Effects of a New Benzimidazole Derivative, NC-1300-O-3, on Gastric Secretion and Gastroduodenal Lesions in Rats

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ABSTRACT—We examined the effects of a new compound, NC-1300-O-3 \(2-[2-N\)-methyl-N-(2-methylpropyl) amino] benzylsulfinyl benzimidazole\), on the gastric mucosal proton pump (\(H^+,K^+-ATPase\)) activity, gastric secretion and gastroduodenal lesions in rats. The compound potently inhibited the enzyme activity in a concentration-dependent manner, the IC\(_{50}\) being \(5.3 \times 10^{-6}\) M at pH 6.0 and \(1.4 \times 10^{-5}\) M at pH 7.4. NC-1300-O-3 markedly and persistently (for more than 24 hr) inhibited basal gastric secretion in male or female animals when administered by the p.o. route. The compound also significantly inhibited gastric secretion by the intraduodenal (i.d.), intragastric (after pylorus ligation) and i.p. routes, but only weakly by the s.c. route. Repeated p.o. administration of the compound for 1 week also significantly inhibited gastric secretion. Histamine-stimulated gastric secretion was also significantly inhibited by the i.d. administration of the compound. NC-1300-O-3, administered p.o., potently prevented water-immersion stress-, histamine-, indomethacin-, prednisolone- and compound 48/80-induced gastric lesions. In addition, it also significantly prevented the formation of gastric lesions induced by various necrotizing agents. Mepirizole- and cysteamine-induced duodenal ulcers were also prevented by the compound. The antisecretory and antilesion activities of NC-1300-O-3, administered p.o., were not altered on its combination with 2% NaHCO\(_3\).

We have reported the effects of gastric mucosal proton pump (\(H^+,K^+-ATPase\)) inhibitors, NC-1300 and NC-1300-B, on gastric secretion and experimental gastric and duodenal lesions in rats (1-5). These compounds were as effective as other pump inhibitors such as omeprazole (6, 7) and AG-1749 (8) in inhibiting gastric secretion, protecting the gastroduodenal mucosa against various acute lesions and accelerating the healing of chronic gastric ulcers in rats. Similar to omeprazole (9), they were quite unstable under acidic conditions, thereby suggesting that the efficacy of these compounds would be greatly reduced when administered by the p.o. route. In order to obtain a much more stable and less toxic compound, a series of compounds were synthesized at Nippon Chemiphar Co., Ltd. In a screening test, we found that one compound, NC-1300-O-3 \(2-[2-N\)-methyl-N-(2-methylpropyl) amino] benzylsulfinyl benzimidazole; molecular weight, 341.47\) (Fig. 1), markedly protected the gastric mucosa against HCl-ethanol-induced lesions in rats. Although its stability under acidic conditions was not better, acute and subacute toxicity studies in-
dicated that this new compound has less adverse effects than the former ones in rats and dogs. The LD_{50} values in male and female rats were more than 5 g/kg after p.o. and s.c. treatment and about 630–720 mg/kg after i.p. treatment. These findings prompted us to evaluate its pharmacological activities in detail and to develop a new antiulcer drug. In this report, we describe the effects of NC-1300-O-3 on the gastric mucosal proton pump, gastric secretion and gastroduodenal lesions in rats.

MATERIALS AND METHODS

Biochemical studies

Preparation of dog gastric membranes enriched in H^+,K^+-ATPase: Gastric H^+,K^+-ATPase was purified from the parietal cell-rich fraction of a dog stomach as described by Saccomani et al. (10), with a slight modification by us (1). The fundic mucosa of the dog stomach was scraped from the underlying muscular layer. The mucosa was suspended in about 10 volumes of a homogenization buffer composed of 250 mM sucrose, 2 mM MgCl_2, 1 mM EGTA and 2 mM Hepes-Tris (pH 7.4). After homogenization at 20,000 × g for 0.5 hr, the resultant supernatant was centrifuged at 78,000 × g for 1 hr to yield the crude microsomal pellet. The 78,000 × g pellet was resuspended in the same solution; and after homogenization it was centrifuged at 105,000 × g for 1 hr in a discontinuous density gradient. Vesicles enriched in the gastric H^+, K^+-ATPase were collected at the interface of the homogenization buffer and 7% Ficoll (w/w) + homogenization buffer layers.

**Assay procedure:** The vesicle (30 µg protein) was preincubated in medium consisting of 4 mM imidazole buffer (pH 6.0 or 7.4) and NC-1300-O-3 or omeprazole at 10^{-7} to 10^{-4} M in a final volume of 1 ml. These agents were dissolved in dimethyl sulfoxide. All incubation mixtures contained less than 1% dimethyl sulfoxide, which had no influence on the assay. The incubation time was 30 min at 37°C, after which the enzyme reaction was started by the addition of 1 ml of a mixture containing 4 mM MgCl_2, 4 mM ATP and 75 mM imidazole buffer (pH 7.4), with or without 10 mM KCl. After incubation for 10 min at 37°C, the reaction was terminated by adding 1 ml of 15% trichloroacetic acid, and then the inorganic phosphate derived from ATP was measured by the method of Fiske and Subbarow (11).

Pharmacological studies

Male and female Sprague-Dawley rats (Nihon Charles-River), weighing 220–260 g, were used. The animals were deprived of food for 24 hr prior to the experiments, unless otherwise noted. Water was given freely during the fasting period, but withheld during the last 2 hr. These animals were kept in raised mesh-bottom cages to prevent coprophagy. Six to sixteen rats were used for each group.

Gastric secretion

**Basal secretion:** Under ether anesthesia, the abdomen was incised and the pylorus ligated. Three hours after the ligation, the animals were killed with an overdose of ether. The gastric contents were collected and analyzed as to volume and acidity. Total acidity was determined by automatic titration of the gastric juice against 100 mM NaOH to pH 7.0 (Hiranuma, Comitite 5). Acid output was expressed as µEq/hr. NC-1300-O-3, suspended in 0.5% carboxymethylcellulose (CMC), was administered at 3–100 mg/kg orally (p.o.) 0.5, 6 or 21 hr before pylorus ligation. In the case of repeated administration, the compound was administered p.o. once daily for 1 week, and then the pylorus was ligated 0.5 hr after the
last dose. The antisecretory effects of the compound administered by other than the p.o. route were also determined. NC-1300-O-3 was administered intraduodenally (i.d.) and intragastrically (i.g.) immediately after pylorus ligation or i.p. 1 hr before or s.c. 0.5 hr before the ligation. Since NC-1300-O-3 was unstable under acidic conditions, its antisecretory effect was determined with its combination with 2% NaHCO₃. The pH of 2% NaHCO₃ (sus- pended in 0.5% CMC) alone and NC-1300-O-3 (100 mg/5 ml) combined with 2% NaHCO₃ was 8.41 and 8.58, respectively. Two percent NaHCO₃ alone or NC-1300-O-3 combined with 2% NaHCO₃ was administered p.o. 0.5 hr before the ligation. The animals were killed 3 hr later, and the gastric contents analyzed as to volume and acidity as described above. The vehicle alone was administered to the corresponding control groups. The volume of NC-1300-O-3 combined with 2% NaHCO₃, 2% NaHCO₃, or the vehicle alone was 1 ml/200 g body wt.

Histamine-stimulated secretion: Under ether anesthesia, the abdomen was incised, and the stomach and duodenum were exposed. A small incision was made in the forestomach and a polyethylene cannula (7 mm inside diameter) was used for drainage of gastric juice (12). The cannula had two small polyethylene tubes, one was inserted into the distal part of the esophagus to exclude saliva from the gastric samples and the other one into the proximal duodenum through the pylorus to administer NC-1300-O-3 or the vehicle alone. The pylorus was then ligated with the tube. After recovery from anesthesia, the animals were placed in Bollman cages and gastric juice was collected. The first 2 hr samples were discarded and the following 2 hr samples used to determine the basal secretion. Histamine-2HCl (Nacalai Tesque), dissolved in 10% gelatin, was injected s.c. twice at 20 mg/kg in a volume of 1 ml/200 g body wt. at every 2 hr after collecting the basal secretion sample. The gastric samples obtained 2 and 4 hr after histamine stimulation were analyzed for volume and acidity. NC-1300-O-3 or the vehicle alone was administered i.d. 3 hr before the first injection of histamine.

Gastric lesions
Water-immersion stress-induced lesions: Rats were placed in a restraint cage (Todai Yakusaku Type, Natume) and then immersed to the level of the xiphoid process in a water bath (23°C) for 8 hr (13). The animals were then killed, and the stomach of each rat was removed, inflated by injecting 8 ml of 2% formalin and immersed in 2% formalin for 10 min to fix the inner and outer layers of the gastric wall. This formalin treatment was performed in all the following experiments. Subsequently, the stomach was incised along the greater curvature and the length (mm) of each lesion in the glandular portion was determined under a dissecting microscope with a square grid (Olympus, 10 ×) and summed per stomach. The person who determined the lesions had no knowledge of which treatment the animals had been administered. NC-1300-O-3 or the vehicle alone was administered p.o. 0.5 hr before the stress.

Histamine-induced lesions: Under ether anesthesia, the abdomen was incised, and then both the gastric artery and vein and the pylorus were ligated. After the abdomen had been closed, histamine-2HCl, dissolved in saline, was administered s.c. at 40 mg/kg (14). The animals were killed 3 hr later. The area (mm²) of each lesion in the glandular portion was determined and summed per stomach. NC-1300-O-3 or the vehicle alone was administered p.o. 0.5 hr before the ligation of vessels.

Indomethacin-induced lesions: Indomethacin (Merck), suspended in saline with a minimal amount of Tween 80, was administered s.c. at 25 mg/kg (15). The animals were killed 7 hr later and their stomachs examined for lesions. The length (mm) of each lesion in the glandular portion was determined and summed per stomach. NC-1300-O-3 or the vehicle alone was administered p.o. 0.5 hr before the indomethacin treatment.

Prednisolone-induced lesions: Prednisolone (Wako), suspended in a Tween 80-saline solu-
tion, was administered s.c. at 50 mg/kg once daily for 4 days to non-fasted rats (16). The animals were killed 24 hr after the final administration of prednisolone. The length (mm) of each lesion in the glandular portion was determined and summed per stomach. NC-1300-O-3 or the vehicle alone was administered p.o. once daily for 4 days 0.5 hr before the prednisolone treatment.

**Compound 48/80-induced lesions:** Compound 48/80 (Sigma), dissolved in saline, was administered i.p. at 0.75 mg/kg once daily (9:30 AM) for 4 days to non-fasted rats (17). The animals were killed 24 hr after the final administration of the agent. The area (mm²) of each lesion in the glandular portion was determined and summed per stomach. NC-1300-O-3 or the vehicle alone was administered p.o. once (9:00 AM) or twice a day (9:00 AM and 6:00 PM) for 4 days.

**Necrotizing agent-induced lesions:** Gastric lesions were produced by administering p.o. 1 ml/200 g body wt. of either 60% ethanol (v/v) in 150 mM HCl (HCl-ethanol) (18), 100% ethanol (v/v) (19), aspirin (150 mg/kg, Nacalai Tesque) in 150 mM HCl (HCl-aspirin) (20), or taurocholate (80 mM, Nacalai Tesque) in 200 mM HCl (HCl-taurocholate) (21). Male and female animals were killed 1.5 and 1.0 hr later, respectively. The stomach and the duodenum were removed, and the gastric contents were expelled through the duodenum by gently pushing the gastric wall. After formalin treatment, the length (mm) of each lesion in the glandular portion was determined and summed per stomach. NC-1300-O-3 or the vehicle alone was administered p.o. once (9:00 AM) or twice a day (9:00 AM and 6:00 PM) for 4 days.

**Duodenal ulcers**

**Mepirizole-induced ulcers:** Mepirizole (Daiichi), suspended in 0.5% CMC, was administered s.c. at 200 mg/kg (22). The animals were killed 24 hr later and examined for ulcers in the proximal duodenum. The area (mm²) of each ulcer was determined and summed per duodenum. NC-1300-O-3 or the vehicle alone was administered p.o. once 0.5 hr before the mepirizole administration.

**Cysteamine-induced ulcers:** Cysteamine (Nacalai Tesque), dissolved in saline, was administered s.c. at 190 mg/kg, twice (the total dose was 380 mg/kg); the second injection was administered 16 hr after the first injection (23). The animals were killed 24 hr after the first injection and their duodenum examined for ulcers. The ulcerated area (mm²) was determined and summed. NC-1300-O-3 or the vehicle alone was administered p.o. once 0.5 hr before the first administration of cysteamine.

**Drugs**

NC-1300-O-3 and omeprazole were provided by Nippon Chemiphar Co., Ltd. (Tokyo).

**Analysis of data**

Student's t-test was employed to determine the statistical significance of the data obtained in this study at the level of P < 0.05. ED₅₀ values (the doses which inhibit gastric acid output and gastric or duodenal lesions by 50%), and 95% confidence limits were calculated by the Litchfield-Wilcoxon method (24).

**RESULTS**

**Effects on gastric H⁺, K⁺-ATPase**

Both NC-1300-O-3 and omeprazole inhibited H⁺,K⁺-ATPase activity in dog stomach in a concentration-dependent manner (Fig. 2). The IC₅₀ values (the concentrations used to obtain 50% inhibition) were 5.3 × 10⁻⁶ M and 1.4 × 10⁻⁵ M at pH 6.0 and 7.4, respectively. The IC₅₀ values of omeprazole were 2.3 × 10⁻⁶ and 2.0 × 10⁻⁷ M at pH 6.0 and 7.4,
Effects on gastric secretion

Basal secretion: Ligation of the pylorus for 3 hr resulted in the accumulation of gastric contents; the volume, acidity and acid output were about 4 ml/stomach, 100–110 mEq/l and 140 μEq/hr, respectively. NC-1300-O-3, administered p.o. 0.5, 6 and 21 hr before the ligation significantly inhibited the gastric secretion in a dose-dependent manner (Table 1). The ED₅₀ values were 29.7, 45.5 and 93.6 mg/kg at 3.5, 9 and 24 hr after administration, respectively. It is noteworthy that significant inhibition of the gastric secretion was even observed 24 hr after a single administration of NC-1300-O-3 at 60 and 100 mg/kg. Repeated administration of NC-1300-O-3 for 1 week also

Table 1. Effects of NC-1300-O-3 on basal gastric secretion in pylorus-ligated rats

| Time before pylorus ligation | Dose (mg/kg) | No. of rats | Volume (ml/rat) | Inhibition (%) | Acid-output (μEq/hr) | Inhibition (%) | ED₅₀ mg/kg (95% confidence limit) |
|-----------------------------|--------------|-------------|-----------------|----------------|----------------------|----------------|----------------------------------|
| Single administration       | Control      | 8           | 3.9 ± 0.3       | 141.3 ± 7.0    |                      |                |                                  |
|                             | 3            | 8           | 3.5 ± 0.2       | 121.5 ± 9.8    | 10.3                 | 29.7           |                                  |
|                             | 10           | 8           | 3.3 ± 0.2       | 106.3 ± 13.9*  | 15.4                 | 24.8           | (21.0–44.0)                       |
| 0.5 hr                      | 30           | 8           | 3.1 ± 0.3       | 72.1 ± 9.9*    | 20.5                 | 49.0           |                                  |
|                             | 60           | 8           | 3.0 ± 0.2*      | 51.8 ± 7.1*    | 23.1                 | 63.3           |                                  |
|                             | 100          | 8           | 3.0 ± 0.2*      | 37.7 ± 5.8*    | 23.1                 | 73.3           |                                  |
| Control                     | 8            | 3           | 3.7 ± 0.3       | 129.2 ± 9.4    | 5.1                  | 64             |                                  |
|                             | 10           | 8           | 3.8 ± 0.3       | 110.6 ± 8.1*   | 2.6                  | 19.9           | 45.5                            |
| 6 hr                        | 30           | 8           | 3.5 ± 0.2       | 81.6 ± 4.4*    | 10.3                 | 40.9           | (35.4–61.8)                      |
|                             | 60           | 8           | 3.3 ± 0.3       | 65.7 ± 4.1*    | 15.4                 | 52.4           |                                  |
|                             | 100          | 8           | 3.4 ± 0.2       | 43.8 ± 2.5*    | 12.8                 | 68.3           |                                  |
| Control                     | 8            | 3           | 3.8 ± 0.3       | 138.0 ± 6.8    |                      |                |                                  |
|                             | 10           | 8           | 3.8 ± 0.3       | 129.2 ± 9.4    | 5.1                  | 64             |                                  |
| 21 hr                       | 30           | 8           | 3.7 ± 0.2       | 148.0 ± 9.1    | 2.6                  | −6.9           | 93.6                            |
|                             | 60           | 8           | 3.6 ± 0.2       | 107.6 ± 8.2*   | 5.3                  | 22.3           | (79.1–121.0)                     |
|                             | 100          | 8           | 3.5 ± 0.3       | 60.8 ± 7.7*    | 7.9                  | 56.1           |                                  |
| Repeated administration     | Control      | 6           | 4.0 ± 0.2       | 141.9 ± 11.9   |                      |                |                                  |
|                             | 3            | 6           | 3.9 ± 0.3       | 120.8 ± 6.2    | 2.5                  | 14.9           |                                  |
|                             | 10           | 6           | 3.8 ± 0.3       | 95.5 ± 5.8*    | 5.0                  | 32.7           | 25.4                            |
| 0.5 hr                      | 30           | 6           | 3.6 ± 0.4       | 62.6 ± 3.7*    | 10.0                 | 55.9           | (20.5–32.2)                      |
|                             | 100          | 6           | 3.4 ± 0.2       | 41.0 ± 2.8*    | 15.0                 | 71.1           |                                  |

The compound was administered p.o. once 0.5, 6 or 21 hr before pylorus ligation. In the case of repeated administration, the compound was administered p.o. once daily for 1 week and the pylorus ligated 0.5 hr after the last dose. The animals were killed 3 hr after the ligation. All values are means ± S.E. *P < 0.05.
significantly inhibited the gastric secretion when the compound was administered 0.5 hr before the ligation. The ED$_{50}$ value was 25.4 mg/kg. NC-1300-O-3 administered i.d. or i.p. also significantly inhibited the gastric secretion in a dose-dependent manner (Table 2). The ED$_{50}$ values after i.d. and i.p. treatment were 21.7 and 52.5 mg/kg, respectively. When the compound was administered s.c., the inhibition of gastric secretion was considerably weaker than that observed with the p.o., i.d. or i.p. route, i.e., the inhibition was only 23.6% even at 100 mg/kg. NC-1300-O-3 also significantly inhibited the gastric secretion when administered at 100 mg/kg, i.g., immediately after the pylorus ligation, the inhibition being 45.1%. In female rats, the compound also significantly inhibited the gastric secretion in a dose-dependent manner (Table 3). The ED$_{50}$ value was 10.9 mg/kg. The p.o. administration of 2% NaHCO$_3$ alone had little or no effect on the volume or acid output 3.5 hr later (Table 4). The administration of NC-1300-O-3 combined with 2% NaHCO$_3$ significantly inhibited the gastric acid output in a dose-dependent manner, the ED$_{50}$ value being 26.6 mg/kg.

**Histamine-stimulated secretion:** Basal secretion in acute fistula rats was almost negligible, the volume and acid output being about 0.2

### Table 2. Effects of NC-1300-O-3 on basal gastric secretion in pylorus-ligated rats

| Route | Dose (mg/kg) | No. of rats | Volume (ml/rat) | Inhibition (%) | Acid-output (μEq/hr) | Inhibition (%) | ED$_{50}$ mg/kg (95% confidence limit) |
|-------|--------------|-------------|----------------|----------------|---------------------|----------------|---------------------------------------|
| Control | 8 | 4.1 ± 0.3 | 141.3 ± 5.5 | 14.6 | 123.8 ± 6.9 | 12.4 | 21.7 |
| i.d. | 3 | 3.5 ± 0.3 | 26.8 | 36.6 | 65.4 ± 7.9 | 53.7 | (17.9–26.3) |
|       | 10 | 3.0 ± 0.3* | 56.1 | 40.6 ± 4.0 | 71.3 | 52.5 |
|       | 30 | 2.6 ± 0.2* | 58.5 | 18.4 ± 4.8 | 87.0 | 52.5 |
|       | 60 | 1.8 ± 0.2* | 58.5 | 18.4 ± 4.8 | 87.0 | 52.5 |
|       | 100 | 1.7 ± 0.3* | 58.5 | 18.4 ± 4.8 | 87.0 | 52.5 |
| Control | 8 | 5.3 ± 0.4 | 127.8 ± 14.4 | 3.8 | 119.2 ± 14.9 | 6.7 | 21.7 |
| i.g. | 3 | 5.1 ± 0.4 | 3.8 | 120.7 ± 17.7 | 5.6 | 21.7 |
|       | 10 | 5.1 ± 0.6 | 0 | 123.0 ± 14.3 | 3.8 | 21.7 |
|       | 30 | 5.3 ± 0.4 | 56.2 | 114.1 ± 11.1 | 10.7 | 21.7 |
|       | 60 | 5.5 ± 0.4 | 56.2 | 114.1 ± 11.1 | 10.7 | 21.7 |
|       | 100 | 5.6 ± 0.4 | 56.2 | 114.1 ± 11.1 | 10.7 | 21.7 |
| Control | 8 | 4.2 ± 0.3 | 136.2 ± 7.0 | 11.9 | 132.7 ± 6.5 | 2.6 | 21.7 |
| i.p. | 3 | 3.7 ± 0.3 | 19.0 | 109.7 ± 9.3* | 19.5 | 52.5 |
|       | 10 | 3.4 ± 0.2 | 35.7 | 80.2 ± 4.5* | 41.1 | (40.9–71.1) |
|       | 30 | 2.7 ± 0.2* | 40.5 | 65.3 ± 4.0 | 52.1 | 52.5 |
|       | 60 | 2.5 ± 0.2* | 40.5 | 65.3 ± 4.0 | 52.1 | 52.5 |
|       | 100 | 2.2 ± 0.1* | 40.5 | 65.3 ± 4.0 | 52.1 | 52.5 |
| Control | 8 | 4.0 ± 0.4 | 139.6 ± 11.1 | 0 | 132.2 ± 12.9 | 5.3 | 21.7 |
| s.c. | 3 | 4.0 ± 0.5 | 5.0 | 126.3 ± 13.6 | 9.5 | 21.7 |
|       | 10 | 3.8 ± 0.5 | 2.5 | 118.8 ± 7.8 | 14.9 | 21.7 |
|       | 30 | 3.9 ± 0.3 | 15.0 | 113.2 ± 10.6 | 18.9 | 21.7 |
|       | 60 | 3.4 ± 0.3 | 20.0 | 106.6 ± 9.5* | 23.6 | 21.7 |
|       | 100 | 3.2 ± 0.3 | 20.0 | 106.6 ± 9.5* | 23.6 | 21.7 |

The compound was administered either intraduodenally (i.d.) or intragastrically (i.g.) immediately after pylorus ligation. In the cases of i.p. and s.c. administration, it was administered 1 and 0.5 hr before pylorus ligation, respectively. The animals were killed 3 hr after the ligation. All values are means ± S.E. *P < 0.05.
Table 3. Effects of NC-1300-O-3 on basal gastric secretion in pylorus-ligated female rats

| Dose (mg/kg) | No. of rats | Volume (ml/rat) Inhibition (%) | Acid-output (μEq/hr) Inhibition (%) | ED50 mg/kg (95% confidence limit) |
|-------------|-------------|---------------------------------|-------------------------------------|-----------------------------------|
| Control     | 8           | 3.4 ± 0.6                        | 120.3 ± 20.3                        |                                   |
| 3           | 8           | 2.7 ± 0.3                        | 20.6                                | 84.7 ± 13.2                      | 29.6                              |
| 10          | 8           | 3.1 ± 0.6                        | 8.8                                 | 77.8 ± 15.6                      | 35.3                              |
| 30          | 8           | 3.0 ± 0.4                        | 11.8                                | 31.5 ± 9.8*                      | 73.8 (6.5–16.0)                   |
| 60          | 8           | 2.9 ± 0.3                        | 14.7                                | 9.9 ± 3.3*                       | 91.8                              |
| 100         | 8           | 2.9 ± 0.4                        | 14.7                                | 6.6 ± 1.4*                       | 94.5                              |

The compound was administered p.o. 0.5 hr before pylorus ligation. The animals were killed 3 hr after the ligation. All values are means ± S.E. *P < 0.05.

Table 4. Effects of 2% NaHCO3 and NC-1300-O-3 combined with 2% NaHCO3 on basal gastric secretion in pylorus-ligated rats

| Treatment | Dose (mg/kg) | No. of rats | Volume (ml/rat) Inhibition (%) | Acid-output (μEq/hr) Inhibition (%) | ED50 mg/kg (95% confidence limit) |
|-----------|-------------|-------------|---------------------------------|-------------------------------------|-----------------------------------|
| Control   | 8           | 3.5 ± 0.5   | 117.1 ± 15.4                    |                                    |                                   |
| 2% NaHCO3 | 8           | 2.9 ± 0.5   | 17.1                            | 103.9 ± 21.7                      | 11.2                              |
| NC-1300-O-3 | 10 7    | 4.0 ± 0.6   | 136.8 ± 23.5                    |                                    |                                   |
| in 2% NaHCO3 | 30 8   | 3.7 ± 0.6   | 7.5                             | 102.6 ± 26.1                      | 25.0                              |
|            | 60 8       | 2.8 ± 0.4   | 30.0                            | 44.5 ± 10.3*                      | 67.5 (11.2–43.7)                  |
|            | 100 8      | 4.0 ± 0.5   | 0                               | 30.3 ± 5.5*                       | 77.9                              |

Each compound was administered p.o. 0.5 hr before pylorus ligation. The animals were killed 3 hr after the ligation. All values are means ± S.E. *P < 0.05.

ml/2 hr and 10 μEq/2 hr, respectively. Histamine significantly stimulated the gastric secretion; i.e., the volume and acid output were about 2.3–2.4 ml/2 hr and 320–350 μEq/2 hr, respectively (Table 5). A single administration of NC-1300-O-3 significantly inhibited the histamine-stimulated gastric secretion for at least 7 hr in a dose-dependent manner. The ED50 values were 9.0 and 7.1 mg/kg, i.d., at 5 and 7 hr after administration of the compound.

Effects on gastric lesions

Water-immersion stress, a single administration of histamine or indomethacin, or repeated administration of prednisolone or compound 48/80 produced apparent gastric mucosal lesions in the glandular portion, the incidence being 100%. Pretreatment with NC-1300-O-3 prevented the development of most of the lesions mentioned above in a dose-dependent manner (Table 6). The inhibition was significant with over 10 or 30 mg/kg. The ED50 values were 32.1, 17.3, 14.2 and 13.5 mg/kg, respectively. The compound tended to prevent compound 48/80-induced lesions when administered once daily. However, it significantly protected the gastric mucosa against the lesions when administered twice daily, the inhibition being 64.4% with 120 mg/kg/day.

Effects on necrotizing agent-induced lesions

The administration of HCl-ethanol, 100% ethanol, HCl-aspirin and HCl-taurocholate in-
Table 5. Effects of NC-1300-O-3 on histamine-stimulated gastric acid secretion in acute fistula rats

| Dose (mg/kg) | No. of rats | Volume (ml/2 hr) | Acid-output (μEq/2 hr) (%) | Inhibition limit | ED₅₀ mg/kg (95% confidence) |
|--------------|-------------|------------------|----------------------------|-----------------|-----------------------------|
| Control      | 3           | 0.2 ± 0.1        | 13.8 ± 4.1                 |                 |                             |
| Basal        | 10          | 0.2 ± 0.1        | 6.5 ± 2.3                  | 52.9            |                             |
| secretion    | 30          | 0.2 ± 0.1        | 24.8 ± 9.0                 | -79.7           |                             |
|              | 100         | 0.2 ± 0.1        | 8.4 ± 2.7                  | 39.1            |                             |
|              |             | 0.2 ± 0.1        | 6.1 ± 2.8                  | 55.8            |                             |

The compound was administered i.d. 3 hr before the first administration of histamine. Gastric secretion was stimulated by administering histamine-2HCl (20 mg/kg, dissolved in 10% gelatin), s.c., twice at 2 hr intervals. All values are means ± S.E. *P < 0.05.

duced multiple, band-like lesions in the glandular stomach, the incidence being 100% 1.5 hr later. Pretreatment with NC-1300-O-3, p.o., markedly protected the gastric mucosa against these lesions in a dose-dependent manner (Table 7). With 30 mg/kg, the inhibition of the above lesions was more than 85%. The ED₅₀ values were 4.4, 8.5, 6.9 and 2.6 mg/kg for the gastric lesions induced by HCl-ethanol, 100% ethanol, HCl-aspirin and HCl-taurocholate, respectively. The compound, administered at 60 mg/kg 24 hr before HCl-ethanol treatment, significantly prevented the lesion formation, the inhibition being 36.7%. The i.p. administration of NC-1300-O-3 at 0.5 hr before HCl-ethanol treatment also significantly prevented the formation of the lesions. The ED₅₀ value was 60.6 mg/kg. However, the s.c. administration of the compound only weakly prevented HCl-ethanol-induced lesions, even with 100 mg/kg, the inhibition being only 31.2%. Similar to in the case of male rats, the development of HCl-ethanol-induced lesions in female rats was significantly prevented by NC-1300-O-3 in a dose-dependent manner (Table 8). The ED₅₀ value was 1.8 mg/kg.

Similar to in the 1.5 hr experiment, the administration of HCl-ethanol had induced extensive gastric lesions 1 hr later. However, the degree of the lesions was somewhat weak compared with that seen in the 1.5 hr experiment. Again, NC-1300-O-3 significantly prevented these lesions in a dose-dependent manner. The ED₅₀ value was 3.3 mg/kg. These lesions were not appreciably influenced by 2% NaHCO₃ alone. NC-1300-O-3, combined with 2% NaHCO₃, also significantly prevented the development of the lesions in a dose-dependent manner (Table 9). The ED₅₀ value was 4.6 mg/kg.

Effects on duodenal ulcers

Both mepirizole and cysteamine induced penetrating duodenal ulcers in the proximal duodenum, the incidence being 100% 24 hr after the first administration. Pretreatment with
Table 6. Effects of NC-1300-0-3 on various gastric lesions in rats

| Lesion models          | Dose (mg/kg) | No. of rats | Length or area of lesions Mean ± S.E. | Inhibition (%) | ED_{50} mg/kg (95% confidence limit) |
|------------------------|--------------|-------------|--------------------------------------|----------------|--------------------------------------|
| Control                | 8            | 18.8 ± 1.6 (mm) | | | |
| Water-immersion stress | 10           | 15.4 ± 2.4 | | | |
|                        | 30           | 11.1 ± 2.2* | | | |
|                        | 60           | 6.5 ± 2.1* | | | |
|                        | 100          | 1.4 ± 1.2* | | | |
| Histamine              | 8            | 115.5 ± 21.0 (mm²) | | | |
|                        | 10           | 92.9 ± 16.3 | | | |
|                        | 20           | 43.3 ± 12.5 | | | |
|                        | 30           | 19.8 ± 5.6* | | | |
|                        | 60           | 11.6 ± 4.5* | | | |
| Indomethacin           | 8            | 30.5 ± 3.8 (mm) | | | |
|                        | 3            | 21.1 ± 3.3 | | | |
|                        | 10           | 18.5 ± 2.8* | | | |
|                        | 30           | 12.9 ± 3.5* | | | |
|                        | 60           | 7.3 ± 2.0* | | | |
| Prednisolone           | 8            | 68.3 ± 6.4 (mm) | | | |
|                        | 3            | 53.6 ± 7.9 | | | |
|                        | 10           | 34.1 ± 5.8* | | | |
|                        | 30           | 33.3 ± 8.0* | | | |
|                        | 60           | 12.9 ± 3.5* | | | |
|                        | 100          | 3.6 ± 1.4* | | | |
| Compound 48/80         | 8            | 98.7 ± 11.3 (mm²) | | | |
|                        | 3            | 93.3 ± 9.2 | | | |
|                        | 10           | 81.4 ± 15.2 | | | |
|                        | 30           | 76.4 ± 12.9 | | | |
|                        | 60           | 76.1 ± 12.9 | | | |
|                        | 100          | 73.5 ± 22.8 | | | |
| Control\(^a\)          | 16           | 92.1 ± 14.6 (mm²) | | | |
|                        | 3            | 100.1 ± 26.8 | | | |
|                        | 10           | 100.1 ± 26.8 | | | |
|                        | 30           | 100.1 ± 26.8 | | | |
|                        | 60           | 100.1 ± 26.8 | | | |

The compound was administered p.o. once 0.5 hr before stress, histamine or indomethacin. In the case of prednisolone-induced lesions, the compound was administered p.o. once daily for 4 days. In the case of compound 48/80, the compound was administered p.o. once (a) or twice (b) daily for 4 days. *P < 0.05.

NC-1300-O-3 significantly prevented the formation of these ulcers (Table 10). The development of mepirizole-induced ulcers was completely prevented by the compound at 20 mg/kg. The ED_{50} value was 4.7 mg/kg in the case of mepirizole-induced ulcers and 9.7 mg/kg in the case of cysteamine-induced ulcers.

DISCUSSION

These results indicate that the new benzimidazole compound NC-1300-O-3 exhibits potential inhibitory activity toward the gastric mucosal proton pump prepared from dog stomachs at pH 6.0 and 7.4. The inhibitory activity was much clearer at pH 6.0, suggesting that
Table 7. Effects of NC-1300-O-3 on various gastric lesions induced by necrotizing agents in rats

| Lesion models    | Route | Dose (mg/kg) | No. of rats | Length (mm) of Lesions Mean ± S.E. | Inhibition (%) | ED$_{50}$ mg/kg (95% confidence limit) |
|------------------|-------|--------------|-------------|-----------------------------------|----------------|----------------------------------------|
| HCl-ethanol      | Control | 6            | 123.8 ± 5.9 | 17.6                              |                |                                        |
|                  | p.o.    | 3            | 6           | 102.0 ± 16.3*                     | 44.5           | 4.4                                    |
|                  |         | 10           | 6           | 45.0 ± 11.3*                      | 63.7           | (2.6–6.9)                              |
|                  |         | 30           | 6           | 7.5 ± 4.6*                        | 93.9           |                                        |
|                  | Control | 8            | 122.3 ± 3.7 |                                    |                |                                        |
|                  | p.o.    | 30           | 8           | 98.3 ± 11.7                       | 19.6           |                                        |
|                  |         | 60           | 8           | 77.4 ± 10.7*                      | 36.7           |                                        |
|                  |         | Control      | 8           | 107.0 ± 12.0                      |                |                                        |
|                  | i.p.    | 3            | 8           | 103.6 ± 12.5                      | 3.2            |                                        |
|                  |         | 10           | 8           | 87.6 ± 12.7                       | 18.1           |                                        |
|                  |         | 30           | 8           | 57.4 ± 13.4*                      | 46.4           | 60.6                                   |
|                  |         | 60           | 8           | 52.4 ± 8.7*                       | 51.0           | (31.9–216.1)                          |
|                  |         | 100          | 8           | 50.0 ± 10.5*                      | 53.3           |                                        |
|                  | s.c.    | 3            | 8           | 102.8 ± 9.1                       | 11.0           |                                        |
|                  |         | 10           | 8           | 102.4 ± 9.4                       | 11.3           |                                        |
|                  |         | 30           | 8           | 95.6 ± 10.4                       | 17.2           |                                        |
|                  |         | 60           | 8           | 92.8 ± 9.9                        | 19.7           |                                        |
|                  |         | 100          | 8           | 79.5 ± 10.0*                      | 31.2           |                                        |
| Ethanol          | Control | 8            | 90.6 ± 12.5 | 14.7                              |                |                                        |
|                  | p.o.    | 3            | 8           | 77.3 ± 11.9                       | 14.7           |                                        |
|                  |         | 10           | 8           | 40.6 ± 16.3*                      | 55.2           | 8.5                                    |
|                  |         | 30           | 8           | 5.9 ± 3.3*                        | 93.5           | (4.9–13.6)                            |
| HCl-aspirin      | Control | 8            | 72.9 ± 11.1 | 8.9                               |                |                                        |
|                  | p.o.    | 3            | 8           | 66.4 ± 14.2                       | 8.9            |                                        |
|                  |         | 6            | 8           | 35.0 ± 6.9*                       | 52.0           | 6.9                                    |
|                  |         | 10           | 8           | 13.1 ± 3.2*                       | 82.0           | (4.2–10.1)                            |
|                  |         | 30           | 8           | 10.3 ± 3.1*                       | 85.9           |                                        |
| HCl-taurocholate | Control | 8            | 53.5 ± 8.5  | 8.5                               |                |                                        |
|                  | p.o.    | 1            | 8           | 41.6 ± 8.3                        | 22.2           |                                        |
|                  |         | 3            | 8           | 22.8 ± 7.0*                       | 57.4           | 2.6                                    |
|                  |         | 10           | 8           | 7.3 ± 2.9*                        | 86.4           | (1.3–4.4)                             |

The compound was administered either p.o., i.p. and s.c. at 0.5 or p.o. 24 hr before administration of the necrotizing agents. The animals were killed 1.5 hr after administration of the necrotizing agents. *P < 0.05.
Table 8. Effects of NC-1300-0-3 on HCl-ethanol-induced gastric lesions in female rats

| Dose (mg/kg) | No. of rats | Length (mm) of Lesions Mean ± S.E. | Inhibition (%) | ED$_{50}$ mg/kg (95% confidence limit) |
|--------------|-------------|-----------------------------------|----------------|----------------------------------------|
| Control      | 8           | 94.0 ± 11.6                       |                |                                        |
| 1            | 8           | 59.5 ± 15.1                       | 36.7           |                                        |
| 3            | 8           | 39.3 ± 8.8*                       | 58.2           | 1.8                                    |
| 10           | 8           | 5.5 ± 2.5*                        | 94.1           | (0.7–3.0)                              |
| 30           | 8           | 0*                                | 100.0          |                                        |

The compound was administered p.o. 0.5 hr before administration of HCl-ethanol solution. The animals were killed 1 hr after the HCl-ethanol administration. *P < 0.05.

Table 9. Effects of NC-1300-0-3, 2% NaHCO$_3$ and NC-1300-0-3 combined with 2% NaHCO$_3$ on HCl-ethanol-induced gastric lesions in rats

| Treatment | Dose (mg/kg) | No. of rats | Length (mm) of lesions Mean ± S.E. | Inhibition (%) | ED$_{50}$ mg/kg (95% confidence limit) |
|-----------|--------------|-------------|------------------------------------|----------------|----------------------------------------|
| Control   | 8            | 111.3 ± 12.8|                                    |                |                                        |
| 1         | 8            | 101.9 ± 12.4| 8.4                                |                |                                        |
| 3         | 8            | 50.5 ± 6.1* | 54.6                               | 3.3            |                                        |
| 10        | 8            | 9.1 ± 2.5*  | 91.8                               | (2.4–4.4)      |                                        |
| 30        | 8            | 0.6 ± 0.6*  | 99.5                               |                |                                        |
| NC-1300-O-3 | 8        | 95.8 ± 12.1|                                    |                |                                        |
| 2% NaHCO$_3$ | 8         | 96.4 ± 12.6| −0.6                               |                |                                        |
| NC-1300-O-3 in 2% NaHCO$_3$ | 1         | 73.5 ± 12.6*|                                    | 31.5           |                                        |
| 3         | 8            | 65.3 ± 12.9*|                                    | 39.1           | 4.6                                    |
| 10        | 8            | 45.5 ± 11.5*|                                    | 57.6           | (1.9–9.6)                              |
| 30        | 8            | 18.9 ± 4.2* | 82.4                               |                |                                        |

Each compound was administered p.o. 0.5 hr before administration of HCl-ethanol. The animals were killed 1 hr after the HCl-ethanol administration. *P < 0.05.

The protonation of the compound is prerequisite for its activity as in the cases of other pump inhibitors (1, 4, 8, 9). The potency of the compound as a pump inhibitor was nearly the same as those of omeprazole, NC-1300 (1) and NC-1300-B (4). Although we initially aimed to obtain a much more stable compound at low pH, NC-1300-O-3 was unstable under acidic conditions. Its half-life at pH 1.2 was 2.0 min. This time is nearly the same as those observed for omeprazole (9), NC-1300 (3) and NC-1300-B (S. Okabe et al., unpublished), the half-lives of which at pH 1.0 are 2.0, 4.2 and 6.0 min, respectively. Despite its instability in an acidic solution, the compound administered p.o. exhibited strong activity as to inhibition of gastric acid secretion and protection of the gastroduodenal mucosa against various damages.

First, NC-1300-O-3 was found to be a long-acting gastric antisecretory compound, its effect persisting for more than 24 hr after a single p.o. administration at 60 mg/kg or over. There was no sex difference as to the anti-
secretory activity of the compound on p.o. administration. The efficacy in female rats, however, was apparently higher than that in male rats. It is unknown whether or not this strong efficacy is related to the better absorption of the compound or higher sensitivity of the proton pump to the compound in female rats. Gastric secretion was also significantly inhibited with the i.p. and i.d. routes, thereby suggesting that the compound acts systemically. Based on the ED$_{50}$ values, the degree of inhibition with the i.d. route was much the same as that with the p.o. route, but that with the i.p. route was about one half that with the p.o. route. Unexpectedly, however, NC-1300-0-3 had only a weak antisecretory effect when administered by the s.c. route, indicating that the absorption of the compound by the subcutaneous area was difficult. It is noteworthy that NC-1300-0-3 administered i.g. immediately after pylorus ligation also significantly inhibited the gastric secretion. This suggests that this compound has the ability to inhibit gastric secretion through a local action. It is unknown whether the compound was absorbed from the stomach into the circulation to exert an antisecretory effect or directly entered the parietal cells through the mucosal surface to inhibit gastric secretion. Konturek et al. (25) reported that in Heidenhain pouch dogs, omeprazole exerted an antisecretory effect even after administration of the compound into the pouch. Repeated administration for 1 week did not reduce the antisecretory activity of the compound, indicating that the parietal cells were not tolerant to it at all.

Since omeprazole is unstable at low pH, we administered it p.o. after combination with 0.2% NaHCO$_3$ to determine its antisecretory and protective activities against various gastric damages (6). In this study, we did not administer NC-1300-O-3 combined with NaHCO$_3$.

The compound was administered p.o. 0.5 hr before mepirizole and cysteamine treatment. The animals were killed 24 hr after the administration of the ulcerogenic agents. *P < 0.05.

**Table 10. Effects of NC-1300-O-3 on duodenal ulcers in rats**

| Ulcer models | Dose (mg/kg) | No. of rats | Area of ulcers (mm$^2$) Mean ± S.E. | Inhibition (%) | ED$_{50}$ mg/kg (95% confidence limit) |
|--------------|--------------|-------------|--------------------------------------|----------------|-----------------------------------------|
| Control      | 8            | 24.3 ± 5.1  |                                       | 3.7            |                                         |
| Mepirizole   | 8            | 23.4 ± 4.2  |                                       |                |                                         |
| Control      | 8            | 18.8 ± 4.5  |                                       | 22.6           | 4.7                                     |
|             | 6            | 12.1 ± 2.6  |                                       | 50.2           | (3.3–6.6)                               |
|             | 10           | 2.9 ± 1.0*  |                                       | 88.1           |                                         |
|             | 20           | 0*          |                                       | 100.0          |                                         |
| Cysteamine   | 8            | 24.8 ± 2.8  |                                       |                |                                         |
|             | 3            | 18.3 ± 3.7  |                                       | 26.2           | 9.7                                     |
|             | 10           | 15.8 ± 3.2  |                                       | 36.3           | (4.9–19.6)                             |
|             | 30           | 3.3 ± 1.3*  |                                       | 86.7           |                                         |

The compound was administered p.o. 0.5 hr before mepirizole and cysteamine treatment. The animals were killed 24 hr after the administration of the ulcerogenic agents. *P < 0.05.
preparation. This finding would suggest that the effect of the compound is potentially greater on stimulated secretion than basal secretion. Previous studies had shown that NC-1300-O-3 at the doses less than $10^{-5}$ M did not compete with histamine at the histamine H2-receptors and cholinergic receptors in guinea pig atrium and ileum (S. Okabe et al., unpublished data). Thus, it is most likely that the mechanism underlying the antisecretory effect of NC-1300-O-3 comprises the inhibition of the gastric mucosal proton pump.

Secondly, NC-1300-O-3 strongly prevented various gastric lesions and the protection was dose-related. Most of the lesion or ulcer models used in this study are generally known to be causally related to gastric acid secretion. Therefore, the mechanism by which NC-1300-O-3 prevents these lesions appears to involve its long-acting antisecretory activity. However, the ED$_{50}$ values of the compound are lower than those observed in the experiments on basal gastric secretion. In particular, the dose (10 mg/kg) which significantly inhibited gastric lesions induced by prednisolone was less effective on basal gastric secretion, when examined 3.5 and 9 hr later. These results clearly suggest that the compound might have some protective effect on the gastric mucosa in addition to its effect on gastric secretion. In contrast to prednisolone-induced lesions which were markedly prevented by NC-1300-O-3, the compound had only a weak effect on compound 48/80-induced lesions when administered once daily. However, the compound significantly prevented the development of the lesions when administered twice daily. It is unknown whether NC-1300-O-3 prevented the lesions through its antisecretory activity or mucosal protective activity. These findings indicate that while a gastric acid factor might play an important role in the pathogenesis of prednisolone-induced lesions, the factor would not contribute substantially to the pathogenesis of compound 48/80-induced lesions.

Furthermore, NC-1300-O-3 markedly protected the gastric mucosa against various necrotizing agent-induced gastric damages. The ED$_{50}$ values for all these lesions were less than 10 mg/kg. Similar to in the case of its antisecretory effect, the compound significantly protected the gastric mucosa against HCl-ethanol-induced lesions for more than 24 hr after administration. This long-acting activity was almost the same as that observed with NC-1300 (1) and NC-1300-B (4). The mechanism underlying this prolonged activity remains unknown. Mattsson et al. (7) reported that omeprazole markedly protected the gastric mucosa against 100% ethanol-induced gastric damage, but had no effect when administered through the parenteral route. In the present study, we found that NC-1300-O-3, administered i.p. or s.c., significantly prevented HCl-ethanol-induced damage, although the degree of the protection was weaker than that seen after p.o. administration. Except for 100% ethanol, the necrotizing agents (HCl-ethanol, HCl-aspirin or HCl-taurocholate) all involved a 150 or 200 mM HCl solution. Thus, the effect of NC-1300-O-3 on the gastric mucosa would not be due to its antisecretory effect but to its mucosal protective effect. The mechanism of action of the compound warrants further investigation.

NC-1300-O-3 also significantly inhibited the development of duodenal ulcers induced by two ulcerogens, mepirizole and cysteamine. These ulcers are presumed to be induced by the inflow of accumulated gastric juice into the proximal duodenum, which has a weakened defensive mechanism (26). Accordingly, the anti-ulcer effect of NC-1300-O-3 is most likely due to its potent and long-acting antisecretory activity. Omeprazole has been reported to have no effect on duodenal HCO$_3^-$ secretion (27). Therefore, it is unlikely that NC-1300-O-3 stimulates duodenal HCO$_3^-$ for protection of the duodenal mucosa.

We conclude that NC-1300-O-3, a new gastric proton pump inhibitor, will be a beneficial drug for the treatment of peptic ulcers by virtue of its potent antisecretory and mucosal protective activities.
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