Effect of NC-1300-0-3 on Healing of Acetic Acid-Induced Gastric Ulcers in Rats

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ABSTRACT—We examined the effect of NC-1300-0-3 on the healing of acetic acid-induced gastric ulcers in male Donryu rats. NC-1300-0-3, administered orally once daily for 2, 4 or 8 weeks after ulceration, significantly accelerated the spontaneous healing of both fresh and unhealed ulcers (induced by pretreatment with indomethacin). The delay in ulcer healing caused by indomethacin was markedly prevented by concurrent administration of NC-1300-0-3 once daily for 4 weeks in a dose-related manner. A bolus administration of NC-1300-0-3 to rats with 5-day-old ulcers potently and persistently (>48 hr) inhibited both the basal and histamine-stimulated gastric secretion. However, the potency and duration of the antisecretory activity of the compound, with or without indomethacin, gradually decreased with the period of treatment. After an 8-week treatment with NC-1300-0-3 alone, the volume of the gastric contents was markedly increased, resulting in an increased acid output. Administration of the compound together with indomethacin for 8 weeks resulted in a significant increase in the volume, no change in acid output and a significant increase in the pH. The spontaneous or delayed healing of gastric ulcers induced 4 weeks after pretreatment with NC-1300-0-3 was also significantly enhanced or prevented with NC-1300-0-3, with a weakened antisecretory activity. Therefore, NC-1300-0-3 seems to promote ulcer healing or prevents delayed healing by its potential antisecretory and/or ulcer-healing activities.

Keywords: NC-1300-0-3, Ulcer (acetic acid-induced), Ulcer healing, Gastric secretion, Indomethacin

We reported that a series of benzimidazole derivatives, exhibiting inhibitory activity toward the gastric proton pump, effectively inhibited gastric secretion, protected the gastroduodenal mucosa against various acute lesions and accelerated the healing of chronic gastric ulcers induced in rats (1-5). Of these derivatives, NC-1300-0-3, 2-[2-\(N\)-methyl-\(N\)-(2-methylpropyl)amino]benzylsulfinyl benzimidazole, is less toxic than the others in both rats and dogs (S. Okabe et al., unpublished data) and exhibits potent antisecretory and antilesion activities in rats (5). The mechanism underlying its antilesion activity appeared to be causally related to its potential antisecretory and mucosal protective activities. It was of interest to determine whether or not such pharmacological properties of the compound have a favorable influence even on the healing of experimental gastric ulcers. We describe herein the effects of NC-1300-0-3 on spontaneous and delayed healing (induced by indomethacin) of penetrating gastric ulcers induced in rats. Attention was also directed to its effect on basal and histamine-stimulated gastric secretion in animals with ulcers following a bolus or repeated oral administration. Abstracts of a part of this study have been published (6, 7).

MATERIALS AND METHODS

Animals

Male Donryu rats (Nihon SLC, Hamamatsu), weighing 240–260 g, were used. In producing gastric ulcers, animals were fasted for 5 hr before injection of acetic acid into the gastric wall. For gastric secretory studies, animals with ulcers were deprived of food for 18 hr prior to experiments. To determine the duration of the antisecretory effect of NC-1300-0-3 (Nippon Chemiphar, Tokyo), it was administered orally to normally fed animals 24 or 48 hr before they began an 18 hr-fast which preceded pylorus ligation and acid secretory studies. Drinking water was freely available to the animals up to 2 hr before the experiments. All animals were kept in raised mesh-bottom cages to prevent coprophagy. The groups consisted of 9–40 rats.
**Induction of acetic acid ulcers**

With the rats under ether anesthesia, the abdomen was incised, and the anterior portion of the stomach exposed. Standard or large gastric ulcers were produced by injecting 0.03 or 0.075 ml of 20% acetic acid (v/v) into the submucosa at the junction of the corpus and antrum, respectively. Acid was injected with a 0.25-ml microsyringe (Terumo, Tokyo) (8, 9). Clearly defined deep ulcers consistently developed 5 days after this injection. Thus, we defined the fifth day after the injection as the day of ulceration. Large ulcers were used to examine the effect on spontaneous healing of prolonged treatment (4 weeks) with NC-1300-O-3. These ulcers are called “fresh ulcers”.

Postoperatively, the animals were maintained on rat chow and water ad libitum.

Daily administration of indomethacin at 1–3 mg/kg for 2 or 4 weeks has previously been shown to markedly delay the healing of acetic acid-induced ulcers in rats (10, 11). Therefore, in additional experiments, indomethacin (1 mg/kg, suspended in saline with a minimal amount of Tween 80; Sigma, St. Louis, MO, USA) was administered subcutaneously at 1 mg/kg once daily (1:00 PM) for 4 weeks after the development of the standard ulcers. These ulcers persisted for an additional 8 weeks without further administration of indomethacin (4). Therefore, these ulcers were designated “unhealed ulcers” to distinguish them from “fresh ulcers”.

In a preliminary study, we found that repeated administration of NC-1300-O-3, at 60 mg/kg once daily orally for 4 weeks, resulted in an attenuated inhibition of basal gastric acid secretion, by the increased volume of gastric juice (S. Okabe et al., unpublished data). Therefore, it was of particular interest to determine whether or not NC-1300-O-3, despite the weakened antisecretory activity after 4-week administration, could accelerate the healing of ulcers produced at that time. NC-1300-O-3 at 60 mg/kg once daily or the vehicle alone was administered orally for 4 weeks, following which standard ulcers were induced in all animals. For 5 days after acetic acid injection, NC-1300-O-3 or vehicle was administered once daily. At this time, ten animals of each group were killed to verify the consistent occurrence of ulcers. The remaining animals given NC-1300-O-3 were randomly divided into two groups to determine the effect of the compound on spontaneous (2-week treatment) and delayed ulcer (4-week treatment) healing. Basal gastric secretion was determined at the end of each experiment.

**Determination of ulcer size**

The animals were killed 24 hr after the final administration of NC-1300-O-3. From rats fasted for 24 hr prior to autopsy, the stomach was removed, inflated by injecting 8 ml of 2% formalin and then immersed in 2% formalin for 15 min. This procedure allows for light fixation of the gastric wall and facilitates examination of the ulcers. The stomach was then opened along its greater curvature and the area (mm²) of ulcers determined under a dissecting microscope (×10; Olympus, Tokyo) with a square grid. The person (S.O.) determining the ulcer size was unaware of the treatment given the animals. When the gastric secretary conditions were determined in rats with ulcers, the stomachs were fixed with formalin following collection of the gastric contents.

**Determination of gastric acid secretion**

We have previously reported the antisecretory effect of NC-1300-O-3 in male Sprague-Dawley rats without gastric ulcers (5). To confirm this effect after a bolus or repeated administration to 2, 4 or 8 weeks to Donryu rats with ulcers, the following experiments were done.

**Basal secretion:** In the first study, the antisecretory effect of NC-1300-O-3 was determined in animals with 5-day-old ulcers (i.e., 10 days after acid injection). At 1, 2 or 44 hr after a bolus oral administration, the pylorus was ligated for 4 hr. Subsequently, the animals were killed, and the gastric contents were collected and analyzed for volume, acidity and pH. Total acidity was determined by automatic titration (Hiranuma, Comitie 5, Tokyo) of the gastric contents against 0.1 N NaOH to pH 7.0. Total acid output (volume × acidity) was expressed as μEq/hr. In the second study, the effect of repeated oral administration of NC-1300-O-3 (once daily, 2:00 PM) on gastric secretion in animals with ulcers was determined after 2-week or 8-week treatment. At 19 hr after the final dose, an additional dose of NC-1300-O-3 was administered orally, and the pylorus was ligated 1 hr later for 4 hr. The gas-
tric samples were analyzed as described above. To examine the persistence of the antisecretory activity after 2-week treatment, the pylorus was ligated 20 hr after the final dose, and then the gastric contents were analyzed for 4 hr. In the third study, NC-1300-O-3 was administrated orally once daily (6:00 PM) for 4 or 8 weeks together with indomethacin. Indomethacin was administrated subcutaneously at 1 mg/kg once daily (5:00 PM) for 4 weeks or twice daily (1 × 2 mg/kg/day, 9:00 AM and 5:00 PM) for 8 weeks. At 16 hr after the final treatment with NC-1300-O-3, an additional dose (without indomethacin) was administrated orally, and the pylorus was ligated 1 hr later. The animals were killed 4 hr after the ligation, and gastric contents and blood were collected to determine acid secretion and fasting serum gastrin. The above experiments were also done on rats pretreated with NC-1300-O-3 for 4 weeks and then treated with the compound for the subsequent 2 or 4 weeks with or without indomethacin.

**Histamine-stimulated secretion:** Similar to the determination of basal gastric secretion, the pylorus was ligated and histamine-HCl (Nacalai Tesque, Kyoto), dissolved in saline, was administrated subcutaneously at 20 mg/kg (as the salt) twice, immediately after ligation and 2 hr later. Gastric samples were collected 4 hr later and analyzed for the volume and acidity. The compounds or vehicle were administrated orally once 1 hr prior to the pylorus ligation. In the case of 2- or 4-week treatment with NC-1300-O-3, an additional dose of the compound was given 19 hr after the final administration, i.e., 1 hr before pylorus ligation.

**Analysis of data**

All data are expressed as means±S.E.M. Dunnett's or Student's t-test was used to determine the statistical significance of the data at the level of P<0.05. ED_{50} values (doses which accelerate ulcer healing, prevent the delay in healing or inhibit gastric acid output by 50%) and 95% confidence limits were calculated by the Litchfield-Wilcoxon method.

**RESULTS**

**Induction of acetic acid ulcers**

Five days after the acid injection, deep and round ulcers consistently developed in all rats, the sizes of which were 33.6±3.1 mm$^2$ (0.03 ml injection, n=10) (Fig. 1A) and 43.0±2.9 mm$^2$ (0.075 ml injection, n=10). These "fresh ulcers" all decreased in size and depth with time, the areas of the ulcers being 6.8±0.7 mm$^2$ (0.03 ml injection, n=40) 2 weeks later and 6.9±1.2 mm$^2$ (0.075 ml injection, n=20) 4 weeks later. The healing of the standard ulcers was clearly delayed on repeated administration of indomethacin for 4 weeks, the areas of the ulcers being about 10–15 mm$^2$ (Fig. 1B). This type of ulcers was designated as "unhealed ulcers" in contrast to the "fresh ulcers".

**Effect of NC-1300-O-3 on ulcer healing**

NC-1300-O-3 administered at 30 mg/kg once daily for 2 weeks to rats with the standard "fresh ulcers" showed a tendency to accelerate the spontaneous healing of ulcers (Fig. 2A). With 60 and 100 mg/kg, however, there was significant acceleration of ulcer healing by 35.3% and

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**Fig. 1.** Gross appearance of acetic acid-induced gastric ulcers in rats. (A) a "fresh ulcer", which was induced 5 days after submucosal injection of an acetic acid solution (20%, 0.075 ml). (B) an "unhealed ulcer", which was observed 4 weeks after daily indomethacin administration (1 mg/kg, subcutaneously) to rats with ulcers (20%, 0.03 ml).
Fig. 2. Effect of NC-1300-O-3 on the spontaneous and delayed healing of acetic acid-induced gastric ulcers in rats. The compound was administered orally once daily for 2 weeks (A) or 4 weeks (B) to rats with “fresh ulcers” produced with 0.03 or 0.075 ml of 20% acetic acid, respectively. In the case of (C), the compound was administered orally once daily for 4 weeks to rats with “unhealed ulcers” (see the text). In the case of (D), the compound was administered orally once daily for 4 weeks after ulceration together with indomethacin (1 mg/kg, subcutaneously). Data are the means ± 1 S.E. for 22 or 40 animals. *Statistically significant at P < 0.05. Note that the ulcers treated with NC-1300-O-3 were smaller compared with those in the corresponding controls.

Fig. 3. Effects of NC-1300-O-3 on basal and histamine-stimulated gastric secretion in pylorus-ligated rats with 5-day-old ulcers. In the case of histamine stimulation, histamine·HCl (20 mg/kg) was administered subcutaneously twice, the first injection immediately after the ligation and the second one 2 hr later. The animals were killed 4 hr after the ligation. NC-1300-O-3 was administered orally once 1, 20 or 44 hr before the ligation. Data are the means ± 1 S.E. for 10 animals. *Statistically significant at P < 0.05.
55.9%, respectively. In the case of the large "fresh ulcers", the compound significantly accelerated the ulcer healing by 85.5% when administered at 100 mg/kg for 4 weeks (Fig. 2B). Seven of twenty-two rats had completely healed ulcers (no healed ulcers in the control group). In the "unhealed ulcer" model, NC-1300-0-3 administered at 100 mg/kg for 4 weeks starting from the next day after the final treatment with indomethacin also markedly accelerated the spontaneous healing of ulcers by 75.7% (Fig. 2C). Seven of twenty-two animals had completely healed ulcers in contrast to 2 of 22 control ones. In all NC-1300-O-3 treated animals, there were no appreciable differences in body weight gain or behavior between treated and control animals for up to 4 weeks.

Repeated administration of indomethacin for 4 weeks apparently delayed the spontaneous healing of the standard ulcers, the ulcerated area being 12.8 ± 1.4 mm² (n=40) in the control group. NC-1300-0-3, administered at 30, 60 and 100 mg/kg together with indomethacin for 4 weeks, significantly prevented this delay in ulcer healing by 39.8%, 47.7% and 79.7%, respectively (Fig. 2D).

Effects of a bolus administration of NC-1300-0-3 on gastric secretion

In rats with 5-day-old ulcers, NC-1300-0-3 showed potent antisecretory activity in a dose-related manner (Fig. 3). At 5 hr after administration, the compound (at > 60 mg/kg) had significantly decreased basal acidity and acid output, without affecting the volume. The ED₅₀ values for acidity and acid output were 37.9 (28.3 – 51.0) mg/kg and 30.6 (17.5 – 47.6) mg/kg, respectively. Twenty-four hours later, the inhibition was 62.7% and 93.6% at 60 and 100 mg/kg, respectively. It should be noted that NC-1300-0-3 administered at 100 mg/kg significantly inhibited the gastric secretion even at 48 hr. The decreases in volume, acidity and acid output amounted to 43.2%, 62.1% and 81.8%, respectively. Much the same results were obtained for histamine-stimulated gastric secretion, although the volume of the gastric contents was significantly reduced with NC-1300-0-3, even at 10 mg/kg (Fig. 3). The ED₅₀ values for acidity and acid output at 5 hr were 19.2 (13.4 – 22.9) mg/kg and 5.7 (2.3 – 9.4) mg/kg, respectively. The antisecretory activity persisted for up to 48 hr after administration of 60 or 100 mg/kg of the compound.

Effect of repeated administration of NC-1300-0-3 on gastric secretion and ulcer healing

After 2-week treatment with NC-1300-0-3, the volumes of the basal and histamine-stimulated gastric contents, determined 1 hr after the additional dose, did not differ from findings in the control group, even with 100 mg/kg of NC-1300-O-3 (Fig. 4). However, acidity and total acid output in the cases of both basal and stimulated secretion were significantly decreased with >30 mg/kg. With 100 mg/kg, the acid output in these groups was inhibited by 65.7 and 59.9%, respectively. The ED₅₀ values for acidity and acid output in the case of basal secretion were 40.3 (20.1 – 54.4) mg/kg and 57.7 (30.3 – 103.8) mg/kg, respectively. Healing of the gastric ulcers was significantly accelerated with 60 and 100 mg/kg of NC-1300-0-3, by >70%, in both the basal and histamine-stimulated groups (Fig. 4). However, even at 100 mg/kg, there was no significant effect of basal secretion at 24 hr after the final dose of NC-1300-O-3, although the ulcer area was diminished in a dose-dependent fashion (Fig. 5). When examined at 24 hr after the final administration, the ulcers had healed with 100 mg/kg of the compound, at a rate of 70.6% (Fig. 5).

In 4-week treated rats (with indomethacin), in the case of basal secretion, the volume tended to increase in response to NC-1300-O-3 (Fig. 6). The acidity and acid output were significantly decreased with >30 mg/kg. With 100 mg/kg, the acid output in these groups was inhibited by 65.7 and 59.9%, respectively. The ED₅₀ values for acidity and acid output in the case of basal secretion were 40.3 (20.1 – 54.4) mg/kg and 57.7 (30.3 – 103.8) mg/kg, respectively. Healing of the gastric ulcers was significantly accelerated with 60 and 100 mg/kg of NC-1300-0-3, by >70%, in both the basal and histamine-stimulated groups (Fig. 4). However, even at 100 mg/kg, there was no significant effect of basal secretion at 24 hr after the final dose of NC-1300-O-3, although the ulcer area was diminished in a dose-dependent fashion (Fig. 5). When examined at 24 hr after the final administration, the ulcers had healed with 100 mg/kg of the compound, at a rate of 70.6% (Fig. 5).
output were significantly reduced by 44.1% and 31.8% with 60 mg/kg and 59.0% and 46.4% with 100 mg/kg, respectively. The ED50 values for acidity and acid output were 73.4 (60.9–96.4) mg/kg and 115.3 (76.0–831.3) mg/kg, respectively. The delay in ulcer healing was significantly prevented by 79.8% with 60 mg/kg and 77.2% with 100 mg/kg. In the case of histamine-stimulated gastric secretion, there was little or no change in the volume of the gastric contents. The acidity was significantly decreased by 20.4%, 43.8% and 27.8% with 30, 60 and 100 mg/kg, respectively. The acid output was inhibited only with 60 mg/kg of the compound by 36.2%. The delay in ulcer healing in these stomachs was significantly prevented by 65.4% and 86.9% with 60 and 100 mg/kg of the compound, respectively.

In the 8-week treated rats (without indomethacin), NC-1300-0-3 markedly increased the volume of the gastric contents and acid output, but significantly decreased the acidity by 26.0% (Fig. 7). The pH value was 2.2±0.2 vs. 2.2±0.1 in the control group (no significant difference). Although the spontaneous healing of ulcers was not significantly accelerated by the compound, completely healed ulcers were observed in 13 of 19 animals in the NC-1300-0-3-treated group vs. 5 of 17 animals in the control group. NC-1300-0-3, administered at 60 mg/kg for 8 weeks together with indomethacin, also markedly increased the volume but significantly reduced the acidity by 46.6%, thereby resulting in unchanged acid output. The pH in the NC-1300-0-3-treated group was 2.4±0.2 vs. 2.0±0.2 in the control, the difference being significantly different. Twice daily administration of indomethacin for 8 weeks markedly delayed the healing of acetic acid-induced ulcers (20.2±2.8 mm², n = 14). There were deep ulcers in 8 of 14 animals. NC-1300-0-3 markedly prevented the delay in ulcer healing caused by indomethacin, the rate being 71.3% (Fig. 7). Completely healed ulcers were observed in 3 of 19 animals.

Effects of NC-1300-O-3 on gastric secretion and healing of "fresh ulcers" produced after 4-week treatment with NC-1300-O-3

The area of 5-day-old standard ulcers in rats administered the vehicle alone or NC-1300-O-3 at 60 mg/kg for 4 weeks was 30.8±1.5 or 31.4±2.3 mm² (n = 10), with no statistical significance. After 2-week treatment with 60 mg/kg of NC-1300-O-3, the spontaneous healing of ulcers was significantly accelerated by 46.8% (Fig. 8). The volume of gastric content was significantly higher than that in the corresponding control group (13.2±0.7 ml/rat vs. 5.8±0.6 ml/rat, n = 28). Although gastric acidity was significantly reduced by 35%, the acid output was not significantly different from the control. The pH value was 2.1±0.1, a value significantly higher than 1.8±0.1 in
Fig. 7. Effects of NC-1300-O-3 on the basal gastric secretion in rats after 8-week treatment (once daily, orally) and spontaneous and delayed healing of acetic acid-induced gastric ulcers. In the case of delayed healing, the compound was administered together with indomethacin (1 mg/kg, twice daily, subcutaneously) for 8 weeks. At 19 hr after the final treatment, an additional dose (without indomethacin) was administered, and then the pylorus was ligated 1 hr later for 4 hr. The numbers above the columns denote the number of animals used. Data are the means ±1 S.E. for 14–19 animals. *Statistically significant at P<0.05.

Fig. 8. Effect of NC-1300-O-3 on healing of acetic acid-induced gastric ulcers and basal gastric secretion in rats which were preliminary treated with the compound (once daily, orally, at 60 mg/kg) for 4 weeks before ulceration. The ulcers were produced one day after the 4-week treatment with NC-1300-O-3. The compound was administered orally once daily for 2 weeks or 4 weeks together with indomethacin (1 mg/kg, subcutaneously) after ulceration. At 19 hr after the final treatment, an additional dose (without indomethacin) was administered and then the pylorus was ligated 1 hr later for 4 hr. All values are the means ±1 S.E. or S.E. of 26–28 animals. *Statistically significant at P<0.05.
the control group. When NC-1300-O-3 was administered at 60 mg/kg for 4 weeks together with indomethacin, the delay in ulcer healing was significantly prevented by 67.3%. Nine out of twenty-six animals treated with the compound had completely healed ulcers in contrast to 0 of 26 animals in the control group. Similar to the 2-week experiment, the volume was significantly increased to 11.5±0.6 ml/rat (n=26) vs. 6.5±0.5 ml/rat (n=26) in the control group, but the acidity was decreased only by 17.6%. Accordingly, the acid output was not significantly different from that in the control group. The pH was 2.0±0.2 in contrast to 1.7±0.1.

DISCUSSION

It is apparent that NC-1300-O-3 has the potential to accelerate spontaneous healing of experimental gastric ulcers and to prevent delayed healing caused by indomethacin.

First, it was demonstrated that the compound significantly and markedly enhanced the spontaneous healing of two ulcer models, i.e., "fresh ulcers" and "unhealed ulcers". The degree of acceleration of the healing of "fresh ulcers" caused by NC-1300-O-3 was slightly higher than that by omeprazole (12). It is conceivable that the potent and persistent inhibition of this compound on gastric secretion, demonstrated previously (5) and confirmed in the present study, greatly contributes to the promotion of ulcer healing. Interestingly, NC-1300-O-3 also significantly accelerated the healing of "unhealed ulcers". The pathological aspects of "fresh ulcers" and "unhealed ulcers" were not evaluated in this study. There are differences among gastric ulcers with regard to the degree of inflammation, granulation tissue formation or angiogenesis in the ulcer base. However, these two ulcer models appear partly to share a common process in healing since NC-1300-O-3 administered for 4 weeks enhanced the healing of these ulcers to the same degree.

Second, NC-1300-O-3 prevented the delay in ulcer healing caused by indomethacin. We have previously postulated that reductions in prostaglandin levels in the gastric mucosa and in the mucosal blood flow around ulcers in response to indomethacin might be partly involved in underlying mechanisms (11, 13). NC-1300, a prototype of NC-1300-O-3, administered at 20 mg/kg, p.o. had no effect on gastric mucosal prostaglandin (E2 and 6-keto-F1α) levels and did not recover the indomethacin reduced levels to the control level (H. Goto et al., personal communication). In addition, we found in a preliminary study that NC-1300-O-3, even at 100 mg/kg, had no influence on gastric blood flow in normal rats (S. Okabe et al., unpublished data). Therefore, it is most unlikely that NC-1300-O-3, from its structural similarity to NC-1300, prevents the delay in ulcer healing through the recovery of reduced prostaglandin levels and mucosal blood flow. We reported that indomethacin administered subcutaneously at 1 mg/kg for 4 weeks had little or no effect on basal gastric acid secretion (14). However, omeprazole, at the antisecretory dosage, potentially prevented delayed ulcer healing (14). Therefore, it is possible that the antisecretory activity of NC-1300-O-3 is partly involved in preventing the delayed ulcer healing.

We reported that a bolus oral administrations of NC-1300-O-3 significantly and persistently inhibited gastric acid secretion in normal Sprague-Dawley rats (5). In the present study, we confirmed the potential and persistent (for >48 hr) antisecretory activity (both basal and histamine-stimulated) of the compound in Donryu rats with 5-day-old gastric ulcers. The inhibitory effect of NC-1300-O-3 on basal and histamine-stimulated secretion (both acidity and acid output) was also observed after 2- or 4-week treatment. However, the degree of inhibition was considerably decreased with the period of treatment, as evidenced by the higher ED50 values than that observed after a bolus administration to rats with 5-day-old ulcers. In the case of 2-week treatment, the basal gastric secretion determined 24 hr after the last dose was not suppressed, even with 100 mg/kg. The reason why the antisecretory effect and duration were attenuated after repeated administration deserves further investigation. Reduced absorption, enhanced metabolism and excretion of NC-1300-O-3 or reduced sensitivity of parietal cells to the compound might be involved after prolonged treatment. Of interest is that NC-1300-O-3 administered for 4 weeks together with indomethacin tended to increase the volume of gastric content, yet both the acidity and acid output were significantly decreased. In contrast, omeprazole, administered at 30 or 100 mg/kg for 4 weeks together with indomethacin to rats with ulcers, had no effect on the volume, yet the acidity and acid output were completely inhibited (14).

After 8-week treatment with NC-1300-O-3, with or without indomethacin, the volume of the basal gastric secretion was markedly increased, but the acidity was slightly reduced. Consequently, the acid output was increased or unchanged as compared to the corresponding control. The spontaneous healing of "fresh ulcers" was enhanced and the delayed healing prevented at the same time. These results also suggest the participation of non-antisecretory activity of NC-1300-O-3 in ulcer healing.

It should be noted that NC-1300-O-3, despite the weakened antisecretory activity by preceding administration for 4 weeks, significantly enhanced the spontaneous healing and prevented the delayed healing. The degree of the effect was much the same as that observed in rats not given pretreatment. As observed in the 8-week experi-
ment, the volume of gastric contents was markedly increased 2 and 4 weeks after NC-1300-O-3 administration. The acidity was only slightly inhibited with the compound, the result being insignificant changes in acid output and pH. These results strongly suggest that NC-1300-O-3 could exert its favorable effect on ulcer healing, even without appreciable suppression of gastric acid secretion. The increased volume caused by a so far unknown mechanism may be involved in the ulcer healing action of NC-1300-O-3. Therefore, the effect of NC-1300-O-3 on ulcer healing consists of the antisecretory activity and/or ulcer healing properties, depending on the period of treatment. Satoh et al. (15) reported that a pump inhibitor AG-1749 significantly accelerated the spontaneous healing of acetic acid ulcers in rats. They also postulated that the mechanism of action of the agent on ulcer healing consists of the potent inhibition of gastric acid secretion, the protection of the mucosa and the trophic effect of the increased serum gastrin.

We conclude that NC-1300-O-3 has unique pharmacological properties and hence may prove to be an effective antiulcer drug for the treatment of human gastric ulcers or acid-related diseases and possibly for the prevention of the noxious effects of non-steroidal anti-inflammatory drugs, including indomethacin, on ulcer healing.

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