 10, 11, 15, 24, 26, 27, 37).

Studies utilizing PAF-receptor antagonists in a variety of injury models (1–5, 29, 30, 42), including during endotoxemia in a variety of species (rats, pigs, sheep), have produced promising results (7, 13, 19, 28, 32, 34, 35). Christman et al. (15) used two different PAF-receptor antagonists, SRI-63-441 and WEB-2086, in sheep during endotoxemia. SRI-63-441 attenuated the early pulmonary hypertension and blocked the reduction in dynamic compliance of the lungs (Cdyn), late neutropenia, and increase in lung lymph flow seen after endotoxin administration (15). WEB-2086 had no significant effect on the early pulmonary hypertension or early changes in lung mechanics observed after endotoxin but did attenuate the late changes in Cdyn and lung lymph flow. These studies were limited by the quality of the PAF-receptor antagonists available at that time (15). SRI-63-441 and WEB-2086 were not potent compared with ABT-299. They had side effects at the higher doses required to block 10-µg bolus injections of PAF. They only partially blocked the in vivo effects of 25-µg bolus injections of exogenous PAF. ABT-299 in the present study, by comparison, abolished the effects of 300-µg bolus injections of PAF while having no direct effect on any of the outcome variables measured. SRI-63-441 and WEB-2086 may have had pharmacological actions unrelated to their ability to block PAF receptors (15).

ABT-299 is a prodrug that is rapidly converted in vivo to A-85783.0 in the presence of plasma esterases. ABT-299 is a highly potent, specific, competitive, reversible, and stereoselective PAF-receptor antagonist. ABT-299 is a potent inhibitor of[^H]PAF binding to receptors on rabbit platelet membranes (inhibitory binding constant = 3.8 nM). ABT-299 has been shown to block the lipopolysaccharide-induced systemic hypotension, vascular leak, disseminated intravascular coagulation, organ damage and/or failure, and high mortality in rats (38). The endotoxin model in sheep has proven to be useful for screening drugs for possible use in human sepsis and ARDS (10, 14, 41). To further study the role of PAF in endotoxin-induced lung dysfunction and to test for the possible utility of ABT-299 in human sepsis and ARDS, we examined the effects of ABT-299 pretreatment on the effects of endotoxin in six chronically instrumented awake sheep.

**METHODS**

Sheep preparation. Yearling sheep (25–35 kg) of both sexes were prepared surgically as previously described (8). Silastic envelopes attached to Silastic tubing were placed in the pleural cavity, and the tubing was externalized for the measurement of pleural pressure. Through a left thoracotomy, Silastic catheters were inserted directly into the main...
pulmonary artery and the left atrium. Vascular catheters were also inserted into the thoracic aorta and superior vena cava through cervical vessels. A tracheostomy was performed. The animals were allowed to recover for several days before investigation. A size-10 Shiley cuffed tracheostomy tube (Shiley, Irvine, CA) was inserted at the time of experimentation.

Lung mechanics. Sheep were studied unanesthetized while they stood in a whole body pressure-compensated integrated-flow plethysmograph. Ropes were used as passive restraints to prevent the sheep from lying down during the experiment. The sheep's tracheostomy tube was connected to an external valve via noncollapsible flexible tubing. A constant bias flow was used to reduce the effective dead space of the tubing. Tidal volume (VT) was measured by pressure compensating for the integrated signal from the plethysmographic pressure transducer. Flow was obtained by electronically differentiating the volume signal. Pleural pressure (Ppl) was measured by a multiple-side-hole catheter positioned 0.5 cm past the distal end of the tracheostomy tube. Transpulmonary pressure (PtP) was measured directly from the Silastic catheter and envelope in the pleural space. Airway opening pressure (Pao) was measured by a Tektronix dual-beam storage oscilloscope (Tektronix, Beaver- ton, OR) and photographed for calculation of Cdyn. Cdyn was calculated as Vt divided by PtP at points of zero flow and expressed in liters per centimeter H2O at BTPS. Functional residual capacity (FRC) was measured by using the Boyle's law method of DuBois et al. (18). The airway was manually obstructed at end expiration. The sheep continued to make respiratory efforts against the obstruction for 1–3 breaths, and a graph of the change in plethysmographic volume against the change in Pao was traced on the oscilloscope and photographed for calculation of FRC.

Mesodynamics. We continuously recorded mean pulmonary artery pressure (Ppa) and mean systemic arterial pressure (Psa) by using fluid-filled pressure transducers (model 1208C, Hewlett-Packard, Palo Alto, CA). The level of the left atrium was the zero reference for all vascular pressure measurements.

Experimental protocol. To ensure that ABT-299 inhibited the action of PAF in sheep, we administered intravascular boluses of C-16 PAF (l-α-lecithin, β-acetyl, γ-O-alkyl, Calbiochem) with and without pretreatment with ABT-299 (loading dose of 1 mg/kg over 10 min followed by a continuous infusion of 0.6 mg·kg−1·h−1). Psa was continuously monitored, and lung mechanics were determined at 1-min intervals for the first 5 min after PAF administration and then every 15 min thereafter.

The effects of ABT-299 on the response to endotoxin were studied in six chronically instrumented awake sheep. Each sheep served as its own control and was studied three times: once with ABT-299 alone, once with endotoxin after ABT-299 pretreatment, and once with the vehicle for ABT-299 (0.9% NaCl) and endotoxin. A minimum of four days was allowed between endotoxin experiments. This protocol has previously been shown to cause reproducible responses to endotoxin (10, 11, 15, 24, 26, 27, 37, 41). The order of experimentation was varied to further exclude the possibility of sequential bias.

At the beginning of each experiment, the physiological measurements were recorded until a stable baseline was obtained for at least 1 h. ABT-299 was prepared by dissolving 203 mg of ABT-299 in 203 ml of sterile 0.9% NaCl. After baseline measurements were taken, infusion of ABT-299 (or an equal amount of vehicle) via the central venous catheter was started as a loading dose of 1 mg/kg over 10 min followed by a continuous infusion of 0.6 mg·kg−1·h−1 through the remainder of the experiment. One hour after drug (or vehicle) infusion began, 0.5 µg/kg Escherichia coli endotoxin in sterile 0.9% NaCl (lipopolysaccharide E. coli O55:B5, Difco Laboratories, Detroit, MI) was infused over 15 min via the pulmonary artery catheter (average total volume 15 ml). Measurements were made for 5 h after the start of the endotoxin infusion.

Throughout the experiment, mean intravascular pressures were continuously recorded and averaged for intervals of 15 min. Lung mechanics were measured every 15 min throughout the experiment. Arterial blood was collected every 30 min for white blood cell (WBC) count and blood-gas determination.

Other methods. WBC were counted by using a Coulter counter (model ZBI, Fear Electronics, Hialeah, FL). Arterial gas tensions for oxygen and carbon dioxide and pH were determined by a Corning blood-gas analyzer (238 pH/blood-gas analyzer, Corning Medical and Scientific, Medfield, MA). The alveolar-arterial PO2 gradient ([A(a)-PO2]) on room air was calculated by using the alveolar gas equation with a fixed respiratory exchange ratio of 0.8.

Statistics. The data were analyzed by using parametric repeated-measures analyses of variance with multiple comparisons by Newman-Keuls test (Statistica/W, StatSoft, Tulsa, OK). A value of P < 0.05 was considered significant.

RESULTS

PAF administration without and with ABT-299 pretreatment. Intravascular bolus administration of 3 µg PAF resulted in an acute nearly fourfold rise in Ppa, with return to baseline Ppa over the subsequent 15 min. The acute rise in Ppa was accompanied by a rapid 70% reduction in Cdyn, which also resolved within 15
min. Pretreatment with ABT-299 completely blocked the effects of 3-, 10-, 30-, 100-, and 300-µg bolus injections of PAF on Ppa and Cdyn. This effect was observed immediately after loading ABT-299, after a 6-h infusion of ABT-299, and after endotoxin.

Ppa. ABT-299 infusion alone did not significantly affect Ppa. As shown in Fig. 1, within 45 min after endotoxin infusion, Ppa increased from a baseline of 17.2 ± 1.7 cmH2O to a peak Ppa of 61.2 ± 4.3 cmH2O and then gradually declined toward baseline but remained significantly elevated for 5 h. After pretreatment with ABT-299, endotoxin administration resulted in an attenuated peak Ppa response, increasing from a baseline of 16.8 ± 0.9 cmH2O to a peak Ppa of 55.8 ± 6.1 cmH2O. Ppa remained significantly elevated from baseline at 1, 2, and 5 h after endotoxin and differed from the endotoxin alone experiments at peak Ppa only.

Psa. ABT-299 infusion had no significant effect on Psa. Endotoxin infusion with or without pretreatment with ABT-299 did not cause any significant change in Psa during the observation period (Fig. 2).

Lung mechanics. As shown in Fig. 3, ABT-299 administration alone did not significantly affect Cdyn. Endotoxin infusion resulted in an acute decline in Cdyn to 30.6 ± 6% baseline within 30 min after endotoxin. Cdyn remained significantly below baseline measurements throughout the remainder of the observation period. Pretreatment with ABT-299 did not significantly affect the response to endotoxin administration with respect to Cdyn. Endotoxin infusion in the presence of ABT-299 resulted in an acute decline in Cdyn to 36.5 ± 8.1% of baseline followed by a gradual return toward baseline, remaining significantly reduced from baseline throughout the remainder of the observation period. There were no statistically significant changes in FRC from baseline values in any of the experimental groups during the course of the experiments (data not shown).

\(\Delta(A-a)PO_2\). As shown in Fig. 4, ABT-299 infusion alone did not significantly affect \(\Delta(A-a)PO_2\). Endotoxin infusion resulted in an increase in \(\Delta(A-a)PO_2\) from a baseline of 26.1 ± 1.6 Torr to a maximum of 44.6 ± 5.7 Torr at 2 h after endotoxin infusion before slowly returning to baseline. Pretreatment with ABT-299 did not significantly alter the effect of endotoxin on \(\Delta(A-a)PO_2\).
WBC. ABT-299 administration alone did not significantly affect WBC (see Fig. 5). Endotoxin infusion resulted in a decline in WBC from a baseline of 8,863 ± 673 to a nadir of 3,049 ± 870 cells/mm³ at 2 h after endotoxin administration before gradually increasing, but the number remained significantly reduced with respect to baseline throughout the remainder of the observation period. Pretreatment with ABT-299 did not significantly alter the leukopenia observed after endotoxin administration.

DISCUSSION

The rationale for believing that PAF is involved in the response to endotoxin is based on the following: 1) PAF levels are elevated after endotoxin administration (12); 2) exogenously administered PAF induces physiological and biochemical responses that are qualitatively similar to those seen after endotoxin administration (16, 33, 36, 40); and 3) PAF-receptor antagonists have attenuated the physiological and biochemical responses to endotoxin administration in several animal models (6, 7, 12, 13, 19–21, 25, 28, 32, 34, 35).

In the experiments reported in this study, ABT-299 had minimal effects on the response to endotoxin administration in chronically instrumented awake sheep. ABT-299 pretreatment reduced the peak Ppa seen after endotoxin administration. ABT-299 had no effect on the response to endotoxin with respect to Psao, Cdyn, FRC, Δ(Pa–a)PO₂, or WBC. ABT-299 clearly was effective in blocking the changes in Ppa and Cdyn elicited by exogenous PAF administration in this animal model (ABT-299 completely blocked the effects of a 2-log-greater dose of exogenous PAF that was nearly lethal in untreated animals), and no untoward effects were apparent during the drug's administration.

The results reported here contradict those of previously published experiments in which other PAF antagonists (WEB-2086, SRI-63-441, SDZ-64-688, BN-52021) were utilized. ABT-299 is a more potent (38) and specific PAF-receptor antagonist than those previously mentioned (15) and is therefore potentially a better experimental tool. It is possible that some of the beneficial effects during endotoxemia reported with other PAF antagonists were the result of unforeseen properties of the drugs that are not associated with the antagonism of PAF receptors per se (15).

The literature contains results from studies with a variety of PAF-receptor antagonists, species, and lung-injury models (7, 13, 15, 19, 27, 32, 34, 35). Differences between the results of these studies and the present work with ABT-299 in the sheep endotoxin model may be the result of non-PAF-receptor-blocking actions of drugs used in the earlier studies, differences between species, or differences between details of the actual models studied. Because ABT-299 attenuates endotoxin-induced injury in rats (37) and pigs (23) but does not have comparable effects in sheep, the role of PAF in endotoxin-induced injury probably varies among species. ABT-299 was highly potent in sheep (blocking the effects of 300-µg bolus injections of PAF) and had no direct effects of its own. The results obtained with ABT-299 in the present study suggest that PAF is not an important mediator of endotoxin-induced lung dysfunction in sheep.

Although these negative results neither will nor should prevent further investigations into the role of PAF in endotoxin-induced lung injury, an argument can be made, on the basis of these results, that it is highly unlikely that PAF-receptor antagonists by themselves will prove beneficial in human sepsis or ARDS. The sheep endotoxin model has proven to be useful for screening drugs for possible use in human sepsis and ARDS. The sheep endotoxin model has proven to be effective in supporting further study in humans (cyclooxygenase inhibitors) (10) or in anticipating negative results in human studies (anti-endotoxin antibodies and steroids) (8, 9, 41). In a situation where bringing new drugs into human use for any indication is exceedingly expensive, we believe that the sheep endotoxin model is a useful tool to employ for making the decision about whether to go ahead with drug development in humans for sepsis and ARDS. ABT-299 did not prove efficacious in the sheep endotoxin model.

In summary, pretreatment with ABT-299 resulted in a small reduction in the peak Ppa seen after endotoxin administration but had no significant effects on any other variables measured. We conclude that PAF does not play an essential role in the sheep's response to endotoxin.

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