NC-1300, a Proton-Pump Inhibitor, Requires Gastric Acid to Exert Cytoprotection in Rat Gastric Mucosa

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Abstract—Effect of gastric acid suppression on the cytoprotective effect of a single dose of NC-1300 given intragastrically was studied. NC-1300 given intragastrically prevented gastric mucosal damage caused by absolute ethanol in rats in a dose-related manner, while the drug given subcutaneously did not. Pretreatment with NC-1300 given subcutaneously to suppress acid secretion abolished the protective effect of NC-1300 given intragastrically, but not in the presence of 0.1 N HCl. Repeated intragastric administration of NC-1300 for 7 days failed to prevent the ethanol damage. These results suggest that NC-1300 requires gastric acid to exert a protective effect against ethanol in rat gastric mucosa.

Keywords: Proton-pump inhibitor, Cytoprotection, Gastric mucosa, Ethanol damage

Omeprazole, a proton-pump inhibitor, prevents damage caused by necrotizing agents such as absolute ethanol and 35% NaCl in rat gastric mucosa (1, 2). This effect is also found in other proton-pump inhibitors (3–5). The mechanism of the cytoprotection is, however, not known. This effect of proton-pump inhibitors is observed only when the drugs are given intragastrically (2–4). So, proton-pump inhibitors might need to come in contact with gastric contents, especially gastric acid, to induce cytoprotection.

This study was done to investigate the effect of gastric acid suppression on the cytoprotective effect of 2-[(2-dimethylaminobenzyl)sulfinyl]benzimidazole (NC-1300), a proton-pump inhibitor developed in Japan, given i.g.

Materials and Methods

Drug

NC-1300 (a gift of Nippon Chemipharm Co., Ltd., Tokyo) was dissolved in 0.5% carboxymethylcellulose immediately before the experiment.

Animals

Male Wistar rats (Nihon Kaei Breeding Laboratory, Shizuoka) weighing about 200 g were housed in individual semi-restraint cages (6) to prevent coprophagia starting 18 hr before the experiments.

Experimental design

In the first experiment, rats were divided into 4 groups (n = 10 each) and were given either NC-1300 in the dose of 3, 10, or 30 mg/kg or vehicle alone, i.g. Four hours later, they were given 2 ml of absolute ethanol, i.g.; they were then killed 30 min later, and gross mucosal lesions in the stomachs were examined. The same experiment except with subcutaneous administration of the drug was also done. Histological evaluation of mucosal damage caused by absolute ethanol was done in rats pretreated with 30 mg/kg of NC-1300 or vehicle alone, i.g. (n = 5 each).

In the second experiment, rats were divided into 4 groups (n = 10 each), and all the animals were pretreated with NC-1300 at the dose of 10 mg/kg, s.c. One hour later, they were given either NC-1300 at the dose of 3, 10, or 30 mg/kg or vehicle alone, i.g.; and 4 hr later, they were given 2 ml of absolute ethanol, i.g. Thirty minutes later, they were killed, and gross mucosal lesions in the stomachs were examined. The same experiment except that the rats were pretreated with vehicle alone, s.c. was done. To see the effect of acidified NC-1300 given i.g. in this experiment, rats were divided into 4 groups (n = 10 each), and all the animals were pretreated with NC-1300 at the dose of 10 mg/kg, s.c. One hour later, they were given either NC-1300 at the dose of 3, 10, or 30 mg/kg or vehicle alone in 0.1 N HCl, i.g. and 4 hr later, they were given 2 ml of absolute ethanol, i.g. Thirty minutes later, they were killed, and gross mucosal lesions in the
stomachs were examined.

In the third experiment, rats were divided into 4 groups (n = 10 each) and were given either NC-1300 at the dose of 3, 10, or 30 mg/kg or vehicle alone, i.g., once a day for 7 days. Then, they were given 2 ml of absolute ethanol, i.g. at 4 hr after the last dose of NC-1300. Thirty minutes later, they were killed, and gross mucosal lesions in the stomachs were examined. The same experiment except that the rats were given vehicle alone, i.g. for the first 6 days was done.

To evaluate the effect of NC-1300 on gastric secretion, 31 rats were given one of the following regimens: 1) a single or repeated (once a day for 7 days) dose of 30 mg/kg of NC-1300 or vehicle alone, i.g.; 2) 30 mg/kg NC-1300 in 0.1 N HCl or vehicle in 0.1 N HCl, i.g.; 3) 30 mg/kg NC-1300 or vehicle alone, s.c. The pylorus was ligated 2 hr after the administration of the drug and 4 hr later, all of the gastric contents were collected for measurements of volume and acidity.

**Assessment of gross mucosal lesions**

Rats were killed, and the stomachs were dissected out and opened along the greater curvature. The number of necrotic lesions in the mucosa was counted blindly. The total number of lesions was used as the lesion score for each stomach.

**Histological evaluation**

Mucosal histology was assessed as described elsewhere (7). The removed stomachs were opened along the greater curvature. Strips of the gastric wall were cut transversely across the middle level of the glandular mucosa. Coded mucosal specimens stained with hematoxylin and eosin were evaluated under light microscopy. The extent of histologic necrosis measuring more than 0.15-mm deep was estimated morphologically with the aid of an ocular micrometer by measuring the length of the mucosal strips and the total length of the necrotic lesions for each strip. In a similar way, the disruption of the surface epithelial layer was estimated by measuring the length of the mucosa devoid of surface epithelium and expressing it as a percentage of the total length of the mucosal strip for each strip studied.

**Analysis of gastric contents**

The gastric contents were measured for volume and acidity with an Autoburet Titlator with a pH meter (PHM62, ABU12, TTT60, and TTA60, Radiometer Copenhagen, Copenhagen, Denmark).

**Statistical analysis**

Differences between experimental groups were evaluated statistically by Mann-Whitney U-test. Probability values of less than 0.05 were considered significant.

**RESULTS**

NC-1300 inhibited the gross mucosal damage caused by absolute ethanol in rat stomach in a dose-related manner.

![Graph 1](attachment:image1.png)

**Fig. 1.** Effect of NC-1300 on gastric mucosal damage caused by absolute ethanol in rats when given i.g. or s.c. Values represent the mean±S.E.M. of 10 rats. Significantly different (*P<0.01 and **P<0.001) from the control values. ○: NC-1300, i.g.; ●: NC-1300, s.c.

![Graph 2](attachment:image2.png)

**Fig. 2.** Effect of pretreatment with NC-1300, s.c. on prevention of ethanol-induced gastric mucosal damage by NC-1300, i.g. with or without 0.1 N HCl in rats. Values represent the mean±S.E.M. of 10 rats. Significantly different (*P<0.01 and **P<0.001) from the control values. ○: vehicle, s.c. + NC-1300, i.g.; ●: NC-1300, 10 mg/kg, s.c. + NC-1300, i.g.; ◦: NC-1300, 10 mg/kg, s.c. + acidified NC-1300, i.g.
when administered i.g. (Fig. 1). The drug, however, did not affect the damage when administered s.c. (Fig. 1). This prevention of mucosal damage by NC-1300 administered i.g. was abolished when the rats were pretreated with NC-1300, s.c. (Fig. 2) or when they were repeatedly administered NC-1300, i.g. for 7 days (Fig. 3). When NC-1300 was given i.g. with 0.1 N HCl, the abolition of the protective effect of NC-1300, i.g. by pretreatment with NC-1300, s.c. was reversed (Fig. 2). Histologically, NC-1300 administered i.g. inhibited deep mucosal necrosis caused by absolute ethanol, but did not prevent disruption of the surface epithelium (Table 1).

NC-1300 at 30 mg/kg reduced the volume of the gastric contents and inhibited acid output either in a single or repeated dose, i.e., in a single dose with 0.1 N HCl, i.e.; or in a single dose, s.c. (Table 2).

**DISCUSSION**

The results of this study show that the protective effect of NC-1300 administered i.g. in rat gastric mucosa against damage caused by absolute ethanol was abolished when the rats were pretreated with the same drug, s.c. at the dose strongly suppressing acid secretion. These findings suggest that NC-1300 needs to come in contact with gastric acid to exert its protective effect. Our finding that acidified NC-1300 works after suppression of acid secretion by pretreatment with NC-1300, s.c. supports this. This evidence may explain why proton-pump inhibitors exert a cytoprotective effect only when given i.g. and not when given parenterally (2-4) as shown again in this study. Abolition of cytoprotection by repeated administration of NC-1300, i.g. may be for the same reason, although involvement of other factors such as morphological changes in gastric mucosa is not yet elucidated.

The role of the acid milieu in cytoprotection by NC-1300 is not known. NC-1300 is mainly degraded to NC-1300-sulfide by acid (5). The degradation products including NC-1300-sulfide are, however, less cytoprotective than NC-1300 by itself (5). The acid milieu might potentiate the effect of NC-1300. Further study is needed to eluci-

### Table 1. Effects of NC-1300 on disruption of surface epithelium and deep necrosis caused by absolute ethanol

|                | No. of rats | Disruption of surface epithelium | Deep necrosis<sub>(&gt;0.15 mm)</sub> |
|----------------|-------------|----------------------------------|--------------------------------------|
| Vehicle        | 5           | 98.3±0.5                         | 24.7±3.7                            |
| NC-1300        | 5           | 97.1±0.9                         | 7.1±2.8<sup>*</sup>                  |

<sup>a</sup>Rats were given 30 mg/kg of NC-1300 or vehicle, intragastrically; and 4 hr later, they were given 2 ml of absolute ethanol. Then, they were killed 30 min later. <sup>b</sup>Percentage of total length of mucosal strip. *Significantly different at P<0.01 from saline controls. Values are mean±S.E.M.

### Table 2. Effects of NC-1300 on gastric secretion in pylorus ligated rats

| Route         | Drug                          | No. of rats | Gastric contents                |
|---------------|-------------------------------|-------------|---------------------------------|
|               |                               |             | volume (ml/4 hr) | acid output (uEq/4 hr) |
| Intragastric  | vehicle                       | 5           | 4.9±0.8           | 145±12               |
|               | NC-1300                       | 4           | 1.5±0.6<sup>**</sup> | 0±0<sup>**</sup>      |
|               | NC-1300×7 days                | 5           | 1.9±0.3<sup>**</sup> | 3±2<sup>**</sup>     |
|               | 0.1 N HCl + vehicle          | 4           | 4.2±0.2           | 179±71               |
|               | 0.1 N HCl + NC-1300          | 5           | 1.9±0.2<sup>**</sup> | 3±3<sup>*</sup>         |
| Subcutaneous  | vehicle                       | 4           | 5.7±1.2           | 151±37               |
|               | NC-1300                       | 4           | 1.4±0.4<sup>**</sup> | 7±7<sup>**</sup>      |

<sup>a</sup>Rats were given 30 mg/kg of NC-1300 or vehicle alone; and 2 hr later, the pylorus was ligated. Then, gastric contents were collected 4 hr later. Statistically significant difference, at <sup>**</sup>P<0.05 and <sup>**</sup>P<0.01, from the controls. Values are means±S.E.M.
date the role of the acid milieu.

Inhibition of acid secretion may not be involved in cytoprotection by NC-1300, because this drug did not prevent mucosal damage caused by absolute ethanol when administered s.c., but did suppress acid secretion to the same extent as the drug administered i.g. It is unlikely that NC-1300 acts as a mild irritant for the reason that this drug administered i.g. no longer exerted cytoprotection when acid secretion was suppressed beforehand. Adaptive cytoprotection by mild irritants such as 20% ethanol and 0.075 M NaOH lasts only for 150 min (6). Cytoprotection by NC-1300 was observed for at least 4 hr after the administration; this also suggests that this drug does not act as a mild irritant. Cytoprotection by acidified NC-1300 is not the effect of 0.1 N HCl, because 0.1 N HCl alone did not inhibit mucosal damage caused by absolute ethanol. Robert et al. (6) have shown that 0.1 N HCl does not act as a mild irritant for cytoprotection. In addition, the present study showed acidified NC-1300 still having an inhibitory effect on acid secretion, suggesting a difference in pharmacological characteristics between omeprazole and this drug.

Lacy and Ito (8) have shown in their histological study that 16,16-dimethyl PGE2 does not inhibit the disruption of the surface epithelium caused by absolute ethanol, but prevents deep necrosis in gastric mucosa. We showed similar histological findings after an ethanol challenge in rats given NC-1300, i.e. beforehand. This similarity is also seen with zinc L-carnosine, a cytoprotective drug, without affecting endogenous PGE2 in gastric mucosa (7). Therefore, this similarity may be prostaglandin-independent.

In conclusion, NC-1300 needs to come in contact with gastric acid to exert its protective effect against ethanol in rat gastric mucosa.

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