PAF increases vascular permeability without increasing pulmonary arterial pressure in the rat

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Clavijo, Leonardo C., Mary B. Carter, Paul J. Matheson, Mark A. Wilson, William B. Wead, and R. Neal Garrison. PAF increases vascular permeability without increasing pulmonary arterial pressure in the rat. J Appl Physiol 90: 261–268, 2001.—In vivo pulmonary arterial catheterization was used to determine the mechanism by which platelet-activating factor (PAF) produces pulmonary edema in rats. PAF induces pulmonary edema by increasing pulmonary microvascular permeability (PMP) without changing the pulmonary pressure gradient. Rats were cannulated for measurement of pulmonary arterial pressure (Ppa) and mean arterial pressure. PMP was determined by using either in vivo fluorescent videomicroscopy or the ex vivo Evans blue dye technique. WEB 2086 was administered intravenously (IV) to antagonize specific PAF effects. Three experiments were performed: 1) IV PAF, 2) topical PAF, and 3) Escherichia coli bacteremia. IV PAF induced systemic hypotension with a decrease in Ppa. PMP increased after IV PAF in a dose-related manner. Topical PAF increased PMP but decreased Ppa only at high doses. Both PMP (88 ± 5%) and Ppa (50 ± 3%) increased during E. coli bacteremia. PAF-receptor blockade prevents changes in Ppa and PMP after both topical PAF and E. coli bacteremia. PAF, which has been shown to mediate pulmonary edema in prior studies, appears to act in the lung by primarily increasing microvascular permeability. The presence of PAF might be prerequisite for pulmonary vascular constriction during gram-negative bacteremia.

platelet-activating factor; septic shock; gram negative; lung injury; pulmonary edema; videomicroscopy; pulmonary arterial catheterization

PLATELET-ACTIVATING FACTOR (PAF), a biologically active phospholipid, is proposed to be a major mediator of acute lung injury (ALI) during sepsis. In experimental models, exogenously administered PAF produces ALI characterized by neutrophil activation, alveolar capillary damage, and pulmonary edema (27). PAF levels increased in lungs of rats during endotoxemia (13). PAF-receptor antagonism inhibits hypoxemia, pulmonary edema, and changes in microvascular permeability during endotoxin-induced ALI (29) and after intestinal ischemia and reperfusion (11). Clinical studies in intensive care unit patients and basic studies in dogs demonstrate that PAF metabolites in circulating blood increased during acute respiratory distress syndrome (ARDS) after trauma, sepsis, or intestinal ischemia-reperfusion (2, 9, 17, 19, 21).

The mechanism by which PAF induces pulmonary edema is not clear. Reports in the literature support three basic mechanisms for the generation of pulmonary edema (1, 35). 1) PAF increases pulmonary arterial hydrostatic pressure (Ppa), creating a pressure gradient across the pulmonary vasculature (20). 2) PAF increases vascular permeability by causing structural changes to endothelial cells (8). 3) Changes in both pressure and vascular permeability might occur. Although assessment of pulmonary edema in small animals such as mice or rats is feasible, measurement of Ppa in vivo is technically challenging in animals of this size. Many of the data demonstrating that PAF might increase Ppa are derived from experiments performed in large-animal models (4) or in vitro rodent models (15). Our aim in the present study was to investigate the mechanism of ALI in rats by measuring both microvascular permeability and Ppa in vivo during administration of exogenous PAF or during bacteremia. It is our hypothesis that PAF is a primary mediator of increased microvascular permeability and systemic hemodynamic derangement but does not cause pulmonary arterial (PA) hypertension in the rat.

To study the contribution PAF makes in the pathophysiology of ALI in the rat, we conducted three series of experiments. First, we measured the effect of a 60-min peripheral venous infusion of PAF on Ppa in closed chest, spontaneously breathing animals. Microvascular permeability was determined at the end of each experiment by using the Evans blue dye (EBD) technique. Second, we measured the response to PAF topically applied to the pulmonary surface using in vivo pulmonary fluorescent videomicroscopy (FVM) while measuring Ppa simultaneously. Third, we investigated changes in Ppa and microvascular permeability during 90 min of Escherichia coli bacteremia with and
without pretreatment with WEB 2086. WEB 2086 (apafant), a hetrazepine, is a potent and specific PAF-receptor antagonist (5).

MATERIALS AND METHODS

Animal Preparation

All experimental protocols were approved by the Louisville Veterans Affairs Medical Center Animal Studies Subcommittee of the Research and Development Committee. Pathogen-free male Sprague-Dawley rats (290–330 g) were housed with the use of reverse isolation in an animal care facility approved by the American Association for Accreditation of Laboratory Animal Care. Food, but not water, was withdrawn 12 h before experimentation. Animals were anesthetized with urethane (800 mg/kg) and α-chloralose (60 mg/kg ip). Repeated doses of urethane (33 mg/kg) were given intraperitoneally as needed to maintain anesthesia. Body temperature was measured with a rectal probe and maintained at 37°C with a heating pad.

Tracheotomy was performed with PE-240 polyethylene tubing. The right femoral artery and vein were cannulated with PE-50 and stretched PE-90, respectively. The femoral artery was connected to a pressure transducer (TXD-310, Digi-Med, Louisville, KY) and a blood pressure analyzer (Digi-Med). Normal saline (1 ml) was injected subcutaneously at the time of anesthesia to compensate for evaporative fluid losses during the surgery.

Closed Chest PA Catheterization

We modified the closed chest PA catheterization technique originally described by Herget and Paleček (24). Briefly, a 13-cm-long Silastic PA catheter (0.012 ID, 0.025 OD) and introducer (7.5-cm PE-90 tube with the tip turned anteriorly 30°) were passed via the right jugular vein into the right ventricle. The Silastic catheter was advanced into the PA, and the introducer was removed from the atrium. The PA catheter was attached to a pressure transducer (TXD-310, Digi-Med) and low-pressure analyzer (LPA-2000, Digi-Med). A calibrated oscilloscope (Tektronix 2213, London, UK) was used to assess the hemodynamic waveform. The catheter position was confirmed in vivo by the characteristic PA wave profile and at autopsy by dissection of the PAs.

Determination of Pulmonary Microvascular Permeability

EBD technique. EBD, 6,6′-[(3,3′-dimethyl[1,1′-biphenyl]-4,4′-diyl])bis[4-amino-5-hydroxy-1,3-napthalenedisulfonic acid], a nonradioactive diazo dye that binds tightly to albumin, is a sensitive marker for early pulmonary edema (30, 36). Prolonged perfusion with EBD does not alter vasoreactivity or water content in isolated, perfused rat lungs (16).

A stock solution of EBD (2.0 g/100 ml, Sigma Chemical, St. Louis, MO) was prepared in sterile water, filtered, and stored at 4°C. In the first series of experiments, animals received 25 mg/kg EBD (0.75 ml) intravenously (IV) after completion of a 60-min infusion of IV PAF. Thirty minutes later, animals were placed on a rodent ventilator (model 683, Harvard Apparatus, South Natick, MA) and ventilated at 70 breaths/min with room air, 2.0-ml tidal volume, and 1-cmH2O positive end-expiratory pressure. After midline thoracotomy, the superior and inferior vena cava were ligated, the aorta was transected, and 20 ml of normal saline solution were injected into the right ventricle at a pressure of 20 cmH2O to wash out the pulmonary intravascular content. A sample of lung tissue was weighed, immersed in formamide, and homogenized. The homogenate was incubated at 50°C for 36 h and centrifuged at 3,900 g for 10 min. The optical density of 1 ml of supernatant was measured at 620-nm wavelength, and the EBD concentration was determined from a standard curve of EBD-formamide solutions. Microvascular permeability was expressed as the ratio of micrograms of EBD per gram lung tissue.

FVM technique. After cannulation, a right thoracotomy was performed using thermal cautery. Animals were ventilated with a rodent ventilator as described above. A lung window designed and provided by Fingar et al. (18) was carefully inserted through the opening in the chest and secured in place with interrupted silk sutures (Fig. 1). Lungs were in contact with and visible through the glass coverslip of the lung window during the remainder of the experiment. The animal was transferred to the microscope stage and placed in the left lateral decubitus position. A continuous IV infusion of 1 mg·kg−1·h−1 vecuronium bromide (Norcuron, Organon, West Orange, NJ) maintained chemical paralysis during the remainder of the experiment. FITC-labeled albumin (FITC-albumin) was stored frozen as aliquots until the day of the experiment (11). Animals received 0.5 ml/kg of FITC-albumin IV after lung window placement. A modified Leitz microscope (Leica, Deerfield, IL) with 100-W mercury epi-illumination and 450- to 490-nm wavelength filter and a special long-distance objective [5 × 0.15] × 2 epi plan; Neuroflex, Zeiss, Germany] with a working distance of 15 mm was used to observe the surface of the upper and middle lobes of the right lung. A high-resolution silicon intensifying target video camera (model C2400, Hamamatsu) was connected to the microscope, and video

Fig. 1. Animal with lung window implanted.
images were recorded on a video cassette recorder (AG-7300, Panasonic, Rockville, MD). The camera voltage was standardized by using a fluorescein-diacetate standard. The pulmonary surface was viewed with a television monitor (model PVM-122, Sony) during data collection. Experiments were performed in a dark room to prevent interference from ambient light.

Two subpleural areas, measuring 0.06375 mm² each, were selected over the middle lobe within the lung window microgrid. For each time point, video recordings were made of the selected areas for 15 s, and data were obtained only from those images that occurred at the peak of inspiration. Care was taken to minimize exposure of the lung to the blue-filtered light to prevent photo bleaching and tissue damage.

The recorded FVM images of the subpleural architecture appear white, wherever FITC-albumin is present (Fig. 2A) (11). Leakage of FITC-albumin into the alveoli (Fig. 2B) was computed off-line using software (Image-1, Universal Imaging, West Chester, PA) that permitted digital analysis of the gray-scale level from videotape images. Four video frames obtained during inspiration were chosen from each recorded observation of the two selected subpleural regions. Each frame was digitized at a 512 × 512-pixel resolution, with each pixel containing a gray-level intensity ranging from 0 for black to 255 for bright white. The spatial average pixel intensity was obtained for each subpleural region in four video frames and averaged for each observation. Because the camera saturates at a maximum pixel intensity of 255, there is the possibility of nonlinearity at the high-intensity range.

Data are reported as means ± SE. Statistical analyses were performed with repeated-measures ANOVA and ANOVA, followed by the Tukey-Kramer honestly significant difference test. The null hypothesis was rejected for P < 0.05.

Data Collection

Hemodynamic variables. After 30 min of hemodynamic stabilization, two baseline measurements of mean arterial pressure (MAP), heart rate (HR), and mean Ppa were obtained at 15-min intervals and averaged (BL-avg). MAP and HR were measured from the femoral arterial line, and Ppa was measured from the PA catheter.

Experiment 1. IV PAF. Aliquots of PAF (Calbiochem-Novabiochem, La Jolla, CA) were prepared in 0.1% BSA and diluted with normal saline to a 1-ml final volume. At time (t) = 0, an IV PAF infusion was begun via the femoral vein at a rate of 1 ml/h and lasting 60 min. Hemodynamic data were measured every 10 min for 90 min, after which pulmonary microvascular permeability was determined by using the EBD technique.

Experimental groups (n = 5, 6, 5, and 5) received increasing doses of IV PAF: 0, 3.3, 16.5, and 33 μg/100 g body wt, respectively. The vehicle control group (sham, 0 μg PAF, n = 5) received a volume of 0.1% BSA equal to that of the highest PAF dose in 1 ml of normal saline.

Experiment 2. Topical PAF. At t = 0, a 50-μl aliquot of PAF in 0.1% BSA was infused over a 10-min interval through a Silastic catheter (0.012 ID, 0.025 OD) positioned at the pleural surface near the region under microscopic observation. Experimental groups (n = 5) each received a single concentration of topical PAF: 0, 30, 80, and 300 nM. A fifth group (300 nM PAF) received 5 mg/kg IV WEB 2086 (courtesy of Boehringer, Ingelheim, Germany) diluted in 1 ml of normal saline 15 min before t = 0 by infusion through the femoral vein. In all groups, hemodynamic and FVM data were recorded at 10-min intervals for 60 min, after which animals were killed.

Experiment 3. Gram-negative bacteremia. Animals (n = 6) received E. coli (10⁹ colony-forming unit/100 g body wt in 1 ml of normal saline) or normal saline alone (sham, n = 4) through the femoral vein over 5 min, beginning at t = 0. Others (n = 4) received 5 mg/kg IV WEB 2086 diluted in 1 ml of normal saline 15 min before infusion of E. coli. Hemodynamic and FVM data were obtained for 90 min at 10-min intervals, after which animals were killed.

RESULTS

Experiment 1. IV PAF

Systemic administration of PAF induced hypotension and tachycardia in a dose-dependent manner (Fig.
Mean Ppa decreased during IV PAF (Fig. 4), with the maximal effect observed in the 16.5 mg/100 g body wt group (21861% vs. BL-avg at t = 40 min). Animals exhibited a tendency toward recovery of MAP, HR, and Ppa once the infusion of PAF ended. In the group receiving the lowest dose of IV PAF (3.3 mg/100 g BW), both MAP and Ppa increased by 962% over BL-avg 30 min after cessation of IV PAF (t = 90 min; Figs. 3 and 4). IV PAF induced a dose-dependent increase in the lung permeability index (Fig. 5) measured by the EBD technique.

Experiment 2. Topical PAF

Topical application of 50 µl PAF decreased peripheral MAP slightly from BL-avg in the 80 and 300 nM groups (Fig. 6, top), an effect that was not observed in animals pretreated with WEB 2086. However, MAP was not different among groups at any time point. HR increased, but this change was also slight (Fig. 6, bottom). Ppa decreased 16 ± 3% from BL-avg in the 300 nM PAF group with a maximal response at t = 60 min (Fig. 7, top). Pretreatment with IV WEB 2086 did not block this Ppa response to 300 nM topically applied PAF.

Pulmonary microvascular leak measured by FVM demonstrated a dose-dependent and time-dependent increase after the topical application of PAF compared with BL-avg (Fig. 7, bottom). The increase in pulmo-

![Fig. 3. Systemic hemodynamic data in animals receiving an intravenous (IV) administration of increasing concentrations of platelet-activating factor (PAF). Top: mean arterial pressure (MAP; mmHg) vs. time (min). Bottom: heart rate (HR; beats/min) vs. time (min). n = 5 For sham and 16.5 and 33 µg PAF/100 g body wt (BW); n = 6 for 3.3 µg PAF/100 g BW. BL, baseline. Significant difference vs. sham, *P < 0.05; vs. BL average (BL-avg), †P < 0.05.](http://jap.physiology.org/)

![Fig. 4. Pulmonary arterial pressure (Ppa; mmHg) vs. time (min) in animals receiving an IV administration of increasing concentrations of PAF. n Values are as in Fig. 3 legend. Significant difference vs. sham, *P < 0.05; vs. BL-avg, †P < 0.05.](http://jap.physiology.org/)

![Fig. 5. Pulmonary vascular permeability [µg Evans blue dye (EBD)/g lung tissue] measured with EBD technique after IV administration of increasing concentrations of PAF. n Values are as in Fig. 3 legend. Significant difference vs. sham, *P < 0.05; vs. 3.3 µg PAF, †P < 0.05.](http://jap.physiology.org/)

![Fig. 6. Systemic hemodynamic data following topical pleural application of 50 µl of increasing concentrations of PAF. Top: MAP (mmHg) vs. time (min). Bottom: HR (beats/min) vs. time (min). n = 5 For sham (0 nM PAF); 30, 80, and 300 nM PAF; and 300 nM PAF + WEB 2086. Significant difference vs. sham, *P < 0.05; vs. BL, †P < 0.05.](http://jap.physiology.org/)
nary microvascular leak in the 300 nM group was diminished but not eradicated with IV WEB 2086 pre-treatment.

Experiment 3. Gram-Negative Bacteremia

MAP initially decreased but soon recovered to values slightly above BL-avg in animals receiving IV E. coli (Fig. 8, top). HR increased noticeably in the E. coli group 10 min after E. coli infusion and reached a maximal value at 70 min (Fig. 8, bottom). There was no difference in HR between sham and E. coli + WEB 2086 groups except at t = 70 min. Ppa (Fig. 9, top) increased steadily in the E. coli group, reaching a maximal value of 50 ± 3% over BL-avg at t = 70 min. Ppa values in sham and E. coli + WEB 2086 groups were not different.

Gram-negative bacteremia induced a time-dependent increase in pulmonary microvascular permeability measured by FVM (Fig. 9, bottom), reaching a maximal increase of 88 ± 5% from BL-avg at t = 90 min. This effect was completely prevented by pretreatment with IV WEB 2086.

DISCUSSION

Previous animal studies suggest that the vasoactive effects of PAF in the pulmonary circulation are responsible for causing pulmonary edema (34). Others suggest that PAF causes increased microvascular permeability (23). Our first series of experiments utilized an IV infusion of increasing concentrations of PAF to assess systemic and pulmonary vascular responses in spontaneously breathing animals. As expected, there was a dose-dependent systemic hypotension and tachycardia during IV PAF infusion that improved after PAF discontinuation. These findings are in agreement with those of others (25). Instead of increasing Ppa, PAF causes pulmonary hypotension during peripheral
PAF increases vascular permeability but not Ppa.

IV infusion at the 16.5 and 33.0 μg/100 g body wt doses. PAF is known to be a pulmonary vasodilator at low doses (28). In the present study, low-dose PAF infusion (3.3 μg/100 g body wt) caused only a trend toward decreased Ppa. On discontinuation, however, Ppa increased by 9 ± 2% from BL-avg. We hypothesize that this “rebound” might represent a secondary response to the return of systemic hemodynamics to normal values.

Despite pulmonary hypotension, IV PAF induced a dose-dependent increase in pulmonary microvascular leak in spontaneously breathing animals, with saturation of the effect at the highest (33.0-μg) dose. Because Ppa was not increased throughout the experiment, we attribute the mechanism for the observed PAF-mediated pulmonary edema to increased pulmonary microvascular permeability.

Our second series of experiments assessed the effect of increasing concentrations of topical PAF applied to the lung surface. The advantage of studying PAF effects when topically applied as opposed to systemically administered is that the confounding effects of activation of systemic inflammation by PAF and effects of systemic hemodynamic derangement are both avoided. Although topically applied PAF induced a slight decrease in systemic blood pressure, MAP was >80 mmHg in all groups. Slight increases in HR in PAF-treated animals were not likely to be physiologically significant. Despite relatively normal systemic hemodynamic values, topical PAF induced a dose-dependent and time-dependent increase in pulmonary microvascular permeability as measured by in vivo FVM. These permeability changes were attenuated, but not completely inhibited, by systemic pretreatment with IV WEB 2086, a specific PAF-receptor antagonist. Topical PAF decreased Ppa from BL-avg only at the maximum dose (300 nM), an effect that was not prevented by IV WEB 2086.

Our findings from the above two experiments suggest that PAF induces pulmonary edema in the rat by increasing microvascular permeability. This is likely due to direct receptor-mediated mechanisms, because topically applied PAF induced pulmonary microvascular edema within only 10 min. Whether or not PAF induces PA hypotension via receptor-mediated mechanisms is less clear.

Our results correlate with data from in vitro studies in cultured umbilical vein endothelial cells that demonstrate that PAF induced changes in cell shape with increased transfer of albumin across endothelial monolayers at nanomolar concentrations (8). These alterations are reversible either by removing PAF from the culture environment or by pretreatment with a PAF-receptor antagonist. PAF modifies endothelial cell-to-cell adhesion properties by inducing hyperpolarization of the endothelium (26). Biochemical changes induced in human umbilical vein endothelial cells by PAF include an increase in intracellular calcium, breakdown of membrane phospholipids with generation of inositol phosphates (3, 6, 7, 22, 31), and translocation of protein kinase C from the cytosol to the membrane (10). Protein kinase C activation induces depolymerization and redistribution of cytoskeletal F-actin and disorganization of vinculin, another cytoskeletal protein. PAF rapidly activates tyrosine kinases that might initiate a cascade of phosphorylation steps that also mediate cytoskeletal rearrangement (10). Hence, our in vivo findings of PAF-induced pulmonary edema without increased Ppa are supported by in vitro studies that demonstrate dose-dependent and receptor-mediated endothelial cytoskeletal changes. Future experiments are underway to quantify cytoskeletal changes induced by PAF in cultured pulmonary microvascular endothelial cells.

Results of our third group of experiments support the hypothesis that PAF is a primary mediator of ALI during sepsis. E. coli bacteremia induced tachycardia, pulmonary hypertension, and a marked increase in pulmonary microvascular leak, all of which are prevented by PAF-receptor antagonism. The exact mechanism of sepsis-induced ALI is complex and likely involves a network of multiple proinflammatory mediators. The fact that pretreatment with a PAF-receptor antagonist prevented the formation of pulmonary edema during E. coli bacteremia cannot be interpreted to mean that PAF is the only mediator of increased pulmonary microvascular permeability during gram-negative sepsis. However, our data do strongly suggest that PAF is a primary mediator of ALI and that PAF-receptor antagonism might be beneficial in the prevention of sepsis-induced pulmonary edema.

The mechanism of pulmonary hypertension during the early phases of sepsis is not completely understood. In the present study, the finding that pretreatment with WEB 2086 blocked the increase in Ppa during E. coli bacteremia suggests that, although PAF itself is not a pulmonary vasoconstrictor in this model, its presence might be necessary for the development of PA hypertension during bacteremia. Others hypothesize that the presence of PAF during bacteremia might correlate with activation of phospholipase A₂, arachidonic acid mobilization, and production of thromboxane A₂, a known pulmonary vasoconstrictor (14). Pulmonary hypertension might be because of humoral mediators rather than cytokines. Recent work by Sheridan et al. (32, 33) demonstrated that endotoxin induces neutrophil accumulation in the pulmonary circulation, inducing dysfunction of cGMP-mediated pulmonary vasorelaxation. Finally, during late phases of pulmonary edema, increased interstitial pressure, decreased alveolar-capillary gas diffusion, hypoxemia, and decreased pulmonary compliance all might further increase Ppa, and hence microvascular leak, contributing to a progressive cascade.

Sepsis and septic shock are significant causes of morbidity and mortality. Present estimates suggest that 500,000 new episodes of sepsis occur in the United States annually, accounting for $5–10 billion in medical care costs (12). ARDS often occurs early in severe sepsis, with prevalence varying from 6 to 42% of cases.
and mortality from 40 to 60%. Human and animal experiments suggest a primary role of PAF during ARDS. Our data support that PAF is a primary mediator in the formation of ALI during sepsis.

In conclusion, in this manuscript, we have provided the first in vivo demonstration that PAF can increase pulmonary vascular permeability without increased Ppa. This finding supports prior in vitro studies which suggest that PAF is capable of altering the pulmonary vascular endothelium’s structure and function. These studies also show that PAF is a mediator of pulmonary inflammation during E. coli bacteremia. We conclude from these experiments that PAF increases pulmonary microvascular permeability and decreases Ppa, whereas gram-negative bacteremia increases both in adult rats. PAF-receptor inhibition prevents these changes in both instances. PAF appears to play an important role in pulmonary edema secondary to sepsis primarily via increased endothelial cell permeability. Although these data show that PAF does not directly increase Ppa, the binding of PAF to its receptor appears to be necessary for the development of pulmonary hypertension during sepsis, because blockade of PAF receptors prevents the increase in Ppa during E. coli bacteremia. PAF-receptor inhibition might be beneficial in the prevention of lung injury after sepsis.

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