Neither platelet activating factor nor leukotrienes are critical mediators of liver injury after lipopolysaccharide administration

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Received 5 September 1996; accepted 12 February 1997

Abstract

The intravenous administration of lipopolysaccharide (LPS) to rats results in liver lesions characterized by the infiltration of both platelets and neutrophils into the liver and by midzonal hepatocellular necrosis. The liver injury is dependent on neutrophils, platelets and the coagulation system, as removal or inhibition of any of these components inhibits the development of hepatocellular necrosis. Platelet activating factor (PAF) and the cysteinyl leukotrienes (LTs) are potent inflammatory lipids that are critical for the development of some LPS-mediated alterations. To test the hypothesis that PAF, alone or in combination with LTs, contributes to the development of liver injury during LPS exposure, we conducted studies with the PAF receptor antagonist, WEB 2086, and the 5-lipoxygenase inhibitor, Zileuton. Female, Sprague-Dawley rats were pretreated with WEB 2086 (10 mg/kg, i.p.) alone or with Zileuton (40 mg/kg, p.o.) 1 h before the administration of LPS (4 mg/kg, i.v.) or its saline vehicle. Treatment with WEB 2086, alone or in combination with Zileuton, did not inhibit LPS-mediated hepatic neutrophil infiltration or liver injury, as assessed by histologic evaluation and increases in plasma alanine aminotransferase activity. Pretreatment with these agents also had no effect on the activation of the coagulation system or on the thrombocytopenia induced by LPS. These results suggest that PAF, alone or in combination with 5-lipoxygenase products, is not a critical mediator of LPS-induced hepatocellular injury in this model. © 1997 Elsevier Science Ireland Ltd.

Keywords: Lipopolysaccharide; Liver; Platelets; Neutrophils; Hepatocellular necrosis

1. Introduction

Exposure to Gram-negative bacterial lipopolysaccharide (LPS) results in numerous patho-
physiologic alterations, including shock, hypotension, disseminated intravascular coagulation (DIC) and multiple organ failure. The liver is one of the organs affected by LPS exposure. Hepatic changes include a pronounced infiltration of both neutrophils and platelets and midzonal hepatocellular necrosis (Hewett et al., 1992; Pearson et al., 1995). Inflammatory mediators are critical for the genesis of hepatic injury, since liver damage is dependent upon neutrophils, platelets, Kupffer cells, tumor necrosis factor-\(\alpha\) and components of the coagulation system (Hewett et al., 1992, 1993; Jaeschke et al., 1991; Pearson et al., 1995, 1996; Iimuro et al., 1994; Hewett and Roth, 1995; Margaretten et al., 1967).

Platelet activating factor (PAF, 1-O-alkyl-2(\(R\))-acyethyl-glycero-3-phosphocholine) is a lipid mediator that is critical for the development of numerous LPS-mediated alterations, including hypotension (Casals-Stenzel and Heuer, 1988), lung injury (Olson et al., 1990) and death (Casals-Stenzel and Heuer, 1988; Salari et al., 1990; Rabinovici et al., 1990). PAF is produced by leukocytes, platelets and endothelial cells and has proinflammatory properties. For example, it can mediate neutrophil chemotaxis and activation (Worthen et al., 1983; Montrucchio et al., 1993; Takahashi et al., 1991), increase vascular permeability and alter vascular tone (Buxton et al., 1986). PAF also stimulates the production of other soluble mediators such as eicosanoids, cytokines and superoxide anion (Snyder, 1990; Takahashi et al., 1991).

PAF can influence the liver, since the intravenous administration of PAF increases hepatic vascular resistance, glucose production, oxygen uptake and free radical generation (Lapointe and Olson, 1989; Zhou et al., 1992). It can influence non-parenchymal cells of the liver by stimulating superoxide production from both Kupffer cells and the endothelium (Gardner et al., 1995). Since PAF alters hepatic functions and can be produced by and stimulate inflammatory cells that are required for the genesis of liver injury, it is possible that PAF may be a critical mediator of hepatocellular injury that occurs during LPS exposure.

A recent study indicated that antagonism of PAF receptors alone is insufficient to prevent liver injury in the rat after the intraperitoneal administration of LPS (Yoshikawa and Goto, 1992). Similarly, administration of a 5-lipoxygenase inhibitor afforded no protection. However, the combination of a PAF receptor antagonist and a 5-lipoxygenase inhibitor prevented hepatic injury and lethality (Yoshikawa and Goto, 1992). This study supported the hypothesis that PAF in combination with LTs is critical for the development of hepatic injury and suggested that PAF and LTs may have redundant actions in this model.

Like PAF, the LTs can mediate inflammatory responses, edema and changes in vascular tone. With regard to other alterations following LPS exposure, the LTs are involved in the development of leukopenia (Cook et al., 1985). In addition, LTC\(_4\) and LTD\(_4\) have been detected in the bile of LPS-treated rats (Hagmann et al., 1985), and the intravenous administration of LPS results in LT formation in vivo through a mechanism that depends upon complement (Jaeschke et al., 1992). The LTs are critical for LPS-induced liver injury in another experimental model of endotoxemia, namely in mice sensitized with galactosamine (Tiegs and Wendel, 1988). However, in the rat the liver injury observed after the intraperitoneal administration of LPS is not mediated by 5-lipoxygenase products, alone, but rather by the combination of LTs and PAF (Yoshikawa and Goto, 1992). Since studies evaluating the role of the cysteinyl LTs appear to be model-dependent, we thought it important to evaluate the role of the LTs in a model of liver injury resulting from the intravenous administration of LPS in the rat.

The role of PAF and LTs in LPS-induced disseminated intravascular coagulation (DIC) is model-dependent as well. DIC is characterized by systemic activation of the coagulation system, which is marked by decreased plasma fibrinogen and blood platelet concentrations, prolonged bleeding times and elevated fibrin degradation products. PAF receptor antagonism attenuates DIC induced by the infusion of LPS in the rat (Imanishi et al., 1991; Imura et al., 1986). However, blockade of PAF’s actions does not inhibit DIC in septic rabbits (Ou et al., 1994) or thrombocytopenia observed in rats after the administra-
tion of large doses of LPS (Rabinovici et al., 1990). Thus, similar to the findings with liver injury, the role of PAF in LPS-induced DIC is model-dependent.

The pathogenesis of hepatocellular injury that follows the intravenous administration of LPS in the rat has been well characterized and is dependent upon neutrophils, platelets, Kupffer cells and tumor necrosis factor-α and requires activation of the coagulation system (Hewett et al., 1992, 1993; Pearson et al., 1995; Iimuro et al., 1994; Hewett and Roth, 1995). Although several studies have implicated PAF and LTs in the development of liver injury in other models, the roles of PAF and LTs appears to be dependent upon species and route of administration of LPS. Therefore, studies were conducted to evaluate the roles of these mediators in the development of liver injury in the rat after the intravenous administration of LPS. To evaluate the roles of PAF and LTs, rats were pretreated with either the PAF receptor antagonist, WEB 2086, alone or in combination with the 5-lipoxygenase inhibitor, Zileuton. We evaluated the effects of the coadministration of WEB 2086 and Zileuton on LPS-induced liver injury, thrombocytopenia and activation of the coagulation system.

2. Materials and methods

2.1. Materials

Lipopolysaccharide (Escherichia coli, serotype 0128:B12) and Kit 59 for determination of plasma alanine aminotransferase activity (ALT) were purchased from Sigma Chemical Company (St. Louis, MO). Dilution of blood and lysis of erythrocytes for platelet enumeration were performed in platelet Unopettes (Baxter Scientific Products, McGaw Park, IL). Plasma fibrinogen concentration was measured in a BBL Fibrometer (Becton, Dickinson and Company, Hunt Valley, MD) using a Data-Fi fibrinogen determination kit (Baxter Scientific Products, McGaw Park, IL). PAF was purchased from Calbiochem (San Diego, CA). WEB 2086 was a generous gift from Boehringer Ingelheim Corporation (Ridgefield, CT), Zileuton was a generous gift from Abbott Laboratories (Abbott Park, IL).

2.2. Animals

Female, Sprague-Dawley rats (Crl:CD BR (SD) VAF/plus, Charles River, Portage, MI) weighing 200–250 g were used in all studies. The animals were maintained on a 12-h light/dark cycle under controlled temperature (18–21°C) and humidity (55 ± 5%). Food (rat chow, Teklad, Madison, WI) and water were allowed ad libitum. All procedures on animals were carried out according to the guidelines of the American Association of Laboratory Animal Sciences and the University Laboratory Animal Research facilities of Michigan State University.

2.3. Verification of drug efficacy

Preliminary studies were conducted to establish an effective dosing regimen for the PAF receptor antagonist, WEB 2086, and the 5-lipoxygenase inhibitor, Zileuton. The administration of WEB 2086 (10 mg/kg, i.p.) inhibited PAF-induced hypotension by 86%. PAF receptor antagonism occurred by 30 min after the administration of WEB 2086 and was maintained up to 8 h thereafter. These results have been published in detail elsewhere (Bailie et al., 1996). The efficacy of 5-lipoxygenase inhibition with Zileuton was verified by evaluating the ex vivo production of LTB₄ after stimulation of whole blood with the calcium ionophore A23187. Six h after the administration of Zileuton (40 mg/kg, p.o.), LTB₄ production in whole blood was inhibited by 85% (Bailie et al., 1995).

2.4. Experimental protocols

To address the role of PAF in liver injury, animals were pretreated with WEB 2086 (10 mg/kg, i.p.) or saline vehicle 1 h before the administration of LPS (4 mg/kg, i.v.) or saline vehicle. Six h after the administration of LPS, animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and a midline laparotomy was performed. Blood was collected into sodium citrate (0.38%
Fig. 1. The effects of LPS and WEB 2086 on plasma ALT activity. Animals were treated with WEB 2086 (10 mg/kg, i.p.) or its saline vehicle 1 h before the administration of LPS (4 mg/kg, i.v.) or its saline vehicle (SAL). Six h after LPS administration, blood samples were collected for measurement of plasma ALT activity as described in Section 2. Results are expressed as mean ± S.E.M., N = 5–8 per group. *Significantly different from respective value in the absence of LPS.

2.5. Statistical analysis

Data were analyzed with a completely random, two-way analysis of variance (ANOVA). Homogeneity of variance was tested with the F-max test prior to analysis (Steel and Torrie, 1980). Non-homogeneous data were log-transformed prior to analysis. Comparisons between treatment means were performed using Tukey’s omega test. The criterion for significance was P ≤ 0.05. Results are expressed as mean ± S.E.M.

3. Results

The administration of LPS to rats resulted in hepatocellular necrosis within 6 h. This liver injury was assessed by elevations in plasma alanine aminotransferase (ALT) activity, an indication of a loss in parenchymal cell integrity. Pretreatment with the PAF receptor antagonist, WEB 2086, did not prevent LPS-induced hepatocellular damage (Fig. 1).

To address the possible redundancy in the roles of PAF and LTs in liver injury, a combination of WEB 2086 and Zileuton was employed. Rats were treated with either WEB 2086 (10 mg/kg, i.p.) or saline vehicle and with either Zileuton (40 mg/kg, p.o.) or 0.2% methylcellulose vehicle 1 h before the administration of LPS (4 mg/kg, i.v.). Six h after LPS administration, animals were anesthetized as described above and blood and liver sections were collected.

Blood platelets were enumerated in a hemacytometer after erythrocyte lysis and dilution in platelet Unopettes. Plasma ALT activity was used as a marker of hepatic parenchymal cell injury and was determined spectrophotometrically using Sigma Kit 59 (Bergmeyer et al., 1978). Plasma fibrinogen concentration was determined from the thrombin clotting times of diluted plasma in a fibrometer (Hewett and Roth, 1995).
Fig. 3. The effects of LPS and WEB 2086/Zileuton on liver injury. Animals were treated with WEB 2086 (10 mg/kg, i.p.) plus Zileuton (40 mg/kg, p.o.) or their respective vehicles (saline and methylcellulose (MC), respectively) 1 h before the administration of LPS (4 mg/kg, i.v.) or its saline vehicle (SAL). Six h after LPS administration, liver sections were collected, fixed and processed for histologic evaluation. LPS administration resulted in a marked sinusoidal neutrophilia and midzonal hepatocellular necrosis. Pretreatment with WEB 2086 and Zileuton did not alter either the neutrophilia or the hepatocellular necrosis observed after LPS administration.
To address the possibility that 5-lipoxygenase metabolites and PAF may have critical but redundant actions, animals were cotreated with WEB 2086 and Zileuton. As before, the administration of LPS produced a significant elevation in plasma ALT activity, indicative of liver injury (Fig. 2). Cotreatment with WEB 2086 and Zileuton did not prevent this liver injury. The findings with ALT activity were consistent with results of histologic evaluation of liver sections. LPS administration resulted in a pronounced infiltration of neutrophils into the liver tissue and multifocal, irregularly shaped areas of midzonal hepatocellular necrosis (Fig. 3). These lesions were characterized by hyper eosinophilic parenchymal cells with small pyknotic nuclei and indistinct cytoplasmic borders. Neither LPS-induced sinusoidal neutrophilia nor hepatocellular necrosis were altered by pretreatment with WEB 2086 and Zileuton.

As reported previously (Hewett and Roth, 1995; Pearson et al., 1995), the intravenous administration of LPS resulted in a decrease in blood platelet concentration (Fig. 4) and plasma fibrinogen concentration (Fig. 5), a marker of activation of the coagulation system. Pretreatment with WEB 2086 and Zileuton did not significantly alter LPS-induced thrombocytopenia (Fig. 4) or the decrease in plasma fibrinogen concentration (Fig. 5).

4. Discussion

Results from this study suggest that PAF, alone or in combination with LTs, is not required for the thrombocytopenia, activation of the coagulation system or liver injury that follows the intravenous administration of *E. coli* LPS to rats. In preliminary studies, the effectiveness of the treatment regimens for WEB 2086 and Zileuton were verified (Bailie et al., 1995, 1996). The results indicated that these drugs effectively antagonized the actions of PAF and LT biosynthesis in vivo. Similar doses of WEB 2086 (1–10 mg/kg) have been used in another rat model in which it protected against the lethal effects of intravenous administration of 15 mg/kg *E. coli* LPS (Casals-Stenzel and Heuer, 1988), supporting the contention that the lack of effect of WEB 2086 on LPS-induced liver injury was not due to insufficient antagonism of PAF.
Our finding that blockade of PAF and LTs did not prevent liver injury in this model is in contrast to results using other models of endotoxemia. For example, pretreatment with the PAF receptor antagonist, CV 3988, in combination with the 5-lipoxygenase inhibitor, ONO-1078, prevented liver injury in the rat after LPS exposure (Yoshikawa and Goto, 1992). The contrasting results may derive from a difference in experimental models. In the study of Yoshikawa and Goto (1992), animals were exposed to a larger dose of LPS (7 mg/kg) by intraperitoneal rather than intravenous injection and liver injury was assessed 3 h, rather than 6 h later. In addition, the liver injury they observed was not as pronounced as that observed in this study. The contrasting nature of the results may be a consequence of differences in the route of administration of LPS and/or the degree of hepatocellular damage between the two studies. In addition, the differences in routes of administration of LPS are associated with temporal differences in the development of injury, since the onset of liver damage after the intravenous administration of 4 mg/kg LPS does not begin until 4 h after exposure (Pearson et al., 1995). Thus, in the study that revealed a role for PAF and LTs in liver injury after the intraperitoneal administration of LPS, hepatocellular injury was evaluated at a time before it appears in response to intravenous administration. This temporal difference suggests that liver injury in the two models arises by different mechanisms, and such a difference may underlie the divergent findings regarding the roles of PAF and LTs.

Another model of LPS-induced liver injury entails mice that are sensitized with galactosamine, a protein synthesis inhibitor. In the galactosamine/LPS model, inhibition of LT biosynthesis prevented liver injury, whereas PAF antagonism with WEB 2086 did not (Tiegs and Wendel, 1988). The contrast between this finding and ours may result from species differences. In addition, the administration of galactosamine may increase the sensitivity of hepatocytes to damage induced by LTs and may also invoke different inflammatory processes. In any case, these differences in results likely arise from biologically significant differences among animal models. If so, these findings emphasize that a specific inflammatory mediator that is critical in one model of endotoxemia may be inconsequential in another.

The administration of PAF results in neutrophil accumulation in lungs of rabbits (Worthen et al., 1983) and rats (Chang, 1994). In addition, PAF receptor antagonism affords protection against pulmonary leukocyte accumulation observed after the intrathoracic administration of E. coli LPS in guinea pigs (Bozza et al., 1994). However, in rats treated with S. enteritidis LPS, PAF blockade does not prevent pulmonary neutrophil sequestration (Chang, 1994). Thus, the role of PAF in LPS-mediated neutrophil accumulation in the lungs is model-dependent.

Compared to the lungs, little is known about possible contributions of PAF and LTs in the sequestration of neutrophils within the liver after LPS exposure. Results from this study suggest that PAF and LTs are not required for sinusoidal neutrophilia during LPS exposure in rats, since histopathologic analysis revealed larger numbers of neutrophils in livers from LPS-treated animals irrespective of pretreatment with WEB 2086 and Zileuton (Fig. 3). In this model, neutrophils appear to accumulate in the liver by a different mechanism, such as the expression of P selectin on sinusoidal endothelium (Coughlan et al., 1994).

Neutropenia is often used as an indicator of neutrophil accumulation in tissues. The role of PAF in neutropenia is controversial, inasmuch as some findings have supported a role for PAF in LPS-induced neutropenia (Coughlan et al., 1994) whereas others suggested that PAF is not involved (Okamoto et al., 1986; Rabinovici et al., 1990). Similarly, the role of LTs in LPS-induced neutropenia is unclear since inhibition of 5-lipoxygenase attenuated neutropenia in the rat after the intravenous administration of 15 mg/kg S. enteritidis LPS (Cook et al., 1985), but did not alter neutropenia in sheep after a 20 min infusion of E. coli LPS (Kuratomi et al., 1993). Our results indicate that, in the rat given LPS intravenously at a relatively small dose, neither LTs nor PAF are responsible for neutropenia. Thus, as for liver injury, the roles of PAF and LTs in LPS-induced neutropenia are highly model-dependent.
The hepatocellular damage after LPS exposure in the model we used is dependent on both platelets and an activated coagulation system (Pearson et al., 1995; Hewett and Roth, 1995). Prior to the onset of liver injury, blood platelet concentration decreases and plasma fibrinogen concentration is reduced, marking activation of the coagulation system (Pearson et al., 1995). Pretreatment with WEB 2086 and Zileuton did not alter LPS-induced thrombocytopenia or the decrease in plasma fibrinogen concentration (Fig. 4 and Fig. 5). This suggests that PAF and LTs are not critical mediators of either of these phenomena in this model. Results of studies evaluating the roles PAF and LTs in LPS-induced thrombocytopenia and activation of the coagulation system have been inconsistent. In a rat model in which LPS was infused over a 4 h period, PAF was critical for the development of thrombocytopenia and activation of the coagulation system (Imanishi et al., 1991; Imura et al., 1986). However, PAF receptor antagonism did not prevent the thrombocytopenia observed after intravenous administration of a larger dose (14.4 mg/kg) of LPS (Rabinovici et al., 1990). Similarly, contrasting results have been reported in models of endotoxemia in rabbits (Ou et al., 1994; Okamoto et al., 1986). As for liver injury, differences in species, LPS source or dose, route of administration or the time of evaluation may underlie the disparities in results.

In conclusion, our results suggest that PAF and the cysteinyl LTs are not critical mediators of the thrombocytopenia, activation of the coagulation system, hepatic neutrophil accumulation or hepatotoxicity that occurs in this model employing intravenous administration of LPS to rats. In light of other published studies of the roles of PAF and LTs, it appears that these lipid mediators contribute to pathophysiologic alterations in some, but not all animal models of endotoxemia. This emphasizes that the role and importance of a specific inflammatory mediator in the pathogenesis of tissue injury may vary with species, nature of LPS exposure or other factors that differ from one animal model to another.

Acknowledgements

R.A. Roth was supported in part by a Burroughs Wellcome Toxicology Scholar Award. M.B. Bailie was supported by an individual National Research Service Award from the NIH. This research was supported by NIH grant ES04139.

References


