BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **231,** 52–55 (1997)
ARTICLE NO. RC966041

Leukocyte Depletion and ONO-5046, a Specific Inhibitor of Granulocyte Elastase, Prevent a Stress-Induced Decrease in Gastric Prostaglandin I_2 in Rats

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Received December 20, 1996

To examine whether activated leukocytes may impair the endothelial production of prostaglandin (PG) I_2, an important cytoprotective agent in gastric mucosa, we investigated the effects of leukocyte depletion and ONO-5046, a specific inhibitor of granulocyte elastase, on the gastric level of this prostaglandin and gastric mucosal injury in rats subjected to water-immersion restraint stress (WIR). Gastric 6-keto-$PGF_1\alpha$ was increased after 30 min of WIR, followed by a decrease to below baseline after 6 h of stress. Gastric levels of 6-keto-$PGF_1\alpha$ in leukopenic animals or animals pretreated with ONO-5046 after 1 h of stress were significantly higher than those of controls, levels after 6 h of stress were not lower than those preceding stress. Leukocytopenia or ONO-5046 significantly inhibited WIR-induced gastric mucosa lesion formation. Iloprost, a stable derivative of PGI_2, prevented stress-induced lesions. These results suggest that activated leukocytes may play an important role in stress-induced gastric mucosal lesion formation by inhibiting production of PGI_2.

Prostaglandins (PGs) play an important role in preventing gastric mucosal injury. (i.e., gastric cytoprotection; 1). Among PGs, PGE_1, PGE_2, and PGI_2 have been shown to prevent gastric mucosal injury induced by various noxious stimuli (2, 3). PGI_2 is synthesized in endothelial cells and regulates physiological processes occurring at the interface between blood and endothelium (4). Since PGI_2 has vasodilatory activity (5) and inhibits platelet aggregation (6), it is possible that PGI_2 enhances gastric mucosal microcirculation, which maintains integrity of the gastric mucosa. The gastric concentration of PGI_2 is decreased in animals with water-immersion restraint stress (WIR)-induced gastric mucosal lesions (7). Zengil et al. (8) have demonstrated that iloprost, a stable derivative of PGI_2, significantly prevented gastric mucosal injury induced by WIR in rats. This suggests that decreased gastric PGI_2 may be an important factor in the development of WIR-induced gastric mucosal lesions. Leukocytes are involved in the development of gastric mucosal injury induced by stress (9), but the mechanism by which activated leukocytes contribute to lesion formation is not fully known. Since activated leukocytes can release a variety of inflammatory mediators, including granulocyte elastase and reactive oxygen species that are capable of damaging the adjacent endothelial cells (10-12), activated leukocyte-induced endothelial cell injury could lead to a microcirculatory disturbance that encourages mucosal lesion formation. Since PGI_2 is synthesized by gastric mucosal endothelial cells (4), activated leukocyte-induced endothelial cell damage may decrease the production of protective PGI_2. The present study was conducted to examine whether activated leukocytes impair the endothelial production of PGI_2, thereby contributing to gastric mucosal injury in rats subjected to WIR.

MATERIALS AND METHODS

Materials

Nitrogen mustard was purchased from Sigma Chemical Co. (St. Louis, MO). ONO-5046 (N-[2-(2,2-dimethylpropionyloxy) phenylsulfonylamino] amino acetic acid) was kindly supplied by Ono Pharmaceutical Co. (Osaka, Japan). Iloprost was kindly supplied by Eizai Pharmaceutical Co. (Tokyo, Japan).

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Abbreviations: PG, prostaglandin; WIR, water-immersion restraint stress; PGI_2, prostaglandin I_2 NM, nitrogen mustard.

0006-291X/97 $25.00

Methods

Water-immersion restraint stress (WIR)-induced gastric mucosal injury in rats. Male Wistar rats weighing 280-320 g were obtained from Nihon SLC (Hamamatsu, Japan) were used in each experiment. The care and handling of the animals were in accordance with the National Institutes of Health guidelines. All experimental procedures described below were approved by the Kumamoto University Animal Care and Use Committee. Before each experiment, rats were deprived of food, but not water, for 18-22 h. Then the animals were placed in a restraint cage and immersed up to the xiphoid process in water at 22°C as described previously (13). After 6 h of WIR, animals were anesthetized by an intraperitoneal injection of sodium pentobarbital (50 mg/kg) and exsanguinated via the abdominal aorta. Their stomachs were removed, inflated with 10 mL of 1% formalin, and immersed in 1% formalin for 24 h. Stomachs then were cut along the greater curvature and examined for mucosal lesions. Since most gastric mucosal lesions were linear and almost always less than 2 mm wide, the total length (mm) of each linear hemorrhagic erosion was measured as the lesion index by an observer blinded to previous treatment (14).

Reduction in the number of circulating leukocytes by nitrogen mustard. Rats were made leukocytopenic by the intravenous injection of nitrogen mustard under light ether anesthesia, at a dose of 1 mg/kg for 2 days prior to the day of the experiment (15). Controls were similarly injected but with normal saline. Blood was collected from the tail vein under light ether anesthesia immediately before stress, smeared on a glass slide, and stained with Wright-Giemsa stain. The slides were coded to avoid observer bias and examined under a light microscope with a × 100 objective. The mean leukocyte count in animals with nitrogen mustard treatment [n = 6, $2.72 \pm 0.22 \times 10^3$ cells/mL (mean ± SD), $P<.01$] was decreased compared with that in control animals (n = 6, $13.22 \pm 1.79 \times 10^3$ cells/mL).

Administration of ONO-5046 to rats subjected to WIR. ONO-5046 was dissolved in bicarbonate-buffered saline and injected intraperitoneally at a dosage of 300 mg/kg as described previously (16). Control animals received intraperitoneal injections consisting of a similar quantity of bicarbonate-buffered saline.

Administration of iloprost to rats subjected to WIR. Iloprost was continuously infused (100 ng/kg/min) via the jugular vein for 6 h after placement of a polyethylene tube under light ether anesthesia. Control animals received normal saline instead of iloprost.

Determination of gastric 6-keto-PGF$_1\alpha$ levels. Gastric levels of 6-keto-PGF$_1\alpha$, a stable metabolite of PGI_2, were determined before and during WIR as described previously (17) by using a specific enzyme immunoassay kit (Amersham, Buckinghamshire, UK). Results were expressed as μg of 6-keto-PGF$_1\alpha$/g tissue. All data are expressed as the mean ± SD. Comparisons among different groups of data were performed using analysis of variance with Scheffé's (post hoc) test for group pairs for multiple comparisons and the unpaired t-test for single comparisons. A P value, less than .05 was considered significant.

RESULTS

Changes in the Gastric 6-Keto-PGF$_1\alpha$ Level and Gastric Lesion Index in Rats Subjected to WIR

Gastric levels of 6-keto-PGF$_1\alpha$ (Fig. 1A) were significantly increased 30 min after WIR compared to pre-WIR levels. The levels began to decrease after 1 h of WIR, and were less than prestress levels after 6 h of WIR (Fig. 1A). The gastric lesion index began to increase after 4 h of WIR, peaking after 6 h of stress (Fig. 1B).

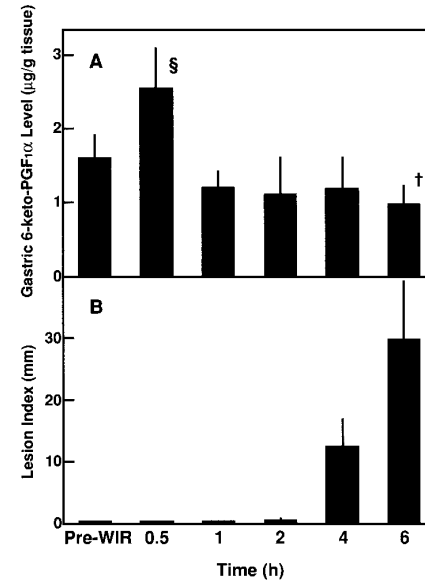

FIG. 1. Changes in the gastric 6-keto-PGF$_1\alpha$ level (A) and gastric lesion index (B) in rats subjected to stress. Changes in the gastric 6-keto-PGF$_1\alpha$ level (A) and gastric lesion index (B) were determined before water-immersion restraint stress (WIR) and after stress at the time points indicated. Results are expressed as the mean ± SD derived from six animal experiments. §, $p<.01$ vs. the pre-WIR values. †, $p<.05$ vs. the pre-WIR values.

Effects of Leukocytopenia and ONO-5046 on Changes in Gastric 6-Keto-PGF$_1\alpha$ Levels in Rats Subjected to WIR

Although prestress levels of 6-keto-PGF$_1\alpha$ were not significantly different in rats with nitrogen mustard-induced leukocytopenia and pretreated with ONO-5046 from those of control animals, the gastric levels after 1 h of WIR were significantly higher than those of controls (Fig. 2). While gastric levels of 6-keto-PGF$_1\alpha$ were lower than prestress levels after 6 h of WIR in controls, the levels at 6 h were not lower than the prestress levels in animals with leukocytopenia and pretreated with ONO-5046 (Fig. 2).

Effects of Leukocytopenia, ONO-5046, and Iloprost on WIR-Induced Gastric Mucosal Lesion Formation in Rats

Gastric mucosal injury after 6 h of WIR in control animals was significantly worse than in animals protected by nitrogen mustard-induced leukocytopenia and administration of ONO-5046 (Fig. 3). Continuous 6-h intravenous infusion of iloprost (100 ng/kg/min), a

stable derivative of PGI_2, prevented the gastric mucosal lesion formation after 6 h of WIR (Fig. 3).

DISCUSSION

Our controls, the gastric level of 6-keto-$PGF_1\alpha$ was found significantly higher than the prestress level after 30 min of WIR, and decreased to a level significantly lower than prestress level after 6 h of WIR. At this time the gastric mucosal lesion was evident. Consistent with these observations is a report by Hamajima et al. (7) demonstrating that the content of 6-keto-$PGF_1\alpha$ in gastric mucosa of rats was significantly decreased 6 h after WIR. Administration of iloprost, a stable derivative of PGI_2, significantly lessened gastric mucosal lesion severity, suggesting that the decrease in the gastric level of PGI_2 might be a cause of stress-induced gastric mucosal lesion formation. The rapid decrease of gastric levels of 6-keto-$PGF_1\alpha$ from the maximum observed at 1 h of WIR was inhibited by nitrogen mustard-induced leukopenia or administration of ONO-5046. Furthermore, leukopenia and ONO-5046 prevented the marked decrease in the gastric level of 6-keto-$PGF_1\alpha$ after 6 h of WIR, suggesting that the activated leukocytes might contribute to the decrease in the endothelial production of PGI_2 in gastric mucosa. Since inflammatory mediators derived from activated leukocytes damage endothelial cells (10-12), they may be responsible for the inhibition of PGI_2 production during stress. Consistent with this notion is a report by LeRoy et al. (18) demonstrating that neutrophil elastase inhibits production of PGI_2 by cultured porcine aortic endothelial cells in response to extracellular ATP. Weksler et al. (19) also demonstrated that neutrophil elastase specifically suppressed thrombin-induced PGI_2 production by cultured human umbilical vein endothelial cells.

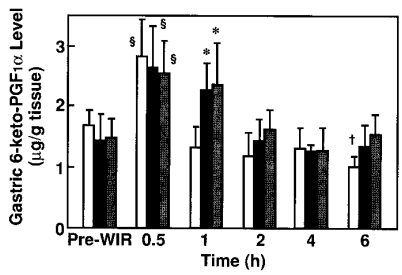

FIG. 2. Effects of nitrogen mustard-induced leukocytopenia and ONO-5046 on changes in the gastric 6-keto-$PGF_1\alpha$ level in stressed animals. Nitrogen mustard was intravenously administered to rats 2 days prior to water-immersion restraint stress (WIR) as described in Materials and Methods. ONO-5046 was administered intraperitoneally to rats 30 min prior to WIR. Gastric levels of 6-keto-$PGF_1\alpha$ were determined in control animals (□), in those with nitrogen mustard-induced leukocytopenia (■), and in those with ONO-5046 (▨) subjected to WIR. Results are expressed as the mean ± SD derived from six animal experiments. §, $p<.01$ vs. the pre-WIR values. †, $p<.05$ vs. the pre-WIR values. *, $p<.01$ vs. the values of control animals.

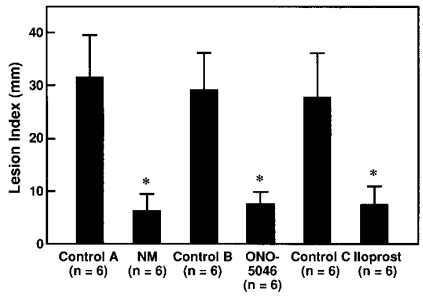

FIG. 3. Effects of leukocyte depletion, ONO-5046, and iloprost on the gastric mucosal injury in stressed rats. Nitrogen mustard (NM) (1 mg/kg) was administered intravenously to rats 2 days prior to the experiments. ONO-5046 was injected intraperitoneally to rats 30 min prior to the experiments. Iloprost was infused continuously at a rate of 100 ng/kg/min for 6 h. Control animals in group A (control A) received saline instead of nitrogen mustard. Control animals in group B (control B) received bicarbonate-buffered saline instead of ONO-5046. Control animals in group C (control C) received the same volume of saline without iloprost for 6 h during water-immersion restraint stress (WIR). After 6 h of WIR, the lesion index was determined as described in the Materials and Methods. Each bar represents the mean ± SD. *, $p<.01$ vs. the values of each control animal group.

Since PGI_2 plays an important role in preventing gastric mucosal injury by increasing gastric mucus secretion, bicarbonate production, and mucosal blood flow (3, 20), impairment by activated leukocytes of the endothelial production of PGI_2 may contribute to mucosal lesion formation. These observations suggest that inhibition of activated leukocytes by therapeutic agents may join inhibition of gastric acid secretion by antiulcer agents in the treatment of gastric mucosal injury.

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