Effects of NC-1300-O-3 on Gastric Mucus Secretion and Prostaglandin Release in Rats

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ABSTRACT—We investigated the effect of NC-1300-O-3 on gastric mucus secretion and prostaglandin release into the gastric lumen in rats. NC-1300-O-3 following single or repeated administration for up to 4 weeks significantly increased the hexose content in the gastric lumen at 10 to 100 mg/kg, p.o. Omeprazole and cimetidine at doses that strongly inhibited gastric acid secretion had no effect on the hexose content following single or repeated administration for 8 days. When administered repeatedly for 8 days, NC-1300-O-3, omeprazole and cimetidine significantly decreased the hexosamine content in gastric surface mucosa, but significantly increased gastric mucus secretion was observed at the same time only with NC-1300-O-3, indicating that this agent has a profile of action on gastric mucus metabolism different from those of omeprazole and cimetidine. NC-1300-O-3 at 10 and 30 mg/kg, p.o. and omeprazole at 30 mg/kg, p.o. increased the release of prostaglandins into the gastric lumen, and this was markedly inhibited by pretreatment with indomethacin, suggesting that these agents may enhance prostaglandin biosynthesis in the gastric mucosa. From these results, it seems that the enhancement of NC-1300-O-3 on gastric mucus secretion and prostaglandin biosynthesis in the gastric mucosa contribute to the antiulcer effect of NC-1300-O-3.

Keywords: NC-1300-O-3, Gastric mucus secretion, Prostaglandin

Okabe et al. have already reported that the new proton pump inhibitor NC-1300-O-3 (2-[[2-(isobutylmethylamino)benzyl]sulfinyl]-1H-benzimidazole) has a potent and persistent antisecretory effect and a potent cytoprotective effect in rats (1). In addition, they also found that this drug markedly prevented development of acute gastric and duodenal lesions and accelerated the natural healing of acetic-acid induced gastric ulcers in rats (1, 2). Delayed healing of acetic-acid induced gastric ulcers caused by indomethacin was also markedly prevented by this agent (2).

The mechanism by which NC-1300-O-3 exerts its antiulcer effects seems to partly involve its potent antisecretory effect, but it is considered to exert enhancing effects on the mucosal defensive function.

In the present study, to elucidate the effects of NC-1300-O-3 on this defensive function, we examined the effects of this drug on gastric mucus secretion and prostaglandin release into the gastric lumen in rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Saitama Experimental Animals Supply Co., Ltd., Saitama) and Donryu rats (Charles River Japan, Atsugi) were housed at a room temperature of 23 ± 2°C and a humidity of 55 ± 10%.

Test compounds

NC-1300-O-3 and omeprazole, which were synthetized by the Research Laboratory of Nippon Chemiphar Co., Ltd., and cimetidine (Sigma, St. Louis, MO, USA) were suspended in 1% methylcellulose solution and administered in a volume of 0.5 ml/100 g body weight. 16,16-Dimethyl prostaglandin E₂ (diMe-PGE₂; Funakoshi, Tokyo) was diluted in saline.

Gastric mucus glycoprotein content

Single administration study: Under ether anesthesia, SD rats (184–244 g), which had been fasted for 24 hr, were laparatomized and the pylorus ligated. One hour later, the animals were sacrificed and the stomach was removed after clamping the esophagus. After infusing 2 ml
of distilled water into the stomach, the gastric content was collected. After determination of the volume of the supernatant derived from centrifugation at 3,000 rpm for 15 min, the mucus glycoprotein content was measured according to the ethanol precipitation method. Briefly, 0.2 ml of 20% Triton X-100 and 6 ml of 99.6% ethanol was added to 2 ml of the supernatant and the resultant mixture was kept overnight at 4°C. After centrifugation at 10,000 rpm for 30 min, 2 ml of distilled water was added to the precipitate. After sonication, the hexosamine content was determined by phenol-sulfuric acid assay (3) using D-galactose as a standard. Drugs were administered orally 1 hr before the ligation of the pylorus. In another study, the effects of pretreatment with indomethacin or atropine on changes in mucus secretion induced by NC-1300-O-3 were examined. SD rats (195–253 g) were treated with NC-1300-O-3, p.o. at 1 hr before the ligation of the pylorus. Indomethacin (10 mg/kg, Sigma) was administered s.c. at 30 min before administration of NC-1300-O-3; and atropine (5 mg/kg; Wako, Osaka) was administered i.p. at 60 min before the administration of NC-1300-O-3.

Repeated administration study: SD rats (157–180 g) were treated with drugs repeatedly for 7 days and fasted after the last dose. On the following day, rats were again treated with drugs and then sacrificed 1 hr after dosing. The stomach was removed by ligating both the pylorus and the esophagus, and the gastric content was collected after infusion of 2 ml of distilled water. The stomach was then opened along the lesser curvature, and the surface mucosa of the corpus was scraped off with a spatula. The spatula was drawn parallel to the mucosal folds from the oral to the anal side (4). From the same stomach, the deep corpus mucosa was also scraped off with a surgical knife. The hexosamine content in the gastric lumen was determined using the method described above. The removed gastric mucosa was lyophilized; and mucus glycoproteins were extracted according to the method described by Azuumi et al. (5), and the hexosamine content was determined based on the findings of Ishihara et al. (6). Briefly, a portion of powdered sample was suspended in 50 mM Tris-HCl buffer (pH 7.2) and boiled for 3 min and then homogenized. Triton X-100 was added to the resultant homogenate to the final concentration of 2% and incubated at 37°C for 1 hr to accelerate the extraction of mucin. After centrifugation at 9,000 rpm for 30 min, the resultant supernatant was hydrolyzed at 100°C for 4 hr in 3 N HCl. After neutralization, the hexosamine content was determined by the method described by Neuhaus and Letzring (7) using D-glucosamine as a standard. NC-1300-O-3 (p.o.) or omeprazole (s.c.) was administered once a day, and cimetidine was administered p.o. twice daily.

In another study, Donryu rats (7 weeks of age) were given NC-1300-O-3 or omeprazole repeatedly p.o. once a day for 2 weeks or 4 weeks and then fasted after the last dose. On the following day, rats were again treated with drugs and sacrificed 3 hr after dosing. The stomach was removed by ligating both the pylorus and the esophagus, and the gastric content was collected after the infusion of 2 ml of distilled water. The hexosamine content in the gastric lumen was determined using the method described above.

Prostaglandin (PG) release into the gastric lumen
SD rats (204–253 g), which had been fasted for 24 hr, were treated with drugs p.o. 30 min before ligation of the pylorus. One hour after ligation, the animals were sacrificed, and the stomach was removed following clamping of the esophagus. After infusion of 2 ml of phosphate-buffered saline (PBS), the gastric content was collected. Following addition of indomethacin at a concentration of 10⁻⁴ M to 2.5 ml of collected gastric content, the sample was centrifuged at 3,000 rpm for 10 min. The volume of supernatant was measured, and the total volume of collected gastric content was determined together with the remaining gastric content. The extraction of PG was carried out as described below.

According to the method of Jaffe et al. (8), 2 ml of supernatant was shaken with 6 ml of petroleum ether and then centrifuged. The lower phase was extracted with 3 ml of ethyl acetate under acidic conditions at pH 3 or lower. Extraction with ethyl acetate was carried out twice and the organic phase was evaporated using a centrifugal evaporator. The resultant residue was dissolved in ethanol and stored at −20°C. After evaporation, the residue was dissolved in a mixture of methanol:ethyl acetate (1:1) and subjected to TLC (silica gel plate, ethyl acetate : isooctane : acetic acid : H₂O = 11:5:2:10 v/v; organic phase). The individual PG spots were collected and extracted with methanol containing 0.5% acetic acid. The concentrations of PGs were measured by radioimmunoassay (RIA, Amersham RIA kit; Amersham International plc., UK).

In another study, the effect of pretreatment with indomethacin on changes in PGE₂ release induced by drugs was examined. Rats (152–178 g), which had been fasted for 24 hr, were treated with NC-1300-O-3 or omeprazole. Indomethacin at 5 mg/kg was administered s.c. 30 min before the administration of drugs, and PGE₂ was then measured as described above.

Statistical analyses
The results are expressed as means ± S.E. The statistical significance of differences between the groups was determined by Dunnett’s multiple comparison test. Student’s or Aspin-Welch’s t-test was also applied to the comparison between the two groups.
RESULTS

Gastric mucus glycoprotein content

Single administration study: NC-1300-O-3 at 10 and 30 mg/kg, p.o. administered 1 hr before ligation of the pylorus produced a dose-dependent increase in hexose content in the gastric lumen, and this effect was significant at a dose of 30 mg/kg (Fig. 1A). When NC-1300-O-3 was administered intraperitoneally at 30 mg/kg, the hexose content (144.0 ± 9.3 μg/rat, n = 6) was the same as that of the control (151.4 ± 14.7 μg/rat, n = 7). In contrast, cimet-$

Fig. 1. Effects of NC-1300-O-3, omeprazole, cimetidine and 16,16-dimethyl PGE$_2$ on hexose content in the gastric lumen in rats. Each drug was administered 1 hr before the pylorus-ligation. Animals were sacrificed 1 hr after the ligation, and the gastric content was collected. Each column represents the mean ± S.E. *P < 0.05, **P < 0.01: Significantly different from the controls.

Fig. 2. Effect of indomethacin pretreatment on changes in hexose content induced by NC-1300-O-3. NC-1300-O-3 was administered 1 hr before the pylorus-ligation. Animals were sacrificed 1 hr after the ligation, and the gastric content was collected. Indomethacin (10 mg/kg) was administered s.c. 30 min before the administration of NC-1300-O-3. Each column represents the mean ± S.E. **P < 0.01: Significantly different from the controls.

Fig. 3. Effect of atropine pretreatment on changes in hexose content induced by NC-1300-O-3. NC-1300-O-3 was administered 1 hr before the pylorus-ligation. Animals were sacrificed 1 hr after the ligation, and the gastric content was collected. Atropine (5 mg/kg) was administered i.p. 60 min before the administration of NC-1300-O-3. Each column represents the mean ± S.E. **P < 0.01, ***P < 0.001: Significantly different from the controls.
dine at 100 mg/kg, p.o. and omeprazole at 30 mg/kg, p.o. did not significantly affect the hexose content. diMe-PGE2 at 10 μg/kg, p.o. produced a significant increase in hexose content (Fig. 1B).

Following pretreatment with indomethacin, NC-1300-O-3 at 10 and 30 mg/kg, p.o. also produced a dose-dependent increase in hexose content, and the extent of this increase was almost the same as when indomethacin pretreatment was not carried out (Fig. 2). In atropine-pretreated rats, the enhancing effect of NC-1300-O-3 on hexose content was slightly weaker compared to the case in which atropine pretreatment was not carried out, but this drug produced a significant increase in hexose content compared to the atropine-pretreated control (Fig. 3).

Repeated administration study: NC-1300-O-3 administered at 30 and 100 mg/kg, p.o. for 8 days produced a significant and dose-dependent increase in hexose content in the gastric lumen (Fig. 4). Omeprazole at 10 mg/kg, s.c. and cimetidine at 100 mg × 2/kg/day, p.o. for 8 days did not significantly affect the hexose content. In terms of hexosamine content in the gastric surface mucosa, NC-1300-O-3 at 30 and 100 mg/kg significantly decreased the content by 27.3% and 36.6%, respectively (Fig. 5). Omeprazole and cimetidine also significantly decreased the hexosamine content by 24.5% and 26.2%, respectively. However, the hexosamine content in gastric deep mucosa was not significantly affected by these drugs (Fig. 6).

The effect of NC-1300-O-3 on the healing of acetic acid-induced gastric ulcers was investigated using Donryu rats. In this study, the effects of repeated administration of NC-1300-O-3 and omeprazole on hexose content in the gastric lumen were also determined in the same strain. NC-1300-O-3 when repeatedly administered at 100 mg/kg, p.o. for 2 or 4 weeks produced a significant increase in hexose content by almost the same extent as that produced by single administration (Fig. 7). Omeprazole at 30 mg/kg, p.o. did not significantly affect the hexose content.

Prostaglandin (PG) release into the gastric lumen
NC-1300-O-3 at 10 and 30 mg/kg, p.o. significantly increased the release of PGE2 into the gastric lumen in a
dose-dependent manner (Fig. 8). Omeprazole at 30 mg/kg, p.o. also increased the release of PGE2, but to a lesser extent than NC-1300-0-3. NC-1300-0-3 at 10 and 30 mg/kg, p.o. increased the release of 13,14-dihydro-15-keto-PGE2 into the gastric lumen in a dose-dependent manner, and the increase was significant at 30 mg/kg. Omeprazole at 30 mg/kg, p.o. also significantly increased the release of 13,14-dihydro-15-keto-PGE2, but to a slight lesser extent than NC-1300-0-3.

Moreover, NC-1300-0-3 at 10 and 30 mg/kg, p.o. increased the release of 6-keto-PGF1α into the gastric lumen in a dose-dependent manner, but not significantly. Omeprazole at 30 mg/kg, p.o. also increased the release of 6-keto-PGF1α by approximately 4 times that of the control, but this was not significant (Fig. 8).

To examine if the increase in PG release into the gastric lumen reflected PG biosynthesis in the stomach, the effect of pretreatment with indomethacin was studied. In indomethacin-pretreated rats, the increase in PGE2 release induced by NC-1300-0-3 or omeprazole observed in non-pretreated rats was markedly inhibited, and a significant increase at 30 mg/kg NC-1300-0-3 was observed compared to the indomethacin-pretreated control (Fig. 9).

**DISCUSSION**

Mucin, a major component of gastric mucus, is considered to be an important mucosal defensive factor (9). In the present study, single administration of NC-1300-0-3 at 10 and 30 mg/kg, p.o. significantly increased hexose content in the gastric lumen in a dose-dependent manner. Ishihara et al. (10) have found that NC-1300-0-3 at 30 mg/kg, p.o. significantly increases the content of soluble mucin, which is recovered from the gastric luminal content. Therefore, the present results are consistent with those reported by Ishihara et al., although the experimen-
tal conditions differed. Omeprazole at 30 mg/kg, p.o. and cimetidine at 100 mg/kg, p.o., doses at which these drugs strongly inhibit gastric acid secretion, had no effect on the hexose content. However, diMe-PGE2 at 10 pg/kg, p.o., which is a cytoprotective dose, significantly increased the hexose content. From these findings, NC-1300-0-3 appears to have a unique profile of action in enhancing the gastric mucosal defensive function as well as an antisecretory effect.

The mechanism by which NC-1300-0-3 enhances gastric mucus secretion is not clear. In this study, we examined if endogenous PG and/or cholinergic factors are related to drug enhancement of gastric mucus secretion in rats. The enhancing effect of NC-1300-0-3 was not affected by pretreatment with indomethacin or atropine, suggesting that the effect is not related to endogenous PG or cholinergic factors. NC-1300-0-3 has a weak inhibitory effect on gastric volume and/or an increase in gastric volume when administered orally to rats. Moreover, the increase in gastric volume induced by NC-1300-0-3 administered intragastrically after ligation of the pylorus was almost completely abolished by pretreatment with atropine at 5–20 mg/kg, i.p. (H. Matsukura et al., unpublished data). Consequently, the present results also suggest that the enhancement of gastric mucus secretion and the increase in gastric volume are due to different mechanisms of action of this drug. In addition, since NC-1300-0-3 has been found to increase the incorporation of \(^{3}H\)-glucosamine into gastric corpus tissue using tissue culture methods (11), it is considered that the enhancement of mucin biosynthesis is related to the mechanism by which NC-1300-0-3 enhances gastric mucus secretion.

It has been demonstrated that the gastric mucins present in different layers of the gastric mucosa, i.e., the surface epithelial cell-derived mucin and the gastric gland cell-derived mucin, have distinct histochemical (12) and biochemical (13) characteristics, and each appears to have its own physiological role in the gastric mucosal defensive mechanism (14). Ishihara et al. (10) have demonstrated that NC-1300-0-3 increases the soluble mucin content in the gastric lumen, but decreases the mucin content in gastric deep mucosa after single oral administration to rats. They speculate that mucin in the gastric deep mucosa is secreted into the gastric lumen and is not retained in the mucosal gel layer. In the present study, to investigate the changes in mucin metabolism induced by repeated administration of NC-1300-0-3, this drug was orally administered for 8 days to rats, and the results compared with those obtained with omeprazole and cimetidine.

NC-1300-0-3, when repeatedly administered at 30 and 100 mg/kg, p.o. for 8 days, doses that respectively exert potent cytoprotective and strong antisecretory effects in rats, significantly increased the hexose content in the gastric lumen in a dose-dependent manner. In contrast, omeprazole at 10 mg/kg, s.c. and cimetidine 100 mg \(\times\) 2/kg/day, p.o., doses at which these drugs strongly in-
hibit gastric acid secretion, had no effect on hexose content. In terms of mucin content in the gastric surface mucosa, repeated administration of NC-1300-0-3 significantly decreased the content in a dose-dependent manner. Omeprazole and cimetidine also significantly decreased the mucin content, although the content in the gastric deep mucosa was not significantly affected by these drugs.

The decrease in mucin content in the surface mucosa caused by omeprazole or cimetidine is likely to reflect a reduction in mucosal defensive function that is probably related to the persistent antisecretory effects of these drugs. In fact, Hotta et al. (15) reported that omeprazole or cimetidine when orally administered for 7 days decreased mucin biosynthesis in the gastric corpus mucosa in rats. The decrease in mucin in the surface mucosa caused by NC-1300-0-3 appears to reflect the secretion of mucin from the surface mucosa in addition to the effects of the persistent antisecretory effect of the drug, since NC-1300-0-3, which differs from omeprazole and cimetidine, caused an increase in mucin in the gastric lumen. The decreasing effect of NC-1300-0-3 on the mucin content in the gastric deep mucosa observed by Ishihara et al. (10) was not observed in the present study. This is probably due to differences in the dosing period and/or the method of mucin determination.

NC-1300-0-3, when repeatedly administered at 100 mg/kg, p.o. for 2 or 4 weeks, markedly enhanced gastric mucus secretion. This result appears to explain the healing effects of NC-1300-0-3 on gastric ulcers in addition to the findings such as the enhancing the proliferation of gastric mucosal cells (16) and the persistent antisecretory effect of the drug.

In terms of the effects of NC-1300-0-3 on gastric mucosal PG, this drug at 10 and 30 mg/kg, p.o. significantly increased the release of PGE2 and 13,14-dihydro-15-keto-PGE2 into the gastric lumen in a dose-dependent manner and tended to increase the release of 6-keto-PGF1α. Moreover, the increase in PGE2 release caused by NC-1300-0-3 was almost completely inhibited by pretreatment with indomethacin. However, the volume of collected gastric content was almost the same irrespective of indomethacin treatment in the present study, and the increase in gastric volume caused by NC-1300-0-3 was not affected by pretreatment with indomethacin (H. Matsukura et al., unpublished data). Thus, the enhancement of PG release observed following NC-1300-0-3 administration is unlikely to be due to a change in gastric volume. Moreover, it has been shown that NC-1300-0-3 does not decrease the potential difference in rat gastric mucosa when administered intragastrically (19) and is unlikely to increase PG release as a mild irritant.

From the findings that NC-1300-0-3 had a cytoprotective effect and markedly inhibited the delayed healing of acetic acid-induced gastric ulcers even following indomethacin treatment, and that the enhancement of gastric mucus secretion was not affected by pretreatment with indomethacin, it is not clear how the increased PG biosynthesis is related to the antiulcer activity of NC-1300-0-3 when administered orally. Nevertheless, there is a possibility that NC-1300-0-3 exerts an enhancing effect on mucosal defensive function by increasing the biosynthesis of endogenous PG as one of its pharmacological properties.

Konturek et al. (20) has reported that omeprazole has no effect on the biosynthesis of gastric mucosal PG2. Moreover, omeprazole has been reported to have no effects on the biosynthesis of PG in human tissue culture (21) or in isolated gastric mucosal cells from guinea pigs (22). In the present study, however, orally administered omeprazole at 30 mg/kg promoted PG release. These different findings seem to be due to differences in experimental methods and conditions.

Since orally administered NC-1300-0-3 exerts a marked cytoprotective action at a dose lower than that at which the antisecretory effect is observed, the cytoprotective effect of NC-1300-0-3 is thought to be a direct action on the gastric mucosa independent of its antisecretory effect. Moreover, both the cytoprotective and antisecretory effects are considered to comprise the mechanism of the antiulcer action of NC-1300-0-3. It has been demonstrated, however, that omeprazole exerts a cytoprotective effect when orally administered at a higher dose than that producing an antisecretory effect and that cimetidine does not have cytoprotective effects in rats (23). In the present study, neither omeprazole at 30 mg/kg, p.o., a dose that shows a cytoprotective effect in rats, nor cimetidine increased gastric mucus secretion. Moreover, NC-1300-0-3 at 30 mg/kg, i.p. did not increase gastric mucus secretion. Consequently, the enhancement of gastric mucus secretion observed at 10 and 30 mg/kg, p.o. of NC-1300-0-3 appears to be closely related to the cytoprotective effect of this drug in addition to its increasing effect on gastric mucosal blood flow by topical application to the gastric mucosa in rats (19).

In conclusion, it is considered that the increase in gastric mucus secretion and PG biosynthesis in the gastric
mucosa contribute to the antiulcer effect of NC-1300-O-3 in addition to its potent and persistent antisecretory effect. It is also suggested that the effect of this drug in enhancing gastric mucus secretion is not related to endogenous PG or cholinergic activity, and that NC-1300-O-3 may promote gastric mucus secretion rather than increasing mucin content in the gastric mucosal layer. Moreover, NC-1300-O-3 is expected to exert a profile of ulcer healing which differs from that of compounds with only antisecretory actions.

Acknowledgments

We are grateful to Prof. S. Okabe (Kyoto Pharmaceutical University) for his critical review of this manuscript.

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