Biochemical and Pharmacological Analysis of 2-[(2-Dimethylaminobenzyl)Sulfinyl] Benzimidazole (NC-1300), a New Proton Pump Inhibitor

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Abstract—Biochemical and pharmacological properties of a newly synthesized compound, 2-[(2-dimethylaminobenzyl)sulfinyl] benzimidazole (NC-1300), were studied. NC-1300, at pH 6.0, potently inhibited the activity of H⁺K⁺ATPase in the rabbit gastric mucosa, thereby classifying it as a proton pump inhibitor. The inhibitory efficacy of NC-1300 on the pump was much the same as that seen with omeprazole. NC-1300 had no effect on acetylcholine-stimulated ileum contraction in guinea pigs at 10⁻⁵ M, but it non-competitively inhibited the contraction at 10⁻⁴ M. NC-1300 had no effect on histamine-stimulated atrial beating frequency in guinea pigs at 10⁻⁴ or 10⁻⁵ M. NC-1300, given either intraduodenally or orally, had a potent and long-lasting (more than 24 hr) inhibitory effect on gastric secretion in pylorus-ligated rats. Pretreatment with NC-1300 dose-dependently protected the gastric mucosa from damage induced by pylorus ligation, water-immersion stress, aspirin, and indomethacin, and the duodenal mucosa from damage induced by mepirizole in rats. We conclude that the antisecretory activity of NC-1300 appears to be mainly related to an inhibition of H⁺K⁺ATPase, while its antigastric and antiduodenal lesion activities are primarily related to an antisecretory effect.

Omeprazole, a benzimidazole derivative, inhibits gastric secretion of both humans and laboratory animals by inhibition of H⁺K⁺ATPase in the parietal cells (1-5). The agent is thus called a proton pump inhibitor. Because of its antisecretory effect, this agent has a beneficial effect on gastric and duodenal ulcers, in both man and animals (6-9). We found that omeprazole significantly prevents the formation of acutely-induced gastric and duodenal lesions, and it accelerates the healing of pre-existing gastric ulcers induced in rats (10, 11).

We synthesized new compounds with benzimidazole in the chemical structures and tested them for proton pump inhibiting potential. The compound 2-[(2-dimethylaminobenzyl)sulfinyl] benzimidazole (NC-1300, Fig. 1) was found to have a potent anti-proton pump activity. We report herein the biochemical and pharmacological properties of this compound. The antisecretory, antigastric and antiduodenal lesion activities of NC-1300 were compared with these activities seen in the case of the histamine H₂-receptor antagonist cimetidine.

Fig. 1. Structural formula for NC-1300 (C₁₆H₁₇N₅O₅S, MW. 229.4).
Materials and Methods

Biochemical studies

Preparation of rabbit gastric membranes enriched in H⁺K⁺ATPase: Gastric H⁺K⁺-ATPase was purified from the parietal cell-rich fraction of rabbit stomach as described by Saccomani et al. (12), with slight modifications. Briefly, the stomach of a male Japanese white rabbit (1.5–2.5 kg) was dissected out and washed in tap water. The fundic mucosal surface was flooded with cold 1 M NaCl, and most of the surface epithelial cells were removed with a spatula. The remaining fundic mucosa was then scraped from the underlying muscular layer and suspended in about 10 volumes of a cold solution of 250 mM sucrose, 20 mM Tris-HCl buffer at pH 7.4. Homogenization was carried out in a Teflon-glass homogenizer (Potter-Elvehjem type, Takashima) with 10 up-down strokes. The resulting homogenate was centrifuged at 8,000 × g for 10 min. The pellet was washed once, and the combined supernatants were centrifuged at 77,000 × g for 60 min to yield the crude microsomal pellet. The 77,000 × g pellet was resuspended in the same solution, and after homogenization it was distributed by centrifugation at 77,000 × g for 180 min in a discontinuous density gradient. The vesicles enriched in the gastric H⁺K⁺ATPase were collected at the interface of 250 mM sucrose, 7% Ficoll (w/w) +250 mM sucrose layers. The vesicle preparation was stored in 250 mM sucrose (unbuffered) at 4°C. Protein concentration was determined by the microbiuret method (13) using bovine serum albumin as standard. The specific activity of H⁺K⁺ATPase in the final fraction was 18.2 amol Pi/mg of protein/hr at 37°C, after which the enzyme reaction was started by the addition of 0.5 ml of a mixture containing 4 mM MgCl₂, 4 mM ATP, and 80 mM imidazole buffer (pH 7.4), with or without 20 mM KCl. After incubation for 15 min at 37°C, the reaction was terminated by adding 1 ml of 24% trichloroacetic acid, and the inorganic phosphate from ATP was measured by the method of Taussky and Shorr (14). NC-1300 and omeprazole were synthesized by the Chemistry Department of Nippon Chemiphar. Substrates used for the biochemical assays were obtained from Boehringer Mannheim. All of the chemicals used were of reagent grade.

Pharmacological studies

To test the activity of NC-1300 on cholinergic and histamine H₂-receptors, the following experiments were done.

Studies on the ileum contraction: Male guinea pigs (260–350 g) were deprived of food but allowed free access to water for 18 hr. The animals were killed, and 2 cm of the terminal ileum was removed and prepared into strips which were suspended in an organ bath containing Tyrode solution, bubbled with 95% O₂ and 5% CO₂ at 30°C. The contractile response to acetylcholine hydrochloride (Daiichi) was measured isotonically and recorded on a kymograph. NC-1300 sodium and atropine sulfate (Wako) as a reference drug was dissolved in distilled water, and it was added 5 min prior to the cumulative addition of acetylcholine at 10⁻⁵, 3 × 10⁻⁵, 10⁻⁴ M and 10⁻⁸ M, respectively.

Studies on the chronotropic response: Male guinea pigs (280–550 g) were killed, and the right atrium was dissected free and mounted in an organ bath containing Krebs-Henseleit solution, bubbled with 95% O₂ and 5% CO₂ at 37°C. A force displacement transducer (Nihon Koden, TB-611T) was connected to the atrium. The frequency of contraction was recorded by a cardio-tachometer (Nihon Koden, RT-5) triggered by the contractile force under a 400 mg load. After a stable basal beating frequency was established, a cumulative concentration-response curve to histamine dihydrochloride (Wako) was obtained with each preparation. The final concentration of histamine in the bath was increased
gradually from $10^{-7}$ to $3 \times 10^{-4}$ M. NC-1300 sodium salt at $10^{-5}$, $3 \times 10^{-6}$ or $10^{-4}$ M, or cimetidine (Sigma) at $3 \times 10^{-6}$ M, as a reference drug, was added 10 min prior to addition of histamine.

**Gastric secretion:** Male Donryu rats (220–240 g), deprived of food but allowed free access to water for 24 hr prior to experiments, were used. Two kinds of experiments were done. In the first studies, the rats were anesthetized with ether, the abdomen incised, and the pylorus ligated. Four or 14 hr after the pylorus ligation, the animals were killed, and the gastric contents were collected and analyzed for volume, acidity and pepsin activity. Acidity was determined by automatic titration of the gastric juice against 0.1 N NaOH to pH 7.0 (Auto burette, Radiometer). Pepsin activity was determined by Anson’s method using bovine albumin as a substrate (15). Titratable acid and pepsin output were expressed as µEq/hr and mg tyrosine/hr, respectively. NC-1300, cimetidine (Sigma), or the vehicle alone as a control was given intraduodenally immediately after ligating the pylorus. The volume of each drug or vehicle was 0.5 ml/100 g body weight. Either NC-1300 or cimetidine was suspended in 1% carboxymethylcellulose (CMC) or distilled water, respectively. In a second study, NC-1300 or the vehicle alone was first given orally by gastric intubation to 24 hr-fasted rats. Twenty hours later, during which time the animals were still fasted, the pylorus was ligated. The gastric contents were collected 4 hr after the pylorus ligation and analyzed for volume, acidity and pepsin activity as described above. Since the effect of cimetidine disappeared 14 hr after administration, the second experiment was done only with NC-1300.

**Production of gastric and duodenal lesions:** Four different types of gastric lesion models were induced in male Donryu rats (220–260 g), which were deprived of food but allowed free access to water for 15 to 48 hr prior to experiments.

**Shay ulcers:** Under ether anesthesia the abdomen of rats fasted for 48 hr was incised and the pylorus ligated. Fourteen hours later, the animals were killed, and the forestomach was examined for ulcers. Each test drug or the vehicle alone was given orally in a volume of 0.5 ml/100 g body weight 30 min before pylorus ligation.

**Water-immersion stress-induced erosions:** Rats fasted for 15 hr before experiments were placed in a restraint cage, the same as the one described in detail elsewhere (16). Rats were then immersed vertically to the level of the xiphoid process in a water bath (21°C) for 10 hr (17) and killed. The stomach of each rat was removed and inflated by injecting 12 ml of 2% formalin 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 examined for erosions in the glandular portion. Each test drug or the vehicle alone was given orally 10 min before stressing.

**Aspirin-induced erosions:** Under ether anesthesia, the abdomen of rats fasted for 24 hr before experiments was incised and the pylorus ligated. Aspirin (Merck), suspended in 1% CMC, was given orally at 150 mg/kg 5 min after pylorus ligation (18). Seven hours later, the animals were killed, and the stomach was examined for erosions in the glandular portion. Each test drug or the vehicle alone was given orally 30 min before pylorus ligation.

**Indomethacin-induced erosions:** Indomethacin (Sigma), suspended in Tween-saline, was given in a dose of 25 mg/kg subcutaneously to rats fasted for 24 hr before experiments. These rats were killed 7 hr later, and the stomach was examined for erosions in the glandular portion. Each test drug or the vehicle alone was given orally 30 min before the indomethacin treatment.

**Mepirizole-induced duodenal ulcers and gastric erosions:** Since Donryu rats are less sensitive to mepirizole-induced duodenal ulcers (19), male Sprague-Dawley rats (250–270 g) were used in the following study. Mepirizole (Daiichi), suspended in 1% CMC, was given subcutaneously at 200 mg/kg to non-fasted rats, which were then deprived of food and water (20). Twenty-four hours later, the animals were killed and
examined for ulcers in the duodenum and erosions in the antrum. NC-1300 or the vehicle alone was given orally once 30 min before mepirizole administration. Cimetidine or the vehicle alone was given twice, i.e., 30 min before and 9 hr after mepirizole administration.

**Ulcer or erosion index:** The area (mm²) of each Shay ulcer was measured under a dissecting microscope with a square grid (×10), summed, and arbitrarily classified into five degrees as follows:

| Ulcerated area (mm²) | 0 1–8 9–16 17–24 25–32 >32 |
|---------------------|-----------------------------|
| Ulcer index         | 0 1 2 3 4 5 |

Length (mm) of each erosion induced by water-immersion stress, aspirin, or indomethacin was also measured under a dissecting microscope (×10), summed, and used as an erosion index. The areas (mm²) of mepirizole-induced doudenal ulcers and gastric erosions were also measured, summed, and used as an ulcer or an erosion index.

**Analysis of data**

Student's t-test was used to determine the statistical significance of the data, and P<0.05 was regarded as significant. The dose of NC-1300 which inhibited 50% (ED50) of the formation of various gastric and duodenal lesions was calculated by the method of least-squares regression analysis.

**Results**

Effects on H⁺K⁺ATPase: Both NC-1300 and omeprazole concentration-dependently inhibited H⁺K⁺ATPase activity in isolated rabbit gastric mucosa (Fig. 2). The concentration of NC-1300 which inhibited 50% (IC50) of H⁺K⁺ATPase activity in the presence of 10 mM KCl was $6.6 \times 10^{-6}$ M and $2.0 \times 10^{-5}$ M at pH 6.0 and pH 7.4, respectively. The IC50 for omeprazole was $1.0 \times 10^{-5}$ M and $7.2 \times 10^{-5}$ M at pH 6.0 and pH 7.4, respectively.

Effects on ileum contraction to acetylcholine: NC-1300, at a concentration of $10^{-6}$ M or $3 \times 10^{-5}$ M, had little or no effect on ileum contraction, in response to acetylcholine (Fig. 3). At $10^{-4}$ M, however, the contraction was non-competitively inhibited. Atropine sulfate at $10^{-8}$ M caused a rightward shift of the dose-response curve.

Effect on chronotropic response to histamine: NC-1300 at $10^{-5}$ or $3 \times 10^{-5}$ M had no effect on the increased atrial beating.

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![Graph](image-url)

**Fig. 2.** Effects of NC-1300 and omeprazole on H⁺K⁺ATPase obtained from the rabbit gastric mucosa. Enzyme (~80 µg protein) was preincubated with the indicated concentration of NC-1300 or omeprazole at pH 6.0 or 7.4. Remaining enzyme activity was then determined at pH 7.4. Activity without inhibitor was taken as 100%. 
frequency in response to histamine (Fig. 4). In contrast, cimetidine at $3 \times 10^{-5}$ M caused a rightward shift of the concentration-response curve.

**Effects on gastric secretion:** NC-1300, given intraduodenally immediately after pylorus ligation, dose-dependently inhibited gastric secretion (volume, acid and pepsin output) for 4 hr after the administration (Fig. 5). The acid output was inhibited by about 95% at 30 mg/kg of NC-1300 in contrast to the control value. At 100 mg/kg, no titratable acidity was observed in the stomach. Cimetidine, given at 150 mg/kg, also sig-

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**Fig. 3.** Effects of NC-1300 (Na) and atropine sulfate on ileum contraction of guinea pigs in response to acetylcholine.

**Fig. 4.** Effects of NC-1300 (Na) and cimetidine on atrial beating frequency of guinea pigs in response to histamine.
significantly inhibited gastric secretion in the pylorus-ligated rats for 4 hr. The antisecretory activity of NC-1300 persisted for even 14 hr after administration. At 100 mg/kg, the volume, acid and pepsin output were inhibited 59.1%, 93.8% and 77.5%, respectively. The effect of 150 mg/kg of cimetidine on gastric secretion disappeared by the 14th hr after administration. ED50 values of NC-1300 on gastric volume, acid and pepsin output were 14.0, 6.4 and 21.0 mg/kg (4 hr-ligated rats) and 86.0, 30.0 or 48.5 mg/kg (14 hr-ligated rats), respectively.

The antisecretory effect of 30 mg/kg of NC-1300 disappeared when determined between 20 to 24 hr after administration.

**Fig. 5.** Effects of NC-1300 and cimetidine on gastric secretion in pylorus-ligated rats. NC-1300 and cimetidine were given intraduodenally immediately after pylorus ligation. Animals were killed 4 or 14 hr after pylorus ligation.
(Fig. 6). However, 100 mg/kg of NC-1300 had an antisecretory effect even 24 hr later. The inhibition of acid and pepsin output was 97.5% and 52.9%, respectively.

Effects on gastric lesions: All control rats subjected to pylorus ligation, water-immersion stress, aspirin, or indomethacin treatment had gastric ulcers either in the forestomach or gastric erosions in the glandular portion. Pretreatment with NC-1300 dose-dependently inhibited Shay ulcers, water-immersion stress-, aspirin- and indomethacin-induced erosions (Figs. 7 and 8). There was a complete or nearly complete inhibition of Shay ulcers, water-immersion stress-, or aspirin-induced erosions when 30 mg/kg of NC-1300 was administered. Even at 10 mg/kg, water-immersion stress- and aspirin-induced erosions were significantly inhibited by 78.3% and 56.0%, respectively. Indomethacin-induced erosions were not affected with 10 mg/kg of NC-1300, but these erosions were significantly inhibited with 30 mg/kg (63.5%) and 100 mg/kg (99.4%). While 150 mg/kg of cimetidine had no effect on Shay ulcers, it potently inhibited water-immersion stress-, aspirin- and indomethacin-induced erosions. ED50 values of NC-1300 for Shay ulcers, water-immersion stress-, aspirin- and indomethacin-induced erosions were 9.4, 2.2, 7.6 and 31.8 mg/kg, respectively.

Effect on duodenal and gastric lesions: In all control rats given 200 mg/kg of mepirizole, two penetrating ulcers were consistently induced in the proximal duodenum, and there were several small erosions in the antrum 24 hr later. A single oral administration of NC-1300 dose-dependently inhibited the development of mepirizole-induced duodenal ulcers, but not the gastric erosions (Fig. 9). While cimetidine given twice at 100 mg/kg potently inhibited the development of mepirizole-induced duodenal ulcers, it had no effect on gastric erosions. The ED50 value of NC-1300 for mepirizole-induced duodenal ulcers was 6.0 mg/kg.

Discussion

These data indicate that NC-1300 is a newly synthesized proton pump inhibitor with potent antisecretory, antigastric and antiduodenal lesion activities in rats.

The inhibitory activity and properties of the action of NC-1300 on rabbit gastric H⁺K⁺ATPase was much the same as that seen with omeprazole. The IC50 values of NC-1300 and omeprazole are \(6.6 \times 10^{-6}\) M and \(1 \times 10^{-5}\)
M at pH 6.0, respectively. Our results confirmed the findings of Wallmark et al. (21) who found that the activity of omeprazole is pH dependent; the efficacy of omeprazole was potent when the pH of the medium was 6.0, but it became weak at pH 7.4. NC-1300 also showed a pH dependent response; the inhibition was about 3 times more potent at pH 6.0 compared with that obtained at pH 7.4.

The substituted benzimidazoles are completely devoid of any anticholinergic and histamine H₂-receptor blocking activities (22). NC-1300 was also found to have little or no anticholinergic and H₂-receptor blocking actions in in vitro experiments at 10⁻⁵ M which inhibited H⁺K⁺ATPase activity. However, NC-1300 showed a non-competitive inhibition on acetylcholine-induced ileum contraction when 10⁻⁴ M was given. Therefore, it is unknown at the present time whether the antisecretory activity of NC-1300 is due to either the inhibition of H⁺K⁺-ATPase action alone or an anticholinergic activity as well. If NC-1300 at the dose which sufficiently inhibits gastric secretion should be proved to have little or no effect on gastric emptying in an in vivo study, it will be appropriate to state that the antisecretory action of NC-1300 is mainly due to the proton pump inhibitory action. Influence of NC-1300 on gastric emptying will be the

**Fig. 7.** Effects of NC-1300 and cimetidine on Shay ulcers and water-immersion stress-induced erosions in rats. NC-1300 and cimetidine were given orally 30 or 10 min before pylorus ligation or stress, respectively. Animals were killed 14 or 10 hr after pylorus ligation or stress, respectively.

**Fig. 8.** Effects of NC-1300 and cimetidine on aspirin (150 mg/kg, p.o.)-induced gastric erosions in pylorus-ligated rats and indomethacin (25 mg/kg, s.c.) induced gastric erosions in intact rats. NC-1300 and cimetidine were given orally 30 min before pylorus ligation in the case of aspirin-induced erosions and indomethacin treatment. Animals were killed 7 hr after aspirin or indomethacin treatment.
subject of our ongoing studies.

We reported the potential and long-lasting effect of omeprazole on gastric secretion in pylorus-ligated rats (10). In these experiments, the ED50 values of omeprazole on gastric secretion determined in the 7 hr-pylorus ligation preparation were 17.0, 5.2 and 51.5 mg/kg for volume, acid and pepsin output, respectively. The values obtained with NC-1300 in the 4 hr-pylorus ligation preparation were much the same. The present study revealed that the gastric acid output was nearly completely inhibited with NC-1300 even 24 hr after administration. In contrast, the antisecretory effect of omeprazole decreased by about 40% of the control value at 24 hr (data not shown). Other investigators (4, 23) demonstrated that while the plasma half-life of omeprazole is only 1 hr, the duration of antisecretory activity continues for over 24 hr. The half life of plasma NC-1300 after oral administration of 200 mg/kg was 1.36±0.2 hr (S. Okabe et al., unpublished data). Therefore, it is unlikely that the antisecretory effect of NC-1300 as well as omeprazole is related to its plasma concentration, but rather likely that it is related to the irreversible inhibition of H+K+ATPase. The antisecretory effect of cimetidine was observed only for 4 hr after administration. If NC-1300 has such a long lasting effect in humans, then this drug could be used to treat clinical cases of hypersecretion or peptic ulcer diseases and frequent dosing would not be necessary.

Pretreatment of rats with NC-1300 had a potent protective effect on gastric and duodenal mucosa from injury caused by pylorus ligation, water-immersion, aspirin, indomethacin and mepirizole treatment. The formation of these lesions was almost completely inhibited with a dose of 30 mg/kg. All of these acute lesions were induced within 14 hr after pylorus ligation, stress or mucosal irritants. Therefore, the mechanism of action of NC-1300 on these lesions may be primarily caused by its antisecretory effects which persisted for more than 14 hr. While NC-1300 as well as cimetidine potently inhibited the development of various gastric lesions, they had little or no effect on gastric lesions which were induced by mepirizole.
These results suggest that gastric lesions in response to mepirizole are caused by mechanisms other than the gastric acid factor.

The ED50 values of omeprazole on Shay ulcers, water-immersion stress, aspirin and indomethacin-induced gastric lesions were 8.7, 8.7, 0.8 and 1.8 mg/kg, respectively, and on mepirizole-induced duodenal lesions, it was about 5.4×2 mg/kg (10). The ED50 values of NC-1300 on water-immersion stress-induced gastric erosions (2.2 mg/kg) and mepirizole-induced duodenal ulcers (6.0 mg/kg) were smaller than those of omeprazole. However, the ED50 values of NC-1300 on Shay ulcers, aspirin- and indomethacin-induced gastric erosions were larger than those obtained with omeprazole. Since omeprazole is chemically unstable and rapidly degraded in acidic media, it must be prepared as an encapsulated, enteric-coated granulate for clinical studies (3). When we administered omeprazole in our laboratory experiments, we suspended the agent in 1% CMC, the pH of which was adjusted to 9.0 with NaHCO3 and NaOH. NC-1300 is also known to be unstable in acidic media (unpublished data). However, we suspended this agent in 1% CMC, the pH of which was 7.5. Therefore, if the pH of NC-1300 was adjusted to pH 9.0 as in the case of omeprazole, the ED50 values will be much smaller.

Mattsson et al. (8) reported that oral administration of omeprazole significantly inhibited ethanol-, HCl-aspirin- or 35% NaCl-induced gastric erosions in rats. In addition, Konturek et al. (9) also demonstrated that oral, but not subcutaneous, omeprazole at nonantisecretory doses significantly protected the gastric mucosa from ethanol- and HCl-aspirin-induced injuries. These results suggest that omeprazole has a cytoprotective activity on the gastric mucosa. We reported that prolonged administration of omeprazole for up to 8 weeks significantly accelerated the healing of acetic acid-induced gastric ulcers in rats (11). Whether or not NC-1300 also has a cytoprotective effect and accelerates healing of chronic gastric ulcers is a subject of our ongoing studies.

We conclude that NC-1300, a new proton pump inhibitor, is equally effective as omeprazole with regard to inhibiting gastric secretion and the formation of gastric and duodenal lesions in rats.

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