Platelet Activating Factor Receptor Antagonist Improves Survival and Attenuates Eicosanoid Release in Severe Endotoxemia

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Exogenous platelet activating factor (PAF) causes hypotension, plasma extravasation, metabolic acidosis, and death. These effects are similar to those of endotoxin as well as the eicosanoids. A specific PAF receptor antagonist, BN52021, was used to determine its effects on the hemodynamic events, the eicosanoid production, and on survival in severe rat endotoxemia. Endotoxin alone significantly produced hypotension, prostaglandins (TxB_2, PGE_2) release, and death. In contrast pretreatment with BN52021, a specific PAF receptor antagonist, significantly altered the hypotension, significantly attenuated the eicosanoid release, and improved the survival rate ($p < 0.01$). These findings suggest that PAF receptor activation is an early event in endotoxemia. Eicosanoid release in endotoxemia could be related to PAF synthesis and PAF receptor activation. These findings support the hypothesis that there may be an intimate relationship between PAF and the eicosanoids and that in endotoxemia some of the effects of PAF may be mediated *via* the cyclo-oxygenase pathway.

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SEPSIS CONTINUES TO BE a significant clinical problem after trauma or elective surgery. The morbidity and mortality rates remain increased despite aggressive surgical treatment, antibiotics, and pharmacologic intervention.[1,2] The basic mechanisms of cellular injury are related to the elaboration and release of mediators; however their interrelationships remain unknown. One proinflammatory mediator that imitates many of these effects is the ether-phospholipid, platelet activating factor (PAF).[3] PAF is released from leukocytes, platelets,[4] kidneys, lungs,[5] and endothelium[6] after physiologic stimulus or agents, such as endotoxin, that injure the cell.[4] PAF is released in gram-negative sepsis and in endotoxemia.[7-9] The potential importance of PAF in mediating events in endotoxin or sepsis is that exogenous PAF in animals causes decreased peripheral resistance,[10] systemic hypotension,[7,11] pulmonary artery hypertension,[12,13] plasma extravasation,[14,15] and death.[7,16] PAF receptor antagonists attenuate many of the events that are observed in endotoxemia in animals and significantly improve the survival rate.[7,17]

The infusion of PAF into isolated organ systems and whole animals[16,18] induces changes that are similar to those observed with endotoxin and similar to the reported effects of the eicosanoids.[3] Because PAF and arachidonic acid release follows phospholipase A_2 activation in early cell injury,[4,19] and phospholipase A_2 values are increased in gram-negative sepsis,[20] we hypothesized that an intimate relationship between PAF and the eicosanoids may exist in endotoxemia. The purposes of the present investigation were to determine the effects of a specific PAF receptor antagonist, BN52021, on the hemodynamic events and survival in rat endotoxemia, and to evaluate the effects, if any, of the PAF receptor antagonist on eicosanoid production in endotoxemia.

Methods

Male Sprague-Dawley rats (250 to 300 g) (Charles River Laboratories) were stabilized from 2 to 4 days before experimentation. They were maintained in a 12-hour light-dark sequence and allowed water and antibiotic-free chow *ad libitum*. On the day of the experiment, animals were placed in individual cages. Each animal was lightly anesthetized with halothane/oxygen mixture, had an anterior neck incision, followed by insertion of catheters (PE50) into the left carotid artery and left jugular vein. Catheters

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were flushed with heparinized saline, tunneled to the dorsal neck of the rat, and secured. The jugular vein catheter was used for injections of saline or pharmacologic agents. Rats were allowed to recover from the effects of anesthesia for at least 1 hour before baseline parameters were measured. At the completion of the experiments, animals were killed.

Hemodynamic measurements were obtained by connecting the carotid catheters to a Gould Brush physiograph (RS2300) *via* a PE23 transducer (Statham, Oxnard, CA). Thromboxane and PGE_2 were measured in plasma specimens collected at baseline, then at 5, 30, 60, and 120 minutes after endotoxin in coincidence with hemodynamic measurements. Analyses were performed by radioimmunoassay. Cross reactivity of the antibodies is less than 3% with other eicosanoid metabolites. Radiolabeled TxB_2 and PGE_2 were obtained from New England Nuclear (Boston, MA). Authentic eicosanoids TxB_2 and PGE_2 were supplied by Dr. John Pike (Upjohn Co., Kalamazoo, MI). *E. coli* endotoxin (055:B5, Difco Laboratories, Detroit, MI) was reconstituted in saline (10 mg/mL) on the day of the experiment. The PAF receptor antagonist, BN52021, was provided by Dr. Pierre Braquet, Institut Henri Beaufour, Le Plessis Robinson, France. BN52021 was diluted in a phosphate solution (pH 7.3) on the day of the experiment (10 mg/mL).

Experimental Design

All experiments were performed in the American Association for Accredidation for Laboratory Animal Care-approved facility and according to the National Institutes of Health guidelines for animal use. Rats for hemodynamic and eicosanoid measurements were studied in individual cages. Indwelling catheters were flushed with sterile heparinized saline. There was a 30-minute (−30 to 0 minutes) basal period and a 2-hour (0 to 120 minutes) experimental period during which hemodynamic measurements were recorded continuously. These consisted of measurements of heart rate and mean arterial pressure (MAP). At time 0, the animals received an I.V. bolus injection of endotoxin. In studies in which the effect of PAF antagonists were examined, the antagonists were given at specified time points before the time 0 minute.

For the survival studies, male rats were randomized into two groups. Group 1 (n = 10) and group 2 (n = 13) received *E. coli* endotoxin (20 mg/kg) IP at time 0 minute. Group 1 animals received diluent (0.5 mL) for the BN52021 subcutaneously at t = −30 minutes, whereas group 2 animals received BN52021 (25 mg/kg) subcutaneously at t = −30 minute. The animals had free access to food and water. Survival rates were determined at 24, 48, and 72 hours. Permanent survival was determined at 72 hours and is reported as a percentage.

The animals for hemodynamic and eicosanoid measurements were randomized into two groups after catheter insertion: group 3 (n = 5) received the diluent of BN52021 at t = −30 minute I.V., then *E. coli* endotoxin (20 mg/kg) I.V. at t = 0 minutes, whereas group 4 (n = 5) received BN52021 (5 mg/kg, I.V.) at t = −30 minutes, then the *E. coli* endotoxin (20 mg/kg) I.V. at t = 0 minutes. Blood samples for eicosanoid analysis were collected in heparinized tubes containing indomethacin (100 μg), the blood was centrifuged, and the plasma was removed and stored at −80 C until assayed. For each milliliter of blood removed, 1 mL of 0.9% saline was returned to the animal *via* the arterial catheter. After completion of the experiment, the animals were killed.

The selection of doses of endotoxin were determined with dose-response studies in our laboratory. Control studies in animals not given endotoxin were performed in rats with the following groups: anesthesia alone (n = 4); anesthesia plus incision and ligation of vessels (n = 5); anesthesia, incision, catheter placement, and vehicle administration (saline) for the endotoxin studies (n = 5); and the diluent for the BN52021 studies (n = 5). None of the perturbations had any significant effect on either the hemodynamic or eicosanoid changes and thus were not included.

Data analysis was accomplished using paired t tests for values within the same group, and ANOVA was used for comparison of values between the groups. Differences in survival were determined by the chi square method. A p value less than 0.05 was considered significant.

Results

Endotoxin-induced hypotension in conscious catheterized rats is shown in Table 1. Endotoxin alone significantly decreased the MAP for each observation point when compared with the baseline value. With BN52021 pretreatment, MAP values were better maintained throughout the study. Only at the 120-minute time point was there a significant ($p < 0.05$) MAP difference in the BN52021 group when compared with the endotoxin-alone group. There were no significant differences in the observed heart rates from the baseline in either group or between the groups at any observation point during the study (data not included).

The effects of the PAF receptor antagonist, BN52021, on eicosanoid synthesis/release in endotoxemia are seen in Table 2. Endotoxin alone significantly increased the production of TxB_2 and PGE_2 as early as 5 minutes after endotoxin. Peak values for TxB_2 occurred at 60 minutes after endotoxin, whereas the peak values for PGE_2 occurred 5 minutes after endotoxin (Table 2). Pretreatment with BN52021 significantly attenuated the synthesis and/or release of TxB_2 and PGE_2 after endotoxin at least for 60 minutes.

TABLE 1. *Effects of Endotoxin on Mean Arterial Pressure with and Without BN52021*

	Time (minutes)				
Group	0	5	30	60	120
EN	112 ± 7	88 ± 3*	90 ± 3*	81 ± 4*	74 ± 7*
BN + EN	110 ± 5	90 ± 8	94 ± 5	95 ± 5	100 ± 4†

Mean ± SE, n = 5; torr.
* $p < 0.05$, significant from base.
† $p < 0.05$, significant from endotoxin alone.
EN, endotoxin alone; BN + EN, PAF receptor antagonist BN52021 plus EN.

BN52021 pretreatment significantly ($p < 0.01$) improved the permanent survival (11 of 13 animals, 85%) when compared with the survival in endotoxin alone (2 of 10 animals, 20%).

Discussion

In this experiment the endotoxin caused a gradual decline in mean arterial pressures, significantly increased the plasma eicosanoids, and produced a significant rate of mortality. Pretreatment with the PAF receptor antagonist, BN52021, significantly attenuated the fall in mean arterial pressure, the increase in the plasma eicosanoids, and improved the permanent survival.

The effects of endotoxin on the hemodynamic events, eicosanoid production, and on survival in this study are similar to those reported by others.[21–23] That PAF receptor antagonists improve the hemodynamic events and survival in endotoxemia as seen in this investigation is in accord with other reports[17,24,25] and suggests that PAF may play an important role in the pathophysiology of this entity. The exact role of PAF on the complex cardiovascular events in endotoxemia is unclear. Exogenous PAF decreases the peripheral vascular resistance,[10] as well as increases plasma extravasation[11] and could be responsible for the hemodynamic changes observed in this study. Additional studies are needed to better define the mechanisms by which BN52021 had these effects. The PAF receptor antagonist BN52021 may have other unknown qualities that could alter eicosanoid synthesis or release.

The rationale for evaluating the effects of a PAF receptor antagonist on the eicosanoid metabolites was that several of the effects of exogenous PAF in animals are similar to those documented for the eicosanoids.[3] Interestingly acute circulatory collapse in dogs induced by exogenous PAF is associated with significantly increased plasma values of TxB_2 and 6-keto-PGF1α.[18] These authors suggest that exogenous PAF stimulates the arachidonic acid cascade *via* platelet aggregation and the release reaction. The effects of PAF on coronary artery blood flow have been related to the release of TxB_2.[26,27] Pulmonary arterial hypertension and increased vascular permeability in the lung induced by exogenous PAF may be caused by a cyclo-oxygenase-dependent and a cyclo-oxygenase-independent (permeability) mechanism.[15,13] Recently the injection of PAF into an isolated gastric perfusion model significantly increased the leukotrienes, in addition to 6-keto-PGF1α and TxB_2. Cyclo-oxygenase inhibition and inhibitors of leukotriene synthesis did not significantly alter the PAF-induced vasoconstriction; however the specific PAF receptor antagonist, BN52021, attenuated the flow reduction caused by PAF. These authors further demonstrated that BN52021 significantly inhibited PAF-induced eicosanoid release in their *in vitro* model.[28]

That an intimate relationship exists between PAF and eicosanoid synthesis after endotoxin in rats is supported by the unexpected finding in this study that a PAF receptor antagonist, BN52021, significantly attenuated the plasma concentrations of the eicosanoids TxB_2 and PGE_2. Although there are no reports in endotoxemia that indicate that PAF receptor blockade attenuates eicosanoid release,

TABLE 2. *Effects of Endotoxin on TxB_2 or PGE_2 Synthesis/Release with and Without BN52021*

	Time (minutes)				
Group	0	5	30	60	120
			TxB_2		
EN	424 ± 56	989 ± 22*†	1165 ± 299*‡	1561 ± 146*§	1256 ± 333*
BN+EN	368 ± 88	340 ± 67	437 ± 106	388 ± 23	734 ± 93*
			PGE_2		
EN	27.3 ± 6.0	100 ± 32*†	84 ± 15*†	92 ± 14*§	35 ± 7
BN+EN	24.2 ± 4.7	24 ± 4	32 ± 7	14 ± 2	20 ± 4

pg/mL mean ± SEM; n = 5 per observation.
* $p < 0.001$ *vs.* baseline.
† $p < 0.05$.
‡ $p < 0.025$.
‖ $p < 0.005$.
§ $p < 0.001$.
EN, endotoxin alone; BN + EN, PAF receptor antagonist BN52021 plus EN.

there are several relevant reports about the biochemical relationships of PAF to the eicosanoids.

Albert and Snyder[29] indicate that a phospholipid, l-alkyl-2-acyl-6PC, exists in the cell membrane and is a precursor of both PAF and arachidonic acid metabolites in human platelets,[30] rat[29] and rabbit[31] alveolar macrophages, peritoneal macrophages from guinea pigs, and rabbit neutrophils.[32] Chilton et al.[33] demonstrated *in vitro* the release of arachidonate from cellular phospholipids in cytochalasin B-treated rabbit polymorphonuclear leukocytes. The phospholipid sources of the arachidonate were phosphatidyl-inositol and phosphatidyl choline (about 50% from each). Interestingly the PAF-stimulated production of arachidonate metabolites and the degranulation response were blocked by eicosatetraenoic acids and nordihydroquaiaretic acid (lipooxygenase inhibitors). These investigators imply that the bioactions of PAF on polymorphonuclear leukocytes may be mediated, in part, by the release of arachidonic acid resulting in production of mono- and dihydroxy-eicosatetraenoic acids (5 HETE; LTB_4). In additional experiments they demonstrated that the metabolic fate of PAF included an incorporation of arachidonate in rabbit and human PMNs; in human PMN, 75% to 80% of the added PAF is reacylated with arachidonate.[34,35] The most recent report indicates that PAF precursors (1-0-alkyl-2-arachidonyl-GPC) are a common source of both PAF and arahidonate through the action of phospholipase A_2.[36]

In support of the *in vitro* findings of Chilton et al.,[36] Haroldsen et al.[37] demonstrated, in the intact lung, that 20% to 23% of the administered PAF resulted in 1-0-hexadecyl-2-arachidonyl-GPC. These data support the hypothesis that a relationship between PAF and arachidonate metabolism exist in the intact organ.

The preceding information suggests that exogenous PAF elicits the production of both the lipooxygenase and the cyclo-oxygenase products presumably from arachidonic acid. In some instances the effects of PAF are dose-dependent, and some of the effects of PAF are mediated *via* the effects of the eicosanoid metabolites. The fundamental cellular interrelationships are less certain. When cellular injury occurs (by many mechanisms), phospholipase A_2 is activated;[19] therefore the potential for simultaneous release of arachidonate (fatty acids) and PAF is present. The studies by Chilton[33,36] and Haroldsen[37] indicate that PAF precursors may be partly responsible for a portion of the arachidonate released *in vitro* and *in vivo* and suggest that there is a very intimate relationship between the two. Additional studies are needed to determine the definitive relationships of these potent proinflammatory mediators.

The findings of the present study suggest that PAF receptor activation occurs after endotoxemia and that its activation is related to the eicosanoid synthesis. Furthermore the improvement in survival in severe rat endotoxemia when a PAF receptor antagonist is used indicates a role for PAF in the pathophysiology of this entity. The fact that a specific PAF receptor antagonist attenuated the eicosanoid release after endotoxin administration was unexpected and suggests that these agents could help elucidate the fundamental cellular mechanisms that are critical in the early events in endotoxemia.

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