Role of Platelet-Activating Factor in Pathogenesis of Galactosamine–Lipopolysaccharide-Induced Liver Injury

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In an attempt to clarify the role of platelet-activating factor (PAF) in the pathogenesis of hepatic injury induced by galactosamine (GalN) plus lipopolysaccharide (LPS), effects of WEB 2086 (PAF receptor antagonist) on hepatic injury in vivo as well as on neutrophil adherence to hepatic endothelial cells in vitro have been investigated, as we have recently clarified the role of neutrophils in this experimental model of hepatic injury. Although an enhanced serum TNF-α level after GalN-LPS administration was not reduced by WEB 2086, hepatic injury and hepatic neutrophil accumulation in the liver after GalN-LPS administration were attenuated by WEB 2086. An in vitro study revealed that an enhanced neutrophil adhesion to hepatic endothelial cells by stimulation with the sera that were collected from the GalN-LPS-treated rats, was reduced in the presence of WEB 2086 in a dose-dependent manner. In addition, LPS, TNF-α, and PAF were found to enhance the neutrophil adherence to hepatic endothelial cells, which was reduced in the presence of WEB 2086. These results suggest that PAF play an important role in the GalN-LPS induced hepatic injury and that PAF receptor antagonist reduces the neutrophil adherence to hepatic endothelial cells in the liver.

KEY WORDS: galactosamine; lipopolysaccharide; platelet-activating factor; WEB 2086; PAF receptor antagonist; liver injury.

Liver injury induced by lipopolysaccharide (LPS) administration in several experimental models is mediated by Kupffer cells (1, 2); deterioration or amelioration of hepatic injury is induced by activation or depression of Kupffer cells, respectively, indicating that activation of Kupffer cells is the major cause of liver injury in experimental animal models, but that LPS itself is not toxic (3–6). Different cell types in the liver, when incubated with LPS, did not show signs of injury, unless incubated together with Kupffer cells (6). Recent studies revealed that cytokines such as TNF-α released from macrophages play an important role in the pathogenesis of liver injury (7, 8). In addition, neutrophils are supposed to aggravate liver injury when adhering to endothelial cells and participating in releasing noxious products (9, 10).

Endotoxin is known to enhance the production of platelet-activating factor (PAF) from various cells such as neutrophils, macrophages, vascular endothelial cells, and platelets (11). PAF is an endogenously produced phospholipid mediator contributing to the pathogenesis of inflammation, endotoxin shock, and...
tissue injury induced by LPS or TNF-α (12–16). LPS is also known to induce the production of TNF-α and PAF from Kupffer cells (17). Recent studies revealed that neutrophil adhesion to vascular endothelial cells is induced by biologically active mediators such as TNF-α, PAF, and thrombin (18–23) and that neutrophil adhesion to vascular endothelial cells may play a crucial role in acute inflammatory responses (24).

WEB 2086, hetrazopine, is a PAF receptor antagonist and is found to attenuate the functions of PAF in several organs (15, 16). Since neutrophil adhesion to hepatic endothelial cells and the role of neutrophils in the pathogenesis of hepatic injury induced by GalN-LPS were elucidated in our recent study (10), the role of PAF in the pathogenesis of liver injury induced by GalN-LPS has been investigated in the present study using the PAF receptor antagonist WEB 2086.

MATERIALS AND METHODS

Experimental Animals

Male Sprague-Dawley rats (Doken, Ibaraki, Japan), 8 weeks old and weighing 250–270 g, were used. They were maintained under standard laboratory conditions with chow diet and water ad libitum. The animals received humane care in compliance with the institution’s guidelines of experimental animal care.

Treatment of Animals

Rats received intraperitoneal administration of d-galactosamine hydrochloride (GalN; 500 mg/kg rat body weight; Nakarai Chem, Kyoto, Japan) in combination with intravenously administered of lipopolysaccharide (LPS; E. coli 026; 0.1 μg/kg rat body weight; Sigma, St. Louis, Missouri). These substances were dissolved in physiological saline.

Effect of WEB 2086 on Hepatic Injury. To clarify the role of PAF in the mechanism of liver injury, rat received an intraperitoneal injection of 5 mg/kg rat body weight of WEB 2086 (a kind gift from Boehringer Ingelheim KG, Ingelheim, Germany) 1 hr before administration of GalN plus LPS.

Estimation of Liver Injury

Hepatic injury was estimated by measuring serum ALT concentrations using a transaminase kit (Wako Chem, Osaka, Japan) and by histology of the liver 24 hr after GalN-LPS administration.

Assessment of Neutrophil Kinetics in the Liver

The number of neutrophils infiltrating into the hepatic sinusoidal spaces was counted after GalN-LPS administration in control and WEB 2086-treated rats. In brief, liver specimens were perfused and fixed in 10% neutral buffered formalin (pH 7.4) at 1, 2, 6, and 24 hr after administration. Paraffin-embedded sections were stained with hematoxylin and eosin, and the number of neutrophils per 1000 hepatocytes was counted under a light microscope.

Measurement of Tumor Necrosis Factor (TNF)-α in Serum

Sera were collected from the control or WEB 2086-treated rats at 1, 2, 6, and 24 hr after GalN-LPS administration. TNF-α concentrations in sera were measured using a TNF-α ELISA kit (Genzyme, Cambridge, Massachusetts).

Neutrophil Adherence to Hepatic Endothelial Cells

Preparation of Hepatic Endothelial Cells. Hepatic sinusoidal endothelial cells were prepared from untreated rats by perfusion of the liver with 0.05% collagenase (Wako Chem.), followed by centrifugation over discontinuous metrizamide gradients (15% and 11% w/v; Sigma) at 2100 rpm for 20 min. The cells at the lower layer were collected, suspended in RPMI 1640 medium (Gibco) containing 20% fetal calf serum (FCS; Gibco) at a cell density of 1 x 10⁶ cells/ml. After removing the contaminated Kupffer cells by the dish adherence procedure at 37°C for 60 min, the nonadherent cells (0.2 ml) were seeded on collagen-coated 96-well plates (Corning 25860 COL 1; Iwaki Glass, Tokyo, Japan). After 18 hr, 90% of the cultured cells showed incorporation of fluorescent-labeled acetylated LDL as well as characteristic features of hepatic endothelial cells such as pore and sieve plates when examined by scanning electron microscopy. Contamination of Kupffer cells was less than 5% as shown by peroxidase staining.

Preparation of Rat Neutrophils. Rat neutrophils were prepared from peritoneal fluid 10 hr after an intraperitoneal injection of 10 ml of 1% casein sodium (Wako Chem.). After washing with PBS, the cells were suspended in RPMI 1640 medium at a density of 3 x 10⁶ cells/ml. Purity of the isolated neutrophils was >95% by Giemsa staining.

Preparation of Serum from GalN-LPS-Treated Rats. Serum was collected from the rats that were sacrificed at 1, 2, and 4 hr after GalN-LPS administration.

Neutrophil-Adherence Assay. Adherence of neutrophils to hepatic endothelial cells was measured according to the method of Gamble et al (25). Briefly, hepatic endothelial cells cultured in collagen-coated 96-well plates for 24 hr were incubated with neutrophils at 37°C for 30 min in the presence or absence of the 3% GalN-LPS-treated serum. After washing with medium, the number of neutrophils adhering to hepatic endothelial cells was estimated by measuring peroxidase activities of neutrophils according to the method of Henson et al (26). The number of neutrophils in the presence of 1 μg/ml of phorbol myristate acetate (Sigma) was estimated as a maximal adhesion of neutrophils, and the number of neutrophils in the presence of control serum collected from untreated rats was estimated as a background value. The assays were performed in quadruplicate, and relative adherence was calculated as follows:

$$\frac{OD_{\text{test}} - OD_{\text{background}}}{OD_{\text{PMA}} - OD_{\text{background}}} \times 100$$