Healing-Promoting Action of the Zinc-Cimetidine Complex on Acetic Acid-Induced Gastric Ulcers in Rats

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ABSTRACT—We investigated the effect of the zinc-cimetidine complex on the healing of acetic acid-induced gastric ulcers in rats. When the effects of test drugs were assessed on the 15th day after acetic acid injection, the zinc-cimetidine complex at oral doses of 15.0 (11.4 mg as cimetidine), 30.0 and 60.0 mg/kg twice daily promoted the ulcer healing in a dose-dependent manner. Cimetidine was effective at oral doses of over 45.4 mg/kg twice daily. ZnCl_2 was ineffective on all ulcer parameters. The effect of the combination of cimetidine and ZnCl_2 was similar to that of cimetidine alone. The zinc-cimetidine complex had already inhibited the increase in thiobarbituric acid reactants in the ulcerated region before the ulcer-healing effect of this compound was recognized. A single oral administration of the complex at 15 and 30 mg/kg to normal rats was ineffective in inhibiting acid secretion and in increasing serum gastrin levels, although cimetidine was markedly effective on both parameters. These results indicate that the zinc-cimetidine complex at about 1/4 the dose of cimetidine was as effective as cimetidine when the ulcer-healing effects of both compounds were compared with the same dose of cimetidine. In addition, the ulcer-healing effect of this complex may be due, at least in part, to the inhibition of lipid peroxidation but not due to the inhibition of acid secretion or the trophic effect of gastrin.

Keywords: Zinc, Cimetidine, Gastric ulcer, Lipid peroxidation

The zinc-cimetidine complex has the chemical structure shown in Fig. 1. Cimetidine, one of the components, is a histamine H_2 -receptor antagonist that has a potent and long-lasting antisecretory action and has been widely used for the treatment of peptic ulcers. On the other hand, the effectiveness of zinc compounds in accelerating the healing of wounds is well established (1, 2). Furthermore, zinc has been shown to have a preventive effect on experimentally induced acute gastric lesions in rats (3–5). It has been suggested that the preventive action of zinc on these gastric lesions may be associated with the inhibition of gastric mucosal mast cell degranulation (6, 7) and the stabilization of lysosomal membranes (8). From the above findings, the zinc-cimetidine complex is expected to possess a more beneficial effect than that of zinc or cimetidine alone on ulcer healing or the inhibition of ulcer formation by a synergistic action of both components.

The aim of the present study was to investigate the effect of the zinc-cimetidine complex in comparison to cimetidine or ZnCl_2 on the healing of acetic acid-induced gastric ulcers in rats with a limited food intake time (9). Furthermore, to elucidate the mechanism of the ulcer

healing-promoting action of the zinc-cimetidine complex, we investigated the effect of this compound on gastric acid secretion and serum gastrin levels. Recently we have demonstrated that oxygen radicals and lipid peroxidation may be involved in the healing of acetic acid-induced gastric ulcers in rats with a limited food intake time (10). In addition, we have demonstrated that the scavenging of

Fig. 1. Chemical structure of the zinc-cimetidine complex.

oxygen radicals in the ulcerated region by free radical scavengers is effective in promoting ulcer healing. It has been shown that polaprezinc, a new antiulcer agent that is a chelate compound of L-carnosine and zinc, possesses a potent antioxidative action (11–13) and is effective in healing chronic gastric ulcers in rats (14). From this finding, the zinc-cimetidine complex may also possess an antioxidative action. Therefore, we examined the effect of the zinc-cimetidine complex on thiobarbituric acid-reactive substances, an index of lipid peroxidation, in the ulcerated region.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley strain SPF rats (Nippon SLC, Shizuoka), weighing 170–180 g, were used in the experiment. The animals were housed in an air-conditioned room at $23 \pm 1^\circ\text{C}$.

Drugs

The drugs used were the zinc-cimetidine complex (The Foundation of Research Institute for Production Development, Kyoto), cimetidine (Sigma Chemical Co., St. Louis, MO, USA), ZnCl_2 (Kanto Chemical Co., Inc., Tokyo) and human Cu, Zn-SOD (superoxide dismutase; Ube Industries, Ltd., Tokyo). The zinc-cimetidine com-

plex and cimetidine were suspended in 1% gum arabic. ZnCl_2 and human Cu, Zn-SOD were dissolved in 1% gum arabic and 0.9% NaCl.

Induction of gastric ulcers in rats with food intake time

The rats were allowed access to commercial food pellets freely only between 9:00–10:00 a.m. and 5:00–6:00 p.m. every day throughout the experimental period from 3 days prior to the ulcer induction. However, tap water was always supplied ad libitum. Gastric ulcers were induced in the rats whose feeding time was limited by injection of 20% acetic acid (v/v) in a volume of 0.05 ml into the submucosal layer at the junction of the fundus and antrum in accordance with the method described by Takagi et al. (15).

Ulcer-healing effects of test drugs

The zinc-cimetidine complex, cimetidine, ZnCl_2 or cimetidine + ZnCl_2 was given orally, twice daily (10:30 a.m. and 6:30 p.m.) in a volume of 0.5 ml per 100 g of body weight for 14 consecutive days from the day (the 1st day) after acetic acid injection. Control animals were given only the vehicle (1% gum arabic) instead of test drugs. On the 15th day, the animals were killed by rapid decapitation. The stomachs were removed, filled with 5 ml of 10% formalin and allowed to stand for 5 min. Then, the stomachs were cut open along the greater curva-

Measurement of ulcerated region

- A: Defect of mucosa
- B: Extent of ruptured muscularis mucosa
- C: Height of marginal mucosa
- BT: Thickness of ulcer base
- DA: Defective area in ulcerated region
- $R_1 + R_2$: Area of regenerated mucosa

Calculation of Indices

$$\text{Index for decrease in exposed ulcer floor} : \frac{B-A}{B} \times 100$$

$$\text{Index for mucosal regeneration} : \frac{R_1 + R_2}{C \times B} \times 100$$

Fig. 2. Method for histological measurements, showing schematic drawings of the ulcerated region.

ture. The longitudinal and abscissal lengths of the upper, opened part of the ulcer were measured with a micrometer that was set on a stereoscopic microscope, and the product of both lengths (mm^2) was expressed in terms of the ulcer index. After the ulcer size was measured, the stomach tissue was again immersed in 10% formalin for 24 hr. The formalin-fixed tissue was then cut so that a little of the normal tissue surrounding the ulcer remained. Thereafter, the central part of ulcer was cut vertically against the serosa along the long diameter. These tissues, cut in half, were embedded in paraffin and cut into 2- to 3- μm -thick sections. The sections were stained with hematoxylin and eosin. Histological measurements were performed under light micrography of the stained preparations as shown in Fig. 2. The ulcerated regions measured are as follows: A: Defect of mucosa, B: Extent of marginal mucosa, DA: Defective area in the ulcerated region, $R_1 + R_2$: Area of regenerated mucosa. On the basis of the above measured values, the following indices were calculated: the index for the decrease in exposed ulcer floor $= (B - A) \times 100/B$ and the index for mucosal regeneration $= (R_1 + R_2) \times 100/(C \times B)$. The healing effect of test drugs was evaluated by comparing the ulcer index, the defective area in the ulcerated region, the index for the decrease in the exposed ulcer floor and the index for the mucosal regeneration of each test drug with those of the respective control.

Effects of test drugs on gastric secretion and serum gastrin levels

The rats were deprived of food but allowed access to water freely for 24 hr. After fasting, the zinc-cimetidine complex, cimetidine, ZnCl_2 or cimetidine + ZnCl_2 was given orally in a volume of 0.5 ml. Control animals were given orally the vehicle (1% gum arabic) only instead of test drugs. At 1 hr after the administration of test drug or the vehicle, the pylorus of each rat was ligated under ether anesthesia. At 3 hr after ligation, the gastric contents were collected. The volume of gastric juice was measured, the acidity was determined by an automatic titrator (ABT-101; Tohadenpa, Tokyo) and the total acid output during the 1-hr period was calculated.

After a 24-hr fast, one of the test drugs described above or the vehicle was given orally. At 1 hr after the administration of test drug or the vehicle, blood samples were collected by decapitation. The serum gastrin levels were determined by using a Gastrin RIA Kit II (Dainabot, Tokyo).

Effects of test drugs on ulcer index and thiobarbituric acid (TBA)-reactive substances in the ulcerated region

Gastric ulcers were induced in the rats with a limited food intake time as mentioned above. The zinc-cimetidine

complex, cimetidine, ZnCl_2 , human Cu, Zn-SOD or vehicle was given orally, twice daily (10:30 a.m. and 6:30 p.m.) for 9 and 14 consecutive days from the day (the 1st day) after acetic acid injection. On the 10th and 15th days, the animals were killed by rapid decapitation. The stomachs were removed and then cut open along the greater curvature. After the longitudinal and abscissal lengths of the upper, opened part of the ulcer for the ulcer index were measured, the stomach tissue from the ulcerated region was punched out with a metallic punch 8 mm in diameter, and the mucosa of the ulcerated region was removed by scraping. TBA-reactive substances in the mucosa were determined by the method of Ohkawa et al. (16). The content of TBA-reactive substances was expressed as nmol of malonaldehyde per mg protein. TBA (Kanto Chemicals Co., Inc.) and 1,1,3,3-tetramethoxypropane (Tokyo Kasei Co., Tokyo) were used for the TBA assay.

Statistical analyses

The results obtained are expressed as the means $\pm$ S.E. The data were analyzed by one-way analysis of variance, and the statistical significance among groups was determined by Duncan's multiple-range test.

RESULTS

Effects on ulcer healing

The zinc-cimetidine complex given orally, twice daily at doses of 15.0 (11.4 mg/kg as cimetidine), 30.0 and 60.0 mg/kg for 14 consecutive days and evaluated on the 15th day after acetic acid injection decreased the ulcer index by 28%, 44% and 49%, respectively, and the defective area of the ulcerated region by 48%, 55% and 50%, respectively (Fig. 3). In addition, this compound at oral doses of 30.0 and 60.0 mg/kg twice daily increased the index for the decrease in the exposed ulcer base by 26% and 50%, respectively, and the index for mucosal regeneration by 50% and 76%, respectively.

Cimetidine at oral doses of 45.4 and 90.7 mg/kg twice daily decreased the ulcer index by 35% and 48%, respectively, and the defective area of the defective area by 44% and 47%, respectively (Fig. 4). Cimetidine at oral doses of 45.4 and 90.7 mg/kg twice daily also increased the index for the decrease in the exposed ulcer base by 43% and 44%, respectively, and the index for mucosal regeneration by 52% and 65%, respectively.

However, ZnCl_2 at oral doses of 12.3 (Zn content in 30 mg/kg of the zinc-cimetidine complex), 24.5 and 49.0 mg/kg twice daily was ineffective on all ulcer parameters (Fig. 5). The ulcer healing effect of the combination of cimetidine and ZnCl_2 was similar to that of cimetidine alone (Fig. 6).

Fig. 3. Effect of the zinc-cimetidine complex on the healing of acetic acid-induced gastric ulcers in rats with a limited food intake time. The zinc-cimetidine complex (15.0 mg of this compound contains 11.4 mg of cimetidine) was given orally, twice daily for 14 consecutive days beginning the day after acetic acid injection. Each column denotes the mean $\pm$ S.E. for 8 rats. Significantly different from the respective control, * $P < 0.05$, ** $P < 0.01$.

Fig. 4. Effect of cimetidine on the healing of acetic acid-induced gastric ulcers in rats with a limited food intake time. Cimetidine was given orally, twice daily for 14 consecutive days beginning the day after acetic acid injection. Each column denotes the mean $\pm$ S.E. for 9 to 10 rats. Significantly different from the respective control, * $P < 0.05$, ** $P < 0.01$.

Fig. 5. Effect of ZnCl_2 on the healing of acetic acid-induced gastric ulcers in rats with a limited food intake time. ZnCl_2 (the Zn content in 12.3 mg of ZnCl_2 is equal to that in 30 mg of the zinc-cimetidine complex) was given orally, twice daily for 14 consecutive days beginning the day after acetic acid injection. Each column denotes the mean $\pm$ S.E. for 8 to 10 rats.

Fig. 6. Effect of the combination of cimetidine (Cim) and ZnCl_2 on the healing of acetic acid-induced gastric ulcers in rats with a limited food intake time. Cim and ZnCl_2 were simultaneously given orally, twice daily for 14 consecutive days beginning the day after acetic acid injection. Each column denotes the mean $\pm$ S.E. for 8 to 10 rats. Significantly different from the respective control, * $P < 0.05$, ** $P < 0.01$.

Effects on gastric acid secretion and serum gastrin levels

A single oral administration of the zinc-cimetidine complex at 60 mg/kg to normal rats slightly (37%), but significantly, inhibited the basal gastric acid outputs (Fig. 7A). However, oral administration of less than 30 mg/kg of this compound did not affect acid secretion. Serum gastrin levels were also significantly elevated only by a single oral administration of 60 mg/kg of the compound to normal rats (a 104% elevation) (Fig. 7A). Cimetidine at oral doses of 45.4 and 90.7 mg/kg markedly inhibited the basal gastric acid outputs by 66% and 91%, respectively (Fig. 7B). In addition, cimetidine at 45.4 and 90.7 mg/kg markedly elevated the serum gastrin levels by 375% and 533%, respectively (Fig. 7B). ZnCl₂ at an oral dose of 24.5 mg/kg (Zn content in 60 mg/kg of the zinc-cimetidine complex) affected neither basal gastric acid outputs nor serum gastrin levels (data not shown). In addition, the effects of the combination of cimetidine (45.4 mg/kg, p.o.) and ZnCl₂ (24.5 mg/kg, p.o.) on basal acid secretion and serum gastrin levels were similar to that of cimetidine alone (data not shown).

Effects on ulcer index and TBA-reactive substances in the ulcerated regions

The zinc-cimetidine complex (30.0 and 60.0 mg/kg), cimetidine (45.4 mg/kg) and Cu, Zn-human SOD (10 mg/kg) given orally, twice daily for 14 consecutive days and evaluated on the 15th day significantly decreased the ulcer index, although these drugs given for 9 consecutive days were ineffective on this index (Fig. 8A). On the 10th and 15th days after acetic acid injection, the TBA-reactive substances in the ulcerated region from control rats were over tenfold higher than those in the un ulcerated region (Fig. 8B). The zinc-cimetidine complex (30.0 and 60.0 mg/kg), cimetidine (45.4 mg/kg) and Cu, Zn-human SOD (10 mg/kg) given orally, twice daily for 9 or 14 consecutive days significantly inhibited the increase in TBA-reactive substances in the ulcerated region (Fig. 8B). However, ZnCl₂ at the oral dose of 24.5 mg/kg twice daily for 9 or 14 consecutive days was ineffective on both the ulcer index and TBA-reactive substances (data not shown).

Fig. 7. Effect of the zinc-cimetidine complex (A) and cimetidine (B) on gastric acid secretion and serum gastrin levels in normal rats. To measure gastric acid secretion, the pylorus of each rat was ligated for 3 hr from 1 hr after oral administration of test drugs. To measure serum gastrin levels, blood samples were collected by decapitation at 1 hr after the administration of the drug. Each column denotes the mean $\pm$ S.E. for 8 rats. Significantly different from the respective control, * $P < 0.05$, ** $P < 0.01$.

Fig. 8. Effects of the zinc-cimetidine complex (Zn-Cim), cimetidine (Cim) and human Cu, Zn-SOD (h-SOD) on ulcer index (A) and thiobarbituric acid (TBA)-reactive substances (B) in the ulcerated region. Zn-Cim, Cim or h-SOD was given orally, twice daily for 14 consecutive days beginning the day after the acetic acid injection. Each column denotes the mean $\pm$ S.E. for 7 to 10 rats. Significantly different from the respective control, * $P < 0.05$, ** $P < 0.01$.

DISCUSSION

The present study indicates that the zinc-cimetidine complex is a new compound having quite different properties from zinc and cimetidine, the components, and it exhibits a more potent healing effect than that of cimetidine on chronic gastric ulcers in rats with a limited food intake time. This study also suggests that the inhibition of lipid peroxidation in the ulcerated region by this compound could be attributed to its ulcer-healing action.

Acetic acid-induced gastric ulcers in rats have been widely used to evaluate the ulcer healing effects of anti-ulcer agents, because histopathological changes in the healing of this ulcer model closely resemble those in human

chronic gastric ulcers (15). Recently, we have demonstrated that cimetidine, a histamine H_2 -receptor antagonist, and omeprazole, a proton pump inhibitor, are more effective in accelerating the healing of acetic acid-induced gastric ulcers in rats with a limited food intake time in comparison to rats with an unlimited food intake time (9). Therefore, in the present study, we used acetic acid-induced gastric ulcers in rats with a limited food intake time to evaluate the effect of the zinc-cimetidine complex on the healing of gastric ulcers.

In the present study, the zinc-cimetidine complex at oral doses of more than 15.0 mg/kg twice daily significantly promoted the ulcer healing. On the other hand, cimetidine was significantly effective at oral doses of more than

45.4 mg/kg, twice daily. These results indicate that the zinc-cimetidine complex at 1/4 the dose of cimetidine is as effective as cimetidine when the ulcer-healing effects of both compounds were compared as the same dose of cimetidine, because 15 mg of the zinc-cimetidine complex contains 11.4 mg of cimetidine. However, ZnCl_2 was ineffective even at an oral dose of 49.0 mg/kg, twice daily (the Zn content in 49.0 mg of ZnCl_2 is equal to that in 120.0 mg of the zinc-cimetidine complex). Frommer (17) reported that zinc sulfate given by mouth (220 mg, three times a day) accelerated the healing of gastric ulcers in humans. This discrepancy between our result and his may be due to the differences in humans and rats or doses of zinc. In addition, the effect of the combination of cimetidine and ZnCl_2 was equal to that of cimetidine alone. These results suggest that the antiulcer effect of the zinc-cimetidine complex may be due to the action of a new chelate compound that consists of cimetidine and zinc rather than to the synergistic action of cimetidine and zinc.

It is generally believed that histamine H_2 -receptor antagonists and proton pump inhibitors accelerate the healing of gastric ulcers by potent and long-lasting antisecretory actions. The other main action of both types of agents is to cause hypergastrinemia. Gastrin has been indicated to possess trophic actions such as inducing the proliferation of gastric mucosal cells (18–20) in addition to stimulation of gastric acid secretion. We have already reported that cimetidine and omeprazole mainly accelerate the healing of gastric ulcers by the trophic action of gastrin via the increase in gastrin secretion (21). Therefore, in the next experiment, we investigated the effects of the zinc-cimetidine complex on gastric acid secretion and serum gastrin levels to clarify the mechanism of the ulcer-healing promoting action of this compound. In the present study, a single oral administration of the zinc-cimetidine complex at 60 mg/kg to normal rats was slightly but significantly effective in inhibiting gastric acid secretion and in elevating serum gastrin levels. However, this compound at doses of 15 and 30 mg/kg, which had been proved to accelerate the ulcer healing, did not affect either parameter. In contrast, cimetidine (45.4 and 90.7 mg/kg, p.o.) markedly inhibited acid secretion and elevated serum gastrin levels. Accordingly, it is unlikely that the ulcer healing-promoting action of the zinc-cimetidine complex is related to the inhibition of acid secretion or to the elevation of gastrin secretion. In addition, this finding also indicates that the zinc-cimetidine complex is a new compound that has lost the properties of histamine H_2 -receptor antagonists. It has been recognized that the absorption of the zinc-cimetidine complex given orally to rats from the gastrointestinal tract is very low, while its excretion into feces is very high (unpublished data, Minobe et al.). It is postulated from these findings that

the antiulcer action of the zinc-cimetidine complex in contrast with cimetidine may be due to its local action.

Recently we demonstrated that neutrophils and TBA-reactive substances were markedly increased in the ulcerated region after acetic acid-induced gastric ulcers in rats with a limited food intake time and then decreased gradually as the day went on (10). This result strongly suggests that neutrophils-generated free radicals are important factors for the delay of the healing of this ulcer model. In the present experiment, human Cu, Zn-SOD (10 mg/kg $\times$ 2/day, p.o.) as well as the zinc-cimetidine complex and cimetidine exhibited a marked ulcer-healing promoting action. These three kinds of drugs had already inhibited the increase in TBA-reactive substances (lipid peroxidation) in the ulcerated region before the ulcer-healing effects of these drugs were recognized. These findings suggest that the ulcer-healing promoting action of the zinc-cimetidine complex may be due to the inhibition of lipid peroxidation in the ulcerated region but not due to the inhibition of acid secretion or the trophic effect of gastrin. However, the mechanism by which this compound inhibits lipid peroxidation remains unclear. Further study is needed to clarify the mechanism. Cimetidine was also effective in inhibiting lipid peroxidation. Cimetidine has been shown to be a powerful hydroxyl radical scavenger (22). Therefore, the hydroxyl radical-scavenging property of cimetidine may be partly related to its ulcer-healing effect.

Recently, it was indicated that *Helicobacter (H) pylori* infection may be associated with the pathogenesis of peptic ulcers (23–25) or relapse of peptic ulcers because the relapse rate is markedly reduced after successful eradication (26, 27). The mechanism by which *H. pylori* causes mucosal damages remains unclear. It has been suggested that free radicals formed by neutrophils infiltrated in the mucosa may be involved in the mucosal damages or ulcer relapse elicited by *H. pylori* (28). Therefore, the free radical-scavenging action of the zinc-cimetidine complex may be also effective in preventing the mucosal damages or ulcer relapse elicited by *H. pylori*.

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