Gastric Bleeding and Increased Gastric Vascular Permeability Induced by Platelet Activating Factor (PAF): Effect of Drugs that Affect Arachidonate Metabolism

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ABSTRACT—The injurious effect of platelet activating factor (PAF) on gastric mucosa was studied by measuring bleeding in the acid perfused stomach of anesthetized rats. The effect of PAF on gastric mucosal vascular permeability (GMVP) was assessed by dye-leakage in the saline perfused stomach of anesthetized rats. Intravenous infusion of PAF (100 ng/kg/min for 20 min) apparently caused gastric bleeding under the gastric luminal perfusion with 150 mM HCl solution, with the peak response at 50–70 min; biopsy indicated the presence of mucosal lesions. GMVP was markedly increased with the peak response at 20–40 min. Pretreatment with CV-3988 (1 or 10 mg/kg, i.v.), a PAF antagonist, dose-dependently blocked the PAF-induced gastric bleeding and increase in GMVP. Pretreatment with hydrocortisone acetate (20 or 40 mg/kg, s.c.) reduced PAF-induced gastric bleeding and increase in GMVP, in contrast to the aggravation by caffeic acid (1 or 5 mg/kg, s.c.). Indomethacin (1 or 5 mg/kg, s.c.) prevented PAF-induced gastric bleeding, and it depressed the increase in GMVP in the case of the lower dose. Prostaglandin E2 (50 or 500 µg/kg, s.c.) significantly reduced PAF-induced gastric bleeding, but had little effect on PAF-induced increase in GMVP. 1-Benzylimidazole (10 or 50 mg/kg, s.c.) also inhibited PAF-induced gastric bleeding and depressed the increase in GMVP at the higher dose. These results suggest that increased GMVP plays a significant role in producing the gastric damage by PAF. Changes in the mucosal level of cyclooxygenase products, especially thromboxanes, by drugs modifying the arachidonate metabolism would be closely associated with their prevention or aggravation of gastric damage induced by PAF.

Platelet activating factor (PAF), a naturally occurring phospholipid, has a wide variety of biological actions, e.g., morphological change or functional stimulation of platelets and leukocytes, hypotension, bronchoconstriction and increase in vascular permeability (1). PAF is a putative mediator of inflammation, asthma and endotoxin shock (1). Furthermore, PAF has been reported to possess a potent ulcerogenic action (2). It is also suggested to be involved in gastric ulceration by bacterial endotoxin (3, 4).

As to regards the etiology of PAF-induced gastric lesions, it was reported that PAF disturbs gastric mucosal defensive mechanisms, as demonstrated by reduction of gastric...
mucosal blood flow (5), with special reference to mechanisms involving capsaicin-sensitive fibers (6, 7). Moreover, pharmacological actions of PAF are known to have an association with arachidonate metabolism. At present, however, findings on the effects of drugs affecting arachidonate metabolism on PAF-induced gastric damage are very controversial. Glucocorticoid steroids such as dexamethasone or prednisolone prevent formation of PAF-induced gastric damage (8) or have no effect (9). Nonsteroidal antiinflammatory drugs (NSAIDs) such as indomethacin or aspirin aggravate PAF-induced gastric damage (2) or produce no change (8). Prostaglandin E₂ (PGE₂) potentiates formation of PAF-induced gastric damage (10). As a result, it remains obscure whether lipoxygenase products or cyclooxygenase products are more responsible for PAF-induced gastric damage.

The present study was carried out to clarify the ulcerogenic activity of PAF in association with arachidonate metabolism by measuring gastric bleeding in the acid perfused stomach of anesthetized rats. The principle of our method for assessing gastric damage is based on the assumption that a decrease in mucosal defensive force will be expressed as gastric bleeding under the gastric perfusion of 150 mM HCl solution which strengthens the aggressive factor. This method permits time-course analysis and quantitative evaluation of gastric damage.

PAF is known to be a potent vasoactive substance which produces an increase in vascular permeability (11). Therefore, we measured gastric mucosal vascular permeability in order to elucidate PAF-induced vascular events leading to gastric bleeding.

MATERIALS AND METHODS

Male, Wistar rats (270–350 g) were deprived of food but not water, overnight. The rats were anesthetized with urethane (1.35 g/kg, i.p.). The trachea was exposed and cannulated to ensure a free airway, and the left femoral vein was cannulated for administration of drugs. The abdomen was opened via a midline incision to expose the stomach, and the gastric contents were washed out with saline through an incision in the forestomach. Then, a push-pull cannula was fixed in the forestomach (Fig. 1). The animal was allowed to equilibrate for 2–3 hr after the operative procedures. PAF (50 or 100 ng/kg/min), lysopAF (100 ng/kg/min) or vehicle was infused via a cannula inserted into the femoral vein for 20 min.

Measurement of gastric bleeding

The stomach was perfused with 150 mM HCl solution (1 ml/min). Gastric perfusates were collected every 10 min for 3 hr, and the amount of blood contained in the perfusate was photometrically assessed by measuring the absorbance at 380 nm.

Measurement of gastric mucosal vascular permeability (GMVP)

The stomach was perfused with saline (0.3 ml/min), and pontamine sky blue (200 mg/kg) was injected intravenously 10 min before the start of PAF infusion. Gastric perfusates were collected every 10 min for 3 hr. Each fraction was alkalinized by adding 0.05 ml of 100 mM NaOH solution, and the amount of dye contained in the perfusate was photometrically assessed by measuring the absorbance at 590 nm according to the method of Whittle (12) with a slight modification.

Drug treatment

The effects of pretreatment with some drugs that affect arachidonate metabolism were studied in both the experiments on gastric bleeding and mucosal vascular permeability. CV-3988 (1 or 10 mg/kg) was intravenously infused for 10 min at 15 min before PAF infusion. Hydrocortisone acetate (20 or 40 mg/kg), caffeic acid (1 or 5 mg/kg), indomethacin (1 or 5 mg/kg), 1-benzylimidazole (10 or 50 mg/kg) and PGE₂ (50 or 500 μg/kg) were subcutaneously administered at 120, 60, 45, 30 and 30 min before PAF infusion, respectively. Drugs used in the present study
were prepared as follows: Platelet activating factor (1-O-alkyl-2-acetyl-sn-glyceryl-3-phosphocholine, Sigma) was diluted in 0.25% (w/v) bovine serum albumin (Nacalai Tesque) in saline. Lyso-PAF (1-O-alkyl-sn-glyceryl-3-phosphocholine, Sigma) was dissolved in 0.25% bovine serum albumin in saline. CV-3988 (gift from Takeda Chemical Industries) was dissolved in saline. Hydrocortisone acetate (Wako Chemicals), indomethacin (Sigma) and 1-benzylimidazole (Aldrich) were suspended in 1% carboxymethyl cellulose. Caffeic acid (Sigma) was dissolved in 2% NaHCO3 in saline. PGE2 (Sigma) was dissolved in absolute ethanol and diluted in saline.

Statistical analysis

All data are expressed as means ± S.E.M. Comparisons between groups were made by the paired t-test or by one way ANOVA followed by Dunnett’s test.

RESULTS

Gastric bleeding

Intravenous infusion of PAF (100 ng/kg/min) for 20 min apparently caused gastric bleeding under the gastric luminal perfusion with 150 mM HCl solution, with the peak response at 50–70 min (Fig. 2). Biopsy revealed the presence of hemorrhagic lesions on the gastric mucosa. In contrast, intravenous infusion of PAF (50 ng/kg/min), lyso-PAF (100 ng/kg/min) or vehicle did not appreciably change gastric bleeding and hemorrhagic lesions (Fig. 2).

GMVP

Intravenous infusion of PAF (100 ng/kg/min) for 20 min produced an apparent increase in GMVP under the gastric luminal perfusion with saline, with the peak response at 20–40 min (Fig. 3). Under this condition, gastric bleeding was not observed. However, PAF (50 ng/kg/min), lyso-PAF (100 ng/kg/min) or vehicle did not increase GMVP.
Effect of CV-3988

Pretreatment with CV-3988 (1 or 10 mg/kg, i.v.), a PAF antagonist, at 10 min before PAF infusion (100 ng/kg/min, i.v., for 20 min) blocked PAF-induced gastric bleeding (Fig. 4a) and inhibited the increase in GMVP (Fig. 4b), in a dose-related fashion.

Effect of drugs that affect to arachidonate metabolism

Pretreatment with hydrocortisone acetate (20 or 40 mg/kg, s.c.) at 120 min before PAF infusion significantly reduced PAF-induced gastric bleeding and depressed the increase in GMVP in a dose-related manner (Table 1). Pretreatment with caffeic acid (1 or 5 mg/kg, s.c.) at 60 min before PAF infusion definitely aggravated PAF-induced gastric bleeding and augmented the increase in GMVP (Table 1). Indomethacin (1 or 5 mg/kg, s.c.) at 45 min before PAF infusion prevented PAF-induced gastric bleeding and depressed the increase in GMVP when it was given at the dose of 1 mg/kg (Table 1). PGE2 (50 or 500 μg/kg,
Fig. 4. Effect of CV-3988 on PAF-induced gastric bleeding (a) and increase in GMVP (b) in rats. In the left panel, the time course of gastric bleeding or dye exudation after a 20-min infusion of PAF (100 ng/kg/min, i.v.) is illustrated. In the right panel, the total amount of blood leakage or dye exudation during a period of 3 hr after PAF infusion is shown. (○) Control (A); (□) CV-3988, 1 mg/kg, i.v. (B); and (■) CV-3988, 10 mg/kg, i.v. (C). Each value represents the mean ± S.E.M. of 4 animals. **P < 0.01, compared with the respective control.

Table 1. Effects of several drugs on gastric bleeding and dye exudation induced by intravenous infusion of PAF in rats

| Treatment                      | Dose (mg/kg) | Bleeding (% control) | Dye exudation (% control) |
|--------------------------------|--------------|----------------------|--------------------------|
| Control (PAF 100 ng/kg/min)    |              | 100.0                | 100.0                    |
| + Hydrocortisone acetate (s.c.)| 20.0         | 19.1 ± 16.7**        | 53.3 ± 6.4**             |
|                                | 40.0         | 8.7 ± 3.0**          | 11.1 ± 6.9**             |
| + Caffeic acid (s.c.)          | 1.0          | 202.9 ± 30.6**       | 150.3 ± 13.2*            |
|                                | 5.0          | 252.1 ± 35.8**       | 226.7 ± 32.8*            |
| + Indomethacin (s.c.)          | 1.0          | 55.8 ± 12.3*         | 51.5 ± 15.8*             |
|                                | 5.0          | 28.1 ± 11.1**        | 103.3 ± 17.9             |
| + Prostaglandin E2 (s.c.)      | 0.05         | 37.1 ± 8.0**         | 123.9 ± 11.0             |
|                                | 0.5          | 20.0 ± 7.2**         | 108.2 ± 27.1             |
| + 1-Benzylimidazole (s.c.)     | 10.0         | 80.7 ± 18.0          | 127.1 ± 35.4             |
|                                | 50.0         | 31.4 ± 21.0*         | 41.7 ± 14.6*             |

Values are means ± S.E.M. of 4 animals. *P < 0.05, **P < 0.01 compared with the respective control.
s.c.) at 30 min before PAF infusion significantly reduced PAF-induced gastric bleeding, but hardly affected PAF-induced GMVP (Table 1). On the other hand, 1-benzylimidazole (10 or 50 mg/kg, s.c.) at 30 min before PAF infusion inhibited PAF-induced gastric bleeding and depressed PAF-induced GMVP (Table 1).

DISCUSSION

Intravenous infusion of PAF apparently caused gastric damage and bleeding under the condition of gastric luminal perfusion with 150 mM HCl solution, with the peak response at 50–70 min. Because the gastric perfusion with 150 mM HCl in itself did not damage the gastric mucosa, treatment with PAF was considered to weaken the gastric mucosal defensive mechanisms and induce gastric mucosal lesions.

Intravenous infusion of PAF also caused a marked increase in GMVP under gastric luminal perfusion with saline. Under this condition, gastric bleeding was not observed. The GMVP was increased with the peak response at 20–40 min, preceding the gastric bleeding. It is suggested that the former is a causative factor for the latter phenomenon.

Gastric mucosal edema induced by PAF has been observed in the rat (2) and has been considered to be mainly due to the increase in GMVP. Gastric mucosal microvascular congestion (2) or reduction in gastric mucosal blood flow (5) induced by PAF in the rat has also been reported. These events were considered to be the results of hemococoncentration induced by the increase in GMVP by PAF.

Intravenous CV-3988, a PAF antagonist, blocked PAF-induced gastric bleeding and GMVP at doses of 1 and 10 mg/kg in a dose-related fashion; it prevented other PAF-induced events, e.g., hypotensive action.

It has been reported that PAF stimulates arachidonate metabolism (13), and the arachidonate metabolites cause the cell activation by PAF (14). The effects of drugs modifying the arachidonate metabolism on the PAF-induced gastric mucosal damage have been reported, but the data have not been consistent with each other, probably because of differences in dose or route of drug administration. For instance, glucocorticoid steroids such as dexamethasone or prednisolone prevent formation of PAF-induced gastric damage (8) or have no effect (9). Moreover, NSAIDs such as indomethacin or aspirin aggravate PAF-induced gastric damage (2) or produce no change (8). On the other hand, 1-benzylimidazole, an inhibitor of thromboxane (TX) synthesis, has no effect on the formation of PAF-induced gastric damage (8). PGE2 and its 16,16-dimethyl analogue potentiate the formation of PAF-induced gastric damage (10).

In the present study, caffeic acid aggravated PAF-induced gastric bleeding and GMVP. Accordingly, two possibilities arise: products of the lipoxygenase pathway prevent PAF-induced gastric damage, while products of the cyclooxygenase pathway, which are possibly increased by caffeic acid induced blockade of the lipoxygenase pathway, aggravate PAF-induced gastric damage. However, the products of lipoxygenase such as leukotrienes have been known to aggravate experimental ulcers (15); thus, it is hardly possible to prevent PAF-induced gastric damage. As a result of the lipoxygenase blockade, PGs and/or TXs which are metabolized from arachidonate in the cyclooxygenase pathway possibly aggravate PAF-induced gastric damage.

Indomethacin prevented PAF-induced gastric bleeding in a dose-related fashion, suggesting that endogenous PGs and/or TXs aggravate PAF-induced gastric damage. On the other hand, PGE2 (50 or 500 μg/kg, s.c.) and 1-benzylimidazole (10 or 50 mg/kg s.c.), a TX synthesis inhibitor, both prevented PAF-induced gastric bleeding. These findings may support the possibility that TX products of the cyclooxygenase pathway are intimately involved in the PAF-induced gastric bleeding. It is also known that local intraarterial infusion of the thromboxane mimetic U-46619 induces gastric damage, characterized as vasocongestion, disruption and hemorrhage in the rat (16). However, these considerations are not
reconciled with the finding of Wallace and Whittle (8) that 1-benzylimidazole did not affect PAF-induced gastric damage. This might largely result from the difference in the methods for evaluating gastric damage induced by PAF.

Reduction of PAF-induced gastric bleeding and increased GMVP by hydrocortisone acetate is considered to be related to inhibition of arachidonate metabolism or stabilization of membranes of lysosomes, leukocytes, etc. (17). The depression of PAF-induced gastric bleeding by hydrocortisone acetate would at least partly result from the inhibition of PAF-induced increase in GMVP.

Conclusively, it is suggested that increase in GMVP plays a significant role in producing the gastric damage by PAF. Changes in the mucosal level of cyclooxygenase products, especially TXs, by drugs modifying arachidonate metabolism would be closely associated with their prevention or aggravation of gastric damage induced by PAF.

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