Nitric Oxide Donating Compounds Inhibit HCl-Induced Gastric Mucosal Lesions Mainly via Prostaglandin

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ABSTRACT—Prostaglandin (PG) and nitric oxide (NO) have been known to inhibit the lesion formation induced by necrotic agents. However, no clear correlation between PG and NO has been shown in the gastroprotective action against necrotic agent-induced gastric mucosal lesions in rats. Thus, the present study was performed to clarify this correlation. Gastric mucosal lesions were induced by the oral administration of 0.6 M HCl in rats. 16,16-Dimethyl PGE\textsubscript{2} (0.3 – 3 mg/kg, p.o.; dim-PGE\textsubscript{2}), sodium nitrite (0.3 and 1 mg/kg, s.c.) and sodium nitroprusside (30 and 100 mg/kg, i.v.; SNP) dose-dependently inhibited the lesion formation. Orally administered sodium nitrite or SNP (3 mg/kg) also significantly inhibited the lesion formation. The gastroprotective action by dim-PGE\textsubscript{2} was not affected by the pre-treatment with N\textsuperscript{G}-nitro-L-arginine methylester (10 mg/kg, i.v.). The gastroprotective effect by sodium nitrite or SNP was markedly attenuated by the pre-treatment with indomethacin (10 mg/kg, s.c.). These findings suggest that NO donating compounds inhibit the HCl-induced mucosal lesions mainly through prostaglandin, but dim-PGE\textsubscript{2} directly inhibits the lesions without involvement of NO in rats.

Keywords: Nitric oxide, Prostaglandin, Gastric mucosal lesion

Gastric mucosal integrity is maintained by many factors, including blood flow, mucus and bicarbonate. Prostaglandins (PG)s, in particular, play an important role in the defensive mechanism (1 – 3). Robert et al. reported that the administration of PG inhibited the lesion formation induced by necrotic agents such as HCl, NaOH or boiling water (4, 5). On the other hand, nitric oxide (NO) also plays many important roles in the stomach (6 – 8). Exogenous administration of NO inhibits the lesion formation induced by necrotic agents (9, 10). These findings suggest that PG and NO play important roles to maintain the integrity of the gastric mucosa.

Recently, many papers suggested the correlation between PG and NO (11 – 14). Uno et al. reported that NO stimulated syntheses of PGs, such as PGE\textsubscript{2} and PGI\textsubscript{2}, in cultured rabbit gastric cells (11). Whittle et al. also reported that endogenous NO formed from L-arginine, acting in concert with PGI\textsubscript{2} and sensory neuropeptides, had a role in modulation of gastric mucosal integrity in anesthetized rats (13). However, there are no reports evaluating the correlation between exogenous NO and PG in the genesis of gastric lesions by necrotic agents in conscious rats.

The present study, therefore, was performed to clarify the correlation between NO and PG in gastric mucosal protection by using the HCl-induced gastric mucosal lesion model in rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats weighing about 250 g were purchased from Charles River (Atsugi) and used after fasting for 24 h, but given drinking water ad libitum.

Gastric lesion formation and its evaluation

Gastric lesions were induced by the oral administration of 0.6 M HCl in a volume of 0.5 ml/100 g body weight. The length of the erosive lesions was measured 1 h after HCl treatment, and the total length of the lesions was taken as the lesion index (mm). Effects of 16,16-dimethyl prostaglandin E\textsubscript{2} (dim-PGE\textsubscript{2}), sodium nitrite and sodium nitroprusside (SNP) on the gastric lesion

Dim-PGE\textsubscript{2} at doses of 0.3 – 3 mg/kg body weight was administered orally 15 min before HCl treatment in the volume of 0.5 ml/100 g body weight. Sodium nitrite at
doses of 0.3 and 1 mg/kg body weight was administered subcutaneously 30 min before HCl treatment in the volume of 0.1 ml/100 g body weight. SNP at doses of 30 and 100 μg/kg was administered intravenously 15 min before HCl treatment in the volume of 0.1 ml/100 g body weight.

**Effect of N^6^-nitro-L-arginine methylester (L-NAME) on the gastroprotective effect by dim-PGE_2**

Effect of L-NAME was evaluated on the gastroprotective effect by dim-PGE_2. L-NAME was administered intravenously 30 min before HCl treatment in the volume of 0.1 ml/100 g body weight. Dim-PGE_2 at doses of 0.3–3 μg/kg body weight was administered orally 15 min before HCl treatment in the volume of 0.5 ml/100 g body weight.

**Effect of indomethacin on the gastroprotective effect by sodium nitrite or SNP**

Effect of indomethacin was evaluated on the gastroprotective effect by sodium nitrite or SNP. Indomethacin at a dose of 10 mg/kg body weight was administered subcutaneously in the volume of 0.1 ml/100 g body weight 1 h before sodium nitrite or SNP treatment. Sodium nitrite at the dose of 1 mg/kg was administered subcutaneously 30 min before HCl treatment, and SNP at a dose of 100 μg/kg body weight was given intravenously 15 min before HCl treatment.

**Effect of sodium nitrite or SNP administered orally on the gastric lesion and effect of indomethacin on the gastroprotective effect by sodium nitrite or SNP**

Sodium nitrite or SNP at a dose of 3 mg/kg body weight was administered orally 30 min before HCl treatment in the volume of 0.5 ml/100 g body weight. In these rats, vehicle (1% gum arabic solution) was administered subcutaneously 1 h before sodium nitrite or SNP treatment in the volume of 0.1 ml/100 g body weight.

To evaluate the effect of indomethacin on the gastroprotective effect by sodium nitrite or SNP, indomethacin at a dose of 10 mg/kg body weight was administered subcutaneously in the volume of 0.1 ml/100 g body weight 1 h before sodium nitrite or SNP treatment.

**Materials**

Sodium nitrite, SNP, L-NAME and indomethacin were purchased from Sigma (St. Louis, MO, USA) and dim-PGE_2 purchased from Nacalai Tesque (Tokyo). Sodium nitrite, SNP and L-NAME were dissolved in water for injection. Dim-PGE_2 was dissolved in a small amount of ethanol and diluted with water for injection at appropriate concentration. Indomethacin was suspended with 1% gum arabic solution. In the control groups, the vehicles were treated in the same way as described above.

**Statistical analyses**

Statistical analysis was performed by Dunnett's multiple comparison test and Student's t-test, and P<0.05 was taken as significant.

**RESULTS**

Gastric erosive lesions were induced by the oral administration of 0.6 M HCl along the gastric mucosal folds.

**Effects of dim-PGE_2, sodium nitrite and SNP on the gastric lesion**

The effect of dim-PGE_2 is shown in Fig. 1. In the control group, the lesion index was 93.3 ± 7.3 mm. Dim-PGE_2 dose-dependently and significantly inhibited the lesion formation at all doses used.

The effect of sodium nitrite administered subcutaneously is shown in Fig. 2. In the control group, the lesion index was 68.8 ± 18.4 mm. Sodium nitrite inhibited the lesion formation and a significant difference was observed at a dose of 1.0 mg/kg.

The effect of SNP administered intravenously is shown in Fig. 3. In the control group, the lesion index was 122 ± 19.5 mm. SNP inhibited the lesion formation and a significant difference was observed at a dose of 100 μg/kg.

**Effects of L-NAME on the gastroprotective effect by dim-PGE_2**

Effect of L-NAME on the gastroprotective effect by dim-PGE_2 is shown in Fig. 4. In the control group, the lesion index was 112 ± 15.7 mm, which is not significantly different from the L-NAME non-treated control shown in Fig. 1.
Dim-PGE\textsubscript{2} dose-dependently and significantly inhibited the lesion formation even under the treatment with L-NAME.

**Effect of indomethacin on the gastroprotective effect by sodium nitrite or SNP**

Effect of indomethacin on the gastroprotective effect by sodium nitrite administered subcutaneously is shown in Fig. 5. In the vehicle-treated control, the lesion index was 81.1 ± 10.6 mm. Sodium nitrite at a dose of 1.0 mg/kg significantly inhibited the lesion formation ($P<0.05$). Pre-treatment with indomethacin aggravated the lesion formation as compared with the vehicle-treated control. However, no significant difference was observed as compared with the vehicle-treated control. Indomethacin treatment almost completely attenuated the gastroprotective effect by sodium nitrite.

Effect of indomethacin on the gastroprotective effect by SNP administered intravenously is shown in Fig. 6. In the vehicle-treated control, the lesion index was...
Effect of sodium nitrite or sodium SNP administered orally on the gastric lesion and effects of indomethacin on the gastroprotective effect by sodium nitrite or SNP

In the vehicle-treated control, the lesion index was 68.8 ± 12.6 mm. Sodium nitrite or SNP significantly inhibited the lesion formation (Fig. 7; \( P<0.05 \) or \( P<0.01 \), respectively).

In the indomethacin-treated control, the lesion index was 58.0 ± 8.2 mm, which was not significantly different from that in the vehicle-treated control. Indomethacin treatment almost completely attenuated the gastroprotective action by sodium nitrite. In the SNP-treated group, the gastroprotective effect by SNP was markedly attenuated by the pretreatment with indomethacin (Fig. 7, \( P<0.05 \)), although a significant difference was observed as compared with the indomethacin-treated control at \( P<0.05 \).

DISCUSSION

For gastric mucosal integrity, PGs play an important role (1 – 3, 15), and the administration of PGs inhibits lesion formation induced by necrotic agents such as ethanol, HCl, NaOH, hypertonic NaCl or boiling water (4, 5). We also reported the inhibitory effect by PGE\(_2\) on ethanol- or HCl-induced gastric lesions in rats (16).

Previously, we reported that capsaicin inhibited the gastric erosive lesions induced by absolute ethanol, and this gastroprotective effect was attenuated by the pre-treatment with indomethacin (6). This finding suggests the involvement of PG even on the inhibitory effect of capsaicin.

87.9 ± 12.9 mm. SNP at a dose of 100 \( \mu g / kg \) significantly inhibited the lesion formation (\( P<0.05 \)). Pre-treatment with indomethacin did not significantly affect the lesion formation. Indomethacin treatment almost completely attenuated the gastroprotective action by SNP.
against absolute ethanol-induced gastric lesions formation in rats. Also in this study, dim-PGE₂ significantly inhibited the lesion formation induced by HCl.

NO is synthesised from L-arginine by NO synthase, and NO synthase exists as two isozymes. One is constitutive NO synthase (c-NOS) (7, 8, 17) and the other is inducible NO synthase (i-NOS) (18). Various cells such as endothelial cells, neurons and mucosal cells contain c-NOS (7).

NO plays many important roles in the stomach including regulation of microcirculation, gastric motility, mucus and acid secretion (19 – 21). Brown et al. reported that the generators of NO stimulated mucus secretion by rat gastric mucosal cells, suggesting the role for NO in mediation of gastric mucous release (20). Kitagawa et al. reported the gastric protective action by sodium nitrite, an NO donor, against the gastric lesions induced by HCl in rats (9). Lopez-Belmonte et al. reported that glycercyl trinitrate, which liberates NO on metabolic transformation, and S-nitroso-N-acetyl-penicillamine, which spontaneously liberates NO, inhibited the lesion formation by endothelin 1 in pentobarbitone-anesthetized rats (10). In the present study, we used sodium nitrite and SNP as NO donating compounds, and these compounds also significantly inhibited the HCl-induced gastric lesion formation in rats. This phenomenon was observed even in the case of sodium nitrite or SNP being administered orally. These findings clearly demonstrate that NO inhibits gastric lesion formation, although the experimental conditions were different from each other.

Recently, Uno et al. reported that NO stimulated synthesis of PGs, such as PGE₂ and PGI₂, in cultured rabbit gastric cells (11). These PGs have been known to inhibit gastric lesions in rats. Tepperman and Whittle reported that endogenous NO and sensory neuropeptides interacted in the modulation of the rat gastric microcirculation (22). We reported that the gastroprotective effect by capsaicin was significantly inhibited by indomethacin or capsaicin-sensitive afferent nerves (6). On the contrary, Tetsuka et al. reported that endogenous PGE₂ down-regulated i-NOS, although the NOS subtype was different from c-NOS (12). These reports showed that some correlation between nitric oxide and PG may exist. However, there is no reports evaluating the correlation between exogenous NO and PG on the genesis of gastric lesions induced by a necrotic agent in conscious rats.

In this study, the gastroprotective effect by dim-PGE₂ against HCl-induced gastric lesions was not affected by the pre-treatment with l-NAME. This finding clearly shows that dim-PGE₂ directly exhibits the gastroprotective action without involvement of NO. On the contrary, the gastroprotective effects by parenterally administered sodium nitrite or SNP and orally administered sodium nitrite were almost completely attenuated by the pre-treatment with indomethacin, suggesting the involvement of PG on the gastroprotective action by NO donating compounds such as sodium nitrite or SNP. However, in the case of orally administered SNP, the gastroprotective effect remained a partial one even under the pretreatment with indomethacin. Therefore, these findings suggest that NO-donating compounds inhibit gastric lesions induced by HCl mainly through PG.

Whittle et al. reported that endogenous NO formed from L-arginine, acting in concert with PGI₂ and sensory neuropeptides, has a role in the modulation of gastric mucosal integrity, although the rats were anesthetized (13). Salvemini et al. also reported that NO directly interacted with COX to cause an increase in the enzymatic activity (14). In addition, Uno et al. also reported that NO stimulated prostaglandin synthesis in cultured rabbit gastric cells (11). These reports are consistent with our results, although the experimental conditions were different from each other.

To clarify the difference in the mechanism of the gastroprotective effect by PGs or NO, the effect of dim-PGE₂ or SNP on the gastric mucosal blood flow was investigated in urethane-anesthetized rats. However, there was no difference between dim-PGE₂ and SNP on the increasing action in gastric mucosal blood flow (data not shown).

In the present study, dim-PGE₂ inhibited the lesions induced by HCl, but this gastroprotective action was not affected by the pre-treatment with L-NAME. Sodium nitrite or SNP also inhibited lesion formation, and these gastroprotective effects were completely attenuated by the pre-treatment with indomethacin. In conclusion, the above findings suggested that nitric oxide inhibited the HCl-induced gastric lesions mainly through PG, and PGE₂ directly inhibits the lesions without involvement of NO in rats.

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