Effects of NC-1300 and Its Degradation Products on Gastric Secretion and HCl-Ethanol-Induced Gastric Lesions in Rats

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Accepted January 16, 1987

Abstract—The proton pump inhibitor NC-1300 has antisecretory and cytoprotective activities in rats. The compound is labile at an acidic pH and degrades into various products. We attempted to identify these products after treatment at different pHs in vitro using HPLC. We also examined whether (A) NC-1300 treated at acidic pHs loses its efficacy on gastric secretion and gastric lesions and (B) whether the degradation products of NC-1300 have pharmacological effects in rats. The acidic degradation products proved to be mainly NC-1300-sulfide and partly *o*-dimethylaminobenzylalcohol (*o*-DMABA) and benzimidazole (BI). NC-1300, pretreated at pH 1.0, 1.25 or 1.5 for 30 min and given p.o. at 30 mg/kg, significantly inhibited gastric acid secretion in pylorus ligated rats and prevented development of HCl-ethanol-induced gastric mucosal lesions. The degree of antisecretory and cytoprotective activities by NC-1300 treated at pH 1.5 was almost the same as that obtained by NC-1300 treated at pH 7.0. NC-1300-sulfide or mixtures of degradation products, with or without unchanged NC-1300, also significantly inhibited the gastric acid secretion and lesion formation. We conclude that while NC-1300 degrades at low pHs, the compound treated at such a low pH exerts pharmacological effects presumably by the unchanged form of NC-1300 and/or its degradation products.

Omeprazole, a gastric proton pump inhibitor, is unstable at acidic circumstances and is rapidly degraded into several products (1, 2). Therefore, the drug is usually given in alkalinized form with sodium bicarbonate to experimental animals or given clinically in enteric-coated granules (3–5). We have developed a new proton pump inhibitor, 2-[(2-dimethylaminobenzyl)sulfinyl] benzimidazole (NC-1300), and determined its effects on gastric acid secretion and experimental ulcers induced in rats (6, 7). NC-1300, given p.o. without alkalinization, exerts potent and long-lasting antisecretory and anti-ulcer effects. The present study was designed to determine (A) the stability of NC-1300 in various pH solutions in vitro and (B) the pH levels in which NC-1300 in solution loses its pharmacological potency on gastric acid secretion and HCl-ethanol-induced gastric mucosal

lesions in rats.

Materials and Methods

Stability test for NC-1300: Stability of NC-1300 at different pH solutions was determined in vitro. NC-1300 was suspended in 0.2% carboxymethylcellulose (CMC) solution (30 mg/ml). One-half ml of the suspension was put into 0.5 ml of solutions of 100 mM HCl (pH 1.0), 56.2 mM HCl (pH 1.25), 31.6 mM HCl (pH 1.5), or pH 7.0 and shaken gently in a water bath for 5, 10, 20 and 30 min at 37°C. These solutions were then mixed with 1 ml of buffer solution (pH 7.4, 0.5 M phosphate buffer—0.126 M NaOH, 1:1, v/v). Subsequently, the solution was mixed with 8 ml of methanol by a vortex mixer and centrifuged at 3,000 rpm for 10 min at 4°C. One ml of the supernatant was mixed with 4 ml of methanol and the preparation recentrifuged

under the same conditions described above. The supernatant (10 μ l) was analyzed using an HPLC (Shimadzu, LC-3A) equipped with a variable wavelength UV monitor (Shimadzu, SPD-2A). NC-1300 gradually degrades into various products such as NC-1300-sulfide, *o*-dimethylaminobenzyl alcohol (*o*-DMABA), benzimidazole (BI), mercaptobenzimidazole (MBI) and NC-1300-sulfone (Fig. 1). The reversed-phase HPLC separation was carried out with Finepak Sil C₁₈ (4.6 ϕ ×250 mm), 5 μ m particles (Jasco), and the UV wavelength was set at 285 nm (for NC-1300, NC-1300-sulfide) and 240 nm (for BI, *o*-DMABA). The mobile phase consisting of methanol—10 mM phosphate buffer (pH 7.5) (65:35, v/v) which contained 10 mM tetra-n-butyl-ammonium-bromide was supplied at a flow rate of 1.0 ml/min at 40°C.

Pharmacological studies: The effects of NC-1300 alone, NC-1300 pretreated at different pHs and then adjusted to pH 7.0 with 0.1 M NaOH, NC-1300-sulfide alone, or a mixture of NC-1300 and/or its degradation products on gastric secretion and HCl-ethanol-induced gastric mucosal lesions were studied as follows. Mixtures A and B consist of NC-1300 and its degradation products obtained after treatment at pH 1.0

and pH 1.5 for 30 min. Accordingly, mixture A consists of NC-1300-sulfide (20.1 mg/kg) + *o*-DMABA (4.9 mg/kg) + BI (4.7 mg/kg) and mixture B consists of NC-1300 (12.9 mg/kg) + NC-1300-sulfide (10.4 mg/kg) + *o*-DMABA (3.5 mg/kg) + BI (3.2 mg/kg). All these substances were suspended in 1% CMC before administration to rats.

Male Sprague-Dawley rats (220–250 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 withheld for 2 hr before the start of the experiments. These rats were kept in raised mesh-bottom cages to prevent coprophagy. Twelve animals were used for each study.

Gastric secretion studies were done using pylorus ligation preparations. With the rats under ether anesthesia, the abdomen was incised and the pylorus ligated. Three hr after this ligation, the animals were killed and the gastric contents collected and analyzed for volume and acidity. Acidity was determined by automatic titration of the gastric juice against 0.1 M NaOH to pH 7.0 (Autoburette, Radiometer). Titratable acid output was expressed as μ Eq/hr. NC-1300 and degradation products were given p.o., 0.5 hr before ligating the pylorus. The vehicle alone was

Fig. 1. Chemical structures of NC-1300 and its acidic degradation products. An example of the HPLC elution pattern of NC-1300 and degraded products after treatment at pH 1.25 for 10 min at 37°C.

given p.o. to the control animals. The volume of each compound was 0.5 ml/100 g body wt.

Gastric mucosal lesions were produced by giving p.o. 1 ml/200 g body wt. of 60% ethanol (v/v) in 150 mM HCl (HCl-ethanol) (8, 9), and the animals were killed 1.5 hr later. The stomachs were removed and inflated by injecting 12 ml of 2% formalin to fix the gastric wall for 10 min. Subsequently, the stomachs were incised along the greater curvature and examined for necrotic lesions in the glandular mucosa. The length (mm) of each lesion was macroscopically measured under a dissecting microscope ($\times 10$) with a square grid and summed per stomach. The person (S.O.) who evaluated the lesions had no knowledge of which treatment the animals had been given. NC-1300 and degradation products were given p.o. at 0.5 hr before p.o. administration of HCl-ethanol. The vehicle alone was given to the control animals. The volume of each substances was 0.5 ml/100 g body wt.

Compounds: NC-1300, NC-1300-sulfide,

and o-DMABA were gifts from Nippon Chemiphar Co., and BI 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

Degradation of NC-1300 at acidic pH:

When NC-1300 (30 mg/ml) was mixed in the solution of pH 1.0, it was gradually degraded to NC-1300-sulfide, o-DMABA, and BI (Fig. 2). Thirty min later, the contents of the solution consisted mainly of NC-1300-sulfide (20.1 mg/ml) and partly of o-DMABA (4.9 mg/ml) and BI (4.7 mg/ml). There was no measurable amount of NC-1300 in the solution. Almost the same changes were observed when NC-1300 was mixed in the solution of pH 1.25, although a small amount (2.1 mg/ml) of NC-1300 was detected 30 min later. The remaining contents were NC-

Fig. 2. Concentrations of NC-1300 and its degradation products after treatment in different pH solutions for up to 30 min at 37°C. Degree of degradation of NC-1300 depends on the pH of dissolved or suspended solutions. At pH 7.0, there was a negligible degradation of NC-1300.

Fig. 3. Effects of NC-1300, treated at different pHs for 30 min and then adjusted to pH 7.0, on the gastric secretion in pylorus-ligated rats. The potency of acid inhibitory activity of NC-1300 increased as the compound was treated at a higher pH; the effects of NC-1300 treated at pH 1.5 and 7.0 were almost the same.

1300-sulfide (16.7 mg/ml), o-DMABA (5.7 mg/ml) and BI (5.4 mg/ml). In the solution of pH 1.5, the degradation of NC-1300 was slight compared with that seen in the solution of pH 1.0 or 1.25. The contents of the solution determined 30 min after mixture were NC-1300 (12.9 mg/ml), NC-1300-sulfide (10.4 mg/ml), o-DMABA (3.5 mg/ml) and BI (3.2 mg/ml). The contents of MBI and NC-1300-sulfone in the degraded products under any pH conditions were negligible, as shown in an example of the HPLC elution pattern seen after treatment of NC-1300 at pH 1.25 for 10 min (Fig. 1). The half life of NC-1300 was 4.2, 7.7 and 36.3 min at pH 1.0, pH 1.25 and pH 1.5, respectively. When NC-1300 was mixed in the solution of pH 7.0, there was practically no degradation during 30 min; The contents were NC-1300 (29.1 mg/kg) and a trace of NC-1300-sulfide, o-DMABA and BI.

Effects of NC-1300 treated in different pH solutions on gastric secretion: NC-1300 (30 mg/kg) treated at any pH and given p.o. slightly reduced the volume of gastric juice, although reduction by the compound treated at pH 1.25 was significantly different from the controls (Fig. 3). NC-1300, treated at pH 1.0, 1.25 or 1.5 significantly reduced the gastric acid output by 36.1%, 51.4% or 89.7%, respectively. NC-1300, suspended at pH 7.0, also significantly and potently reduced gastric acid output by 94.9%.

Fig. 4. Effects of NC-1300, treated at different pHs for 30 min and then adjusted to pH 7.0, on HCl-ethanol-induced gastric mucosal lesions in rats. The potency of preventive activity of NC-1300 increased as the compound was treated at a higher pH; the effects of NC-1300 treated at pH 1.5 and 7.0 were almost the same.

Effect of NC-1300 treated in different pH solutions on HCl-ethanol-induced gastric lesions: In control animals, there were multiple red streak lesions in the glandular stomach (mainly in the corpus mucosa) 1.5 hr after HCl-ethanol administration. NC-1300 (30 mg/kg), treated at pH 1.0 or 1.25, slightly but significantly prevented HCl-ethanol-induced gastric mucosal lesions, the prevention being 27.1% or 32.4%, respec-

tively (Figs. 4 and 5). However, NC-1300 (30 mg/kg), treated at pH 1.5 or 7.0, pronouncedly protected the gastric mucosa against the lesions, the rate being 87.3% or 94.9%, respectively (Fig. 5).

Effects of NC-1300 and degradation products on gastric secretion: Pretreatment with NC-1300 (29.1 mg/kg) reduced the volume of gastric juice by 40.0%, and the compound (12.9 or 29.1 mg/kg) reduced the gastric acid output by 72.2% or 95.1%, respectively (Fig. 6). NC-1300 sulfide (10.4 or 20.1 mg/kg) had no effect on the volume of gastric juice, but did reduce the gastric

acid output by 36.5% or 55.6%, respectively. Both mixtures A and B significantly reduced both the volume and acid output. The reduction of volume by mixtures A or B was 35.5% or 40.0%, and the reduction of acid output by mixtures A or B was 61.5% or 93.3%, respectively.

Effects of NC-1300 and degradation products on HCl-ethanol-induced gastric mucosal lesions: Pretreatment with NC-1300 potently and dose-dependently prevented lesion formation induced by HCl-ethanol, the prevention being 81.3% and 97.8% at 12.9 or 29.1 mg/kg, respectively (Fig. 7).

Fig. 5. Gross appearances of the stomachs of rats given 1 ml/200 g body wt. of HCl-ethanol. A: The stomach of a control rat (left), the stomach of a rat given NC-1300 treated at pH 1.0 (right). B: The stomach of a control rat (left), the stomach of a rat given NC-1300 treated at pH 1.5 (right). Note that NC-1300 treated at pH 1.5 potently prevented the development of HCl-ethanol-induced gastric mucosal lesions.

Fig. 6. Effects of NC-1300, NC-1300-sulfide, and mixtures A and B on the gastric secretion in pylorus-ligated rats. Mixture A consists of NC-1300-sulfide (20.1 mg/kg), o-DMABA (4.9 mg/kg) and BI (4.7 mg/kg), and mixture B consists of NC-1300 (12.9 mg/kg), NC-1300-sulfide (10.4 mg/kg), o-DMABA (3.5 mg/kg) and BI (3.2 mg/kg). NC-1300 alone, NC-1300-sulfide alone, and mixtures A and B significantly inhibited the gastric acid secretion.

Fig. 7. Effects of NC-1300 alone, NC-1300-sulfide alone, and mixtures A and B (see the footnote of Fig. 6) on HCl-ethanol-induced gastric mucosal lesions in rats. Except for the low dose of NC-1300-sulfide, all of the compounds significantly prevented development of the lesions.

NC-1300-sulfide (10.4 mg/kg) did not prevent the gastric mucosal lesions, but it (20.1 mg/kg) did prevent the lesion formation at a rate of 41.1%. Mixtures A and B also significantly prevented the formation of gastric mucosal lesions by 42.5% and 68.0%, respectively.

Discussion

This study shows that while NC-1300 is stable at neutral pH, it is unstable in highly acidic conditions and degrades into several products. We reported that the acidic de-

gradation products of NC-1300 are NC-1300-sulfide, mercaptobenzimidazole (MBI) and NC-1300-sulfone (7). However, the present study proved that the contents of MBI and NC-1300-sulfone in the degraded products were much smaller than those of o-DMABA and BI. Therefore, the influence of MBI and NC-1300-sulfone on gastric secretion and gastric mucosal damage was disregarded in this study. In solutions of pH 1.0 or 1.25, NC-1300 was almost completely degraded within 10 to 20 min after mixture, and it was transformed mainly to the reduced

form (NC-1300-sulfide) and partly to its fragments (o-DMABA, BI). However, the half life of NC-1300 at pH 1.5 was over 30 min. These results indicate that NC-1300 is much more stable than omeprazole, the half life at pH 1.0 to 2.0 being about 2 min (2).

In a foregoing study, we demonstrated that NC-1300 has little or no effect on the volume of gastric juice, but does potentially reduce the gastric acid output in pylorus-ligated rats (7). We confirmed these findings in the present study when NC-1300 was given in a suspension form with 1% CMC at pH 7.0. Unexpectedly, NC-1300 treated at low pHs, even pH 1.0, significantly reduced gastric acid output in the rats. The degree of reduction of acid output by NC-1300 treated at pH 1.5 (89.7%) practically equalled that (94.9%) seen with NC-1300 suspended at pH 7.0. NC-1300 (30 mg/ml) treated at pH 1.5 contains unchanged NC-1300 (12.9 mg/ml) and NC-1300-sulfide, o-DMABA and BI. Mixture B whose contents correspond to those of NC-1300 treated at pH 1.5 also reduced gastric acid output to the same degree as observed with NC-1300 treated at pH 1.5. When NC-1300 (12.9 mg/kg) alone was administered, the gastric acid output was reduced by 72.2%. These results indicate that the reduction observed with NC-1300 treated at pH 1.5 is caused by the combined activity of NC-1300 and its degradation products. The proposal that degradation products in themselves have antisecretory activity seems justified because (A) NC-1300 treated at pH 1.0 for 30 min (containing no detectable NC-1300) significantly reduced gastric acid output, and (B) NC-1300-sulfide itself at the dose which is involved in NC-1300 treated at pH 1.0 also reduced gastric acid output. In addition, the mixture A which mimicks the contents of NC-1300 treated at pH 1.0 also significantly reduced gastric acid output. These findings indicate that NC-1300 (with no alkalization) will amply exert antisecretory effects when given to rats with gastric contents of pH 1.5 or over.

We also confirmed our previous findings (7) that NC-1300 given p.o. at pH 7.0 markedly protected the gastric mucosa against HCl-ethanol-induced lesions in rats. As in

the case of gastric acid secretion, NC-1300 treated at low pH (including pH 1.0) significantly prevented development of HCl-ethanol-induced gastric lesions. The degree of prevention by NC-1300 treated at pH 1.5 was almost the same as that of NC-1300 treated at pH 7.0. These results also indicate that it is the unchanged NC-1300 (12.9 mg/ml) which is responsible for the efficacy because NC-1300 (12.9 mg/kg) alone and mixture B also potentially prevented lesion formation. However, the significant protection afforded by NC-1300 treated at pH 1.0 appears to be induced by the degradation products, because (A) there was no detectable NC-1300 in the test solution, (B) NC-1300-sulfide alone (20.1 mg/kg) significantly prevented lesion formation, and (C) mixture A (containing degradation products only) also significantly prevented lesion formation. Again, these findings suggest that NC-1300 (without alkalization) exerts cytoprotective effects when the stomachs have contents with a pH of 1.5 or over.

Brändström and Lindberg (10) reported that both omeprazole and its reduced form (omeprazole-sulfide) have an antisecretory activity when determined *in vivo*, and omeprazole alone (but not its sulfide form) has an antisecretory effect *in vitro*. They suggested that omeprazole-sulfide undergoes oxidation when given *in vivo* and hence became active in reducing gastric acid secretion. NC-1300-sulfide itself had no proton pump inhibitory activity determined using purified hog gastric H^+K^+ -ATPase (S. Okabe et al., unpublished data). Therefore, it is possible that the antisecretory effects of NC-1300-sulfide are caused by *in vivo* oxidation of a certain amount of the compound to NC-1300 itself. We reported that the i.p. administration of NC-1300 is as effective in inhibiting gastric acid secretion as is the p.o. administration (7). The question arises as to whether the cytoprotective effect of NC-1300-sulfide is also caused by *in vivo* oxidation of the compound to NC-1300. NC-1300, however, exerts its cytoprotective effect only when given p.o. (no effect by i.p. administration), thereby suggesting that NC-1300 acts locally and not systemically (7). It is thus unlikely that NC-1300-sulfide

exerts its protective effect systemically after being changed to NC-1300 in the body. NC-1300-sulfide itself appears to have a cytoprotective effect on the gastric mucosa when given locally, however, the degree of cytoprotective effect is not comparable to that of NC-1300.

Based on the above results, we conclude that while NC-1300 is unstable at low pHs, the compound can exert a potent antisecretory and cytoprotective effect even when mixed at pH 1.5, presumably due to the unchanged NC-1300 and to the degradation products.

Acknowledgments: We thank Dr. K. Kitamura for advice on the HPLC analysis of NC-1300 and its degradation products, Y. Awane for technical assistance and H. Wada for secretarial services.

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