Cytoprotective Effects of NC-1300 and Omeprazole on HCl-Ethanol-Induced Gastric Lesions in Rats

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Abstract—NC-1300 (10–100 mg/kg), given p.o. at 0.5, 6, 12 or 24 hr before HCl-ethanol, dose-dependently protected the rat gastric mucosa. This protection was observed even when the gastric contents had been removed before application of HCl-ethanol. NC-1300 (30 mg/kg), given i.p., was without effect on lesion formation in a dose which potently inhibited gastric acid secretion in pylorus-ligated rats. Pretreatment with indomethacin (5 mg/kg, s.c.) resulted in no reduction in the protection by NC-1300, excluding the possible participation of endogenous prostaglandins in the protective mechanism. N-ethylmaleimide pretreatment (10 mg/kg, s.c.) slightly reduced the protective activity of NC-1300, suggesting the partial participation of endogenous sulfhydryl compounds in the NC-1300 protection. NC-1300 sulfide and mercaptobenzimidazole (compounds obtained after mixing NC-1300 with acidic solution) also dose-dependently protected against HCl-ethanol-induced lesions when given p.o. at 0.5 hr before HCl-ethanol. The protection was significant but was considerably reduced in contrast to NC-1300 when the compounds were given 12 hr beforehand. NC-1300 sulfone had no effect on lesion formation. Omeprazole (10, 30 mg/kg), given p.o., also dose-dependently inhibited HCl-ethanol-induced lesions. However, the duration of protection was shorter than that seen with NC-1300, i.e., the effect disappeared 12 hr later. Thus, NC-1300 has a potent and long-lasting activity on HCl-ethanol-induced gastric lesions. The mechanism by which this occurs remains unknown.

We recently reported that NC-1300, a new proton pump inhibitor, is a potent inhibitor of gastric acid secretion in rats and that this action persisted for more than 24 hr after a single p.o. administration (1). In addition, the compound markedly protected the gastric mucosa against injury induced by pylorus-ligation, water-immersion stress, aspirin and indomethacin, and the duodenal mucosa against mepirizole. The mechanism by which NC-1300 inhibits these lesions appears to relate to its antisecretory property.

There are reports that proton pump inhibitors such as omeprazole or timoprazole protect the gastric mucosa against necrotizing agents, including ethanol, HCl, or boiling water (2–4). These findings indicate that such compounds have cytoprotective activity which is unrelated to their anti-

secretory effects. The present study was designed to determine if NC-1300 has also a cytoprotective activity on HCl-ethanol-induced gastric lesions in rats. NC-1300 was mixed in acidic solution and three major compounds were obtained (NC-1300-sulfide, mercaptobenzimidazole, NC-1300-sulfone) (Fig. 1). The effects of these materials on HCl-ethanol-induced lesions were also studied in comparison with those obtained with NC-1300. Omeprazole, an established proton pump inhibitor (5), was used as a reference drug.

Materials and Methods

Male Sprague-Dawley rats (230–270 g, Charles-River Japan) were deprived of food for 24 hr before the experiments. Water was given freely for the initial 22 hr, but was

who determined the lesions had no knowledge of which treatment the animals had been given.

Gastric secretion: Antisecretory activities of NC-1300 and omeprazole were studied using pylorus ligation preparations. Under ether anesthesia, the abdomen was incised, the pylorus ligated, and the animals killed 3 hr later. The gastric contents were collected and analyzed for volume and acidity. Acidity was determined by automatic titration of the gastric juice against 0.1 N NaOH to pH 7.0 (Autoburette). Titratable acid output was expressed as $\mu\text{Eq/hr}$. NC-1300, omeprazole, or the vehicle alone was given p.o. or i.p. at 10 or 30 mg/kg at 0.5 hr before ligating the pylorus.

Drugs: NC-1300, Sulfide, Sulfone and omeprazole were all gifts from Nippon Chemiphar and MBI was purchased from Janssen.

Analysis of data: Student's *t*-test was used to determine the statistical significance of the data, and a $P < 0.05$ value was regarded as significant. All data represent the

mean $\pm$ one S.E.M.

Results

Effects of NC-1300 and omeprazole on HCl-ethanol-induced gastric lesions: In all the control rats given the vehicle alone and HCl-ethanol p.o., severe mucosal lesions developed in the glandular stomach 1.5 hr later. These lesions consisted of elongated bands of necrosis. The mean lesion length was 99.8 ± 9.0 mm. Pretreatment with NC-1300 (3–30 mg/kg), p.o., at 0.5 hr before the HCl-ethanol administration dose-dependently inhibited HCl-ethanol-induced lesions (Fig. 2). With a dose of 30 mg/kg, lesion formation was almost completely inhibited. Omeprazole (30 mg/kg) also significantly inhibited the lesion formation by 74.3%. Neither NC-1300 nor omeprazole (30 mg/kg) protected the surface epithelium of rat gastric mucosa against necrosis induced by HCl-ethanol as determined histologically. However, the deeper layer of the gastric mucosa was protected from HCl-ethanol-induced damage. Pretreatment with NC-

Fig. 2. Effects of NC-1300 and omeprazole on HCl-ethanol-induced gastric lesions in rats. Each compound was given p.o. 0.5 hr before HCl-ethanol administration in a volume of 1 ml/200 g body weight. Animals were killed 1.5 hr after HCl-ethanol administration. Both compounds dose-dependently protected the gastric mucosa from HCl-ethanol-induced gastric lesions. Data represent the mean $\pm$ one S.E.M.

Fig. 3. Effects of NC-1300 and omeprazole on HCl-ethanol-induced gastric lesions in rats. Each compound was given p.o. 6, 12 or 24 hr before HCl-ethanol administration. Note that the protective effect of NC-1300 on HCl-ethanol-induced gastric lesions was evident even 24 hr after a single administration. Data represent the mean $\pm$ one S.E.M.

Fig. 4. Gross appearance of the stomachs of rats treated with the vehicle alone (left) or NC-1300 (right). NC-1300 was given p.o. 12 hr before HCl-ethanol administration. Animals were killed 1.5 hr after HCl-ethanol administration. Protection of the gastric mucosa by NC-1300 was nearly complete, indicating the long-lasting activity of the compound.

1300 (30 mg/kg), p.o., at 6 or 12 hr before HCl-ethanol administration also significantly inhibited lesion formation (Figs. 3 and 4), the inhibition being practically complete or at least 85.6%, respectively. Even 24 hr after the administration of 30 mg/kg, there was a 37.3% inhibition of HCl-ethanol-induced lesions. It seems remarkable that an almost total protection of the gastric mucosa against HCl-ethanol-induced lesions was apparent even 24 hr after the p.o. administration of NC-1300 (100 mg/kg). Omeprazole (30 mg/kg), given 6 hr before HCl-ethanol administration, also inhibited the lesion formation by 52.6%. However, the protection by omeprazole disappeared when given 12 hr beforehand.

Influence of dilution by gastric contents: Removal of the gastric contents 0.5 hr after NC-1300 (10 or 30 mg/kg) or omeprazole (10 or 30 mg/kg) administration did not influence the protective activity of these compounds on HCl-ethanol-induced lesions (Fig. 5). Rather, a significant protection was observed in the dose of 10 mg/kg of each compound, i.e., the inhibition was 73.0%

with the NC-1300 treatment and 52.9% with the omeprazole treatment.

Effects of i.p. administration: The i.p. administration of NC-1300 (10 or 30 mg/kg) and omeprazole (10 or 30 mg/kg) at 0.5 hr before HCl-ethanol administration had little or no effect on the lesion formation (Fig. 6).

Effects of NC-1300 and omeprazole on gastric secretion: The p.o. administration of NC-1300 (10 and 30 mg/kg) had no effect on the volume of gastric contents, but did significantly inhibit gastric acid output for 3 hr (Fig. 7). However, the i.p. administration of NC-1300 (10 and 30 mg/kg) significantly inhibited both the volume and acid output. Omeprazole (10 or 30 mg/kg), whether given p.o. or i.p., also significantly and dose-dependently inhibited gastric acid output for 3 hr. In addition, the volume was also significantly reduced when given either p.o. (10 mg/kg) or i.p. (10 or 30 mg/kg). Gross inspection of gastric samples after the p.o. or i.p. treatment with either NC-1300 or omeprazole (30 mg/kg) indicated an apparent increase in viscosity of the gastric contents.

Fig. 5. Effects of NC-1300 and omeprazole on HCl-ethanol-induced gastric lesions in rats. The gastric contents of rats were removed 0.5 hr after the p.o. administration of each compound. HCl-ethanol was given immediately after removal of the gastric contents. It was evident that the protective effects of NC-1300 and omeprazole were not caused by dilution of HCl-ethanol with gastric contents. Data represent the mean $\pm$ one S.E.M.

Effects of indomethacin and N-ethylmaleimide pretreatment on NC-1300 and omeprazole protection: Pretreatment with indomethacin (5 mg/kg) had no influence on the development of HCl-ethanol-induced gastric lesions (Fig. 8). Protective effects of

Fig. 6. Effects of NC-1300 and omeprazole on HCl-ethanol-induced gastric lesions in rats. Each compound was given i.p. 0.5 hr before HCl-ethanol administration. Neither NC-1300 nor omeprazole showed any protective effects on the lesion formation. Data represent the mean $\pm$ one S.E.M.

Fig. 7. Effects of NC-1300 and omeprazole on gastric secretion in pylorus-ligated rats. Each compound was given either p.o. or i.p. 0.5 hr before pylorus ligation. Animals were killed 3 hr after pylorus ligation. Both compounds dose-dependently inhibited gastric acid output regardless of the route of administration. Data represent the mean $\pm$ one S.E.M.

NC-1300 (10 or 30 mg/kg) and omeprazole were not affected by pretreatment with (10 or 30 mg/kg) on the lesion formation indomethacin. Pretreatment with N-ethyl-

Fig. 8. Effects of indomethacin pretreatment on the protective effects of NC-1300 and omeprazole against HCl-ethanol-induced gastric lesions in rats. Indomethacin was given s.c. at 5 mg/kg 1 hr before NC-1300 or omeprazole administration. The protective effects of both compounds were not altered by the pretreatment with indomethacin. Data represent the mean $\pm$ one S.E.M.

Fig. 9. Effects of N-ethylmaleimide pretreatment on the protective effects of NC-1300 and omeprazole against HCl-ethanol-induced gastric lesions in rats. N-ethylmaleimide was given s.c. at 10 mg/kg 1 hr before NC-1300 or omeprazole administration. The protective effects of both compounds were slightly reduced by pretreatment with N-ethylmaleimide. Data represent the mean $\pm$ one S.E.M.

Fig. 10. Effects of NC-1300, Sulfide, MBI and Sulfone on HCl-ethanol-induced gastric lesions in rats. Each compound was given 0.5 hr before HCl-ethanol administration. NC-1300, Sulfide and MBI dose-dependently protected the gastric mucosa against HCl-ethanol-induced gastric lesions, but Sulfone had no effect on the lesion formation. Data represent the mean $\pm$ one S.E.M.

Fig. 11. Effects of NC-1300, Sulfide and MBI on HCl-ethanol-induced gastric lesions in rats. Each compound was given 12 hr before HCl-ethanol administration. The protective effects of NC-1300 were more potent than those of Sulfide and MBI given in a dose of 30 mg/kg. Data represent the mean $\pm$ one S.E.M.

maleimide (10 mg/kg) had no effects on HCl-ethanol-induced lesions (Fig. 9). In N-ethylmaleimide treated rats, NC-1300 or omeprazole at a dose of 10 mg/kg gave no protection against the lesion formation

induced by HCl-ethanol, but a dose of 30 mg/kg was significantly protective.

Effects of Sulfide, MBI and Sulfone on HCl-ethanol-induced lesions: Sulfide (10 or 30 mg/kg) significantly and dose-dependently protected against the lesion formation when given p.o. at 0.5 hr before HCl-ethanol administration (Fig. 10). The degree of protection was comparable to that observed with NC-1300, i.e., the inhibition was 40.2% at 10 mg/kg and 80.7% at 30 mg/kg. Sulfone (30 mg/kg) had no effect on the lesion formation. Both Sulfide and MBI (30 mg/kg) also significantly protected the gastric mucosa when given 12 hr before HCl-ethanol administration (Fig. 11). However, the effects of these compounds were significantly weaker than those of NC-1300, the inhibition being 37.3% at 30 mg/kg of Sulfide and 27.1% at 30 mg/kg of MBI.

Discussion

We obtained evidence that NC-1300, like other proton pump inhibitors, protects the gastric mucosa from necrotizing agents such as HCl-ethanol. The apparent difference between NC-1300 and other compounds was the duration of this protective effect.

Mattsson et al. (3) reported that the protection by omeprazole (27.5 mg/kg) against absolute ethanol was observed for 3 hr after the p.o. administration, but disappeared 3.5 hr later. Prostaglandins are representative cytoprotective agents against various necrotizing agents (8). The maximal duration of gastric cytoprotection by various agents, including prostaglandins and mild irritants, is reportedly about 1–8 hr after a single administration (8–11). Thus, the protective activity of NC-1300 seen even 24 hr after a single p.o. administration was remarkable. In this regard, NC-1300 seems to be the longest lasting cytoprotective agent so far developed.

The mechanisms related to the extraordinarily long effect include the following. First, this persistent activity of NC-1300 is comparable to the antisecretory activity of the agent. In an earlier study, we found that NC-1300 at the same dose levels used in this study inhibited the gastric acid secretion in pylorus-ligated rats for 24 hr (1). Other investigators (2–4) suggested that the protection by omeprazole or timoprazole appears to be unrelated to its antisecretory activity, because the protection was ineffective when these agents were given parenterally. The antisecretory activity of such agents was practically equal whether given p.o. or parenterally (i.p., s.c., or i.v.). We also found that the i.p. administration of NC-1300 or omeprazole (10 or 30 mg/kg) had no effect on HCl-ethanol-induced lesions. With that dose and route, the gastric acid secretion was potently inhibited for 3 hr. These data suggest that the protective effect of NC-1300 or omeprazole is not due to its antisecretory activity, but rather to a local action. In addition, we induced gastric mucosal lesions by giving an excess amount of HCl together with ethanol directly into the stomach. This experimental condition also rules out the possible involvement of antisecretory effects of these agents in the mucosal protection.

Second, several investigators considered that the possible dilution of necrotizing agents by gastric contents may relate to the cytoprotective effects of various compounds (12–14), because these compounds were usually given p.o. in a volume of 1 ml or more at 0.5 to 1 hr before the agents. Most of

these workers demonstrated that cytoprotection was not caused by a simple physical dilution of necrotizing agents, but rather by a pharmacological effect. We also obtained evidence that the NC-1300 protection is not due to physical dilution of HCl-ethanol, because preliminary removal of gastric contents did not alter the protection; rather, the protection was enhanced at a dose of 10 mg/kg of NC-1300. The mechanism of this increased effect of NC-1300 is not yet clear.

Third, it has been reported that mild irritants such as 25% ethanol or 0.35 N HCl are cytoprotective against strong irritants, possibly through the stimulation of prostaglandin synthesis by the stomach (10, 15). We examined whether NC-1300 acts as a mild irritant mediated by endogenous prostaglandins and found that indomethacin did not block the protection afforded by NC-1300. Therefore, it is likely that endogenous prostaglandins are not involved in the mechanism of the protective action of the agent.

Szabo et al. (16), Ezer (17) and Miller et al. (18) proposed that sulfhydryl compounds in the gastric mucosa play an important role on the cytoprotection seen with several agents. However, there are controversial data about the role of sulfhydryl compounds (mainly reduced glutathione) in the role of gastric cytoprotection. Konturek et al. (19) reported that both sulfhydryl containing compounds such as cysteamine or sulfhydryl blockers such as diethyl maleate have a cytoprotective effect against absolute ethanol. We showed that while N-ethylmaleimide had no effect on the formation of HCl-ethanol-induced lesions, the agent counteracted the cytoprotection by NC-1300 to some extent, thereby suggesting that NC-1300 protection may partly involve sulfhydryl compounds in the stomach.

Omeprazole is readily degraded by acidic pH or metabolized in the body resulting in omeprazole-sulfone or omeprazole-sulfide (20). Since NC-1300 was given p.o. without mixing with NaHCO_3 , there is the possibility that the NC-1300 protection may be due to degradation in the stomach. Actually, two agents (Sulfide and MBI) showed a similar protective effect on HCl-ethanol-induced lesions, although the doses related to the

protection were the same as NC-1300. It seems likely that NC-1300 exerts its protective effect partly by being transformed to Sulfide or MBI. However, when NC-1300, Sulfide or MBI was given 12 hr before HCl-ethanol, only NC-1300 showed a marked inhibitory effect on lesion formation. Protection by Sulfide and MBI was weaker than that seen with NC-1300 itself. These data suggest that the protection by NC-1300 is mainly caused by the agent itself and partly due to its degradation. Whether or not NC-1300 dissolved in an acidic solution is also effective against HCl-ethanol-induced lesions is the subject of an ongoing study.

Since gastric motility in rats was inhibited by NC-1300 (30 mg/kg) for over 0.5 hr (S. Okabe et al., unpublished data), it is unlikely that the protection by the agent is due to the enhanced emptying of HCl-ethanol into the duodenum. Takeuchi and Nobuhara (21) recently reported that gastric hypercontraction elicited by various necrotizing agents, including absolute ethanol, in rats plays an important role in the pathogenesis of lesion formation. In contrast to their findings, HCl-ethanol did not induce any appreciable hypercontraction of the stomach, except for the increase in internal pressure (S. Okabe et al., unpublished data). Therefore, NC-1300 protection may be caused through mechanisms other than the inhibition of the gastric hypercontraction.

Another possibility is that since the viscosity of gastric contents appeared to be increased after NC-1300 treatment, an increase in mucus secretion may be involved in the causal factor. However, the degree of viscosity of gastric contents seems to be fairly equal with p.o. or i.p. drug administrations. This finding may exclude the possible participation of mucus in the cytoprotective effect of NC-1300. The viscosity, mucus secretion and gastric mucosal circulation will be determined quantitatively and reported in detail elsewhere. All those findings taken together suggest that various proton pump inhibitors, including NC-1300, protect the gastric mucosa against necrotic lesions through mechanisms unrelated to proton pump inhibition.

We conclude that regardless of the

mechanism of action, NC-1300 has a potent and long-lasting protective effect on HCl-ethanol-induced gastric lesions in rats.

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