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Influence of aging on gastric ulcer healing activities of cimetidine and omeprazole

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Abstract

In this study, we compared the effects of cimetidine and omeprazole on the healing of acetic acid-induced gastric ulcers in 8-, 48-, and 96-week-old rats. The repeated oral administration of cimetidine or omeprazole for 14 consecutive days markedly accelerated the ulcer healing in 8- and 48-week-old rats. However, both drugs were ineffective in 96-week-old rats. The basal gastric acid secretion of 8-, 48-, and 96-week-old rats decreased with aging. A single oral administration of cimetidine or omeprazole strongly decreased basal gastric acid secretion in the three different ages of rats. Cimetidine and omeprazole produced a potent and sustained serum gastrin-elevating action in 8- and 48-week-old rats. However, the gastrin-elevating actions of both drugs in 96-week-old rats were much weaker than in the 8- and 48-week-old rats. These results indicate that cimetidine and omeprazole have potent gastric ulcer healing actions in 8- and 48-week-old rats, as well as potent serum gastrin-elevating actions, but both drugs are ineffective in 96-week-old rats, which have lost their gastrin-elevating actions.

Keywords: Gastric ulcer; Aging; Cimetidine; Omeprazole; (Rat)

1. Introduction

In general, the incidence of gastric ulcers is relatively higher in elderly people, compared to that of duodenal ulcers (Yamagiwa et al., 1981). Aggressive factors such as gastric acid secretion (Khalil et al., 1988; Majumdar et al., 1988; Ohno et al., 1988) and pepsin activity (Ohno et al., 1988) have been shown to decrease with aging in humans and animals. Defensive factors such as gastric mucosal blood flow (Masuda et al., 1991), gastric mucosal prostaglandin (PG) content (Cryer et al., 1992; Goto et al., 1992; Lee and Feldman, 1994) and bicarbonate secretion (Kim et al., 1990) have been demonstrated to cause an age-related decrease in humans and animals. In general, gastric ulcer patients show no change or a decrease in gastric acid secretion and pepsin activity, compared to healthy subjects (Testino, 1996). Gastric acid hypersecretion is observed only in some duodenal ulcer patients, and most other duodenal ulcer patients secrete normal amounts of gastric acid (McQuaid and Isenberg, 1992). Histamine H_2 receptor antagonists and proton pump inhibitors having potent and sustained acid inhibitory action are now widely used as drugs of first choice in treatment of both gastric and duodenal ulcers, although some patients do not have increased gastric acid secretion. On the other hand, it has been hypothesized that both types of drugs promote gastrin release through the elevation of intragastric pH due to antisecretory action (Peters et al., 1983; Larsson et al., 1986). We have reported that both types of potent acid inhibitors may promote gastric ulcer healing by the trophic action of gastrin rather than by an antisecretory action (Ito et al., 1994a,b).

In general, 7- or 8-week-old rats have been used to assess the anti-ulcer actions and the mechanisms for the action of new compounds. As mentioned above, the incidence of gastric ulcers is relatively high in elderly people. Therefore, in order to preclinically evaluate the anti-ulcer actions of new compounds or clarify the mechanisms of their actions, we need to use aged rats. Furthermore, it is unclear whether histamine H_2 receptor antagonists and proton pump inhibitors exert anti-ulcer actions by inhibiting gastric acid secretion or by increasing gastrin release in elderly gastric ulcer patients with decreased acid secretion. Little is known about the influence of aging on the gastric ulcer healing-promoting actions of histamine H_2 receptor antagonists and proton pump inhibitors.

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Therefore, in the present study, we investigated the influence of aging on the gastric ulcer healing activities of cimetidine, a histamine H_2 receptor antagonist, and omeprazole, a proton pump inhibitor, by using 8-, 48-, and 96-week-old rats. In addition, we investigated the influence of aging on acid secretion-inhibitory actions and serum gastrin-elevating actions of both drugs in rats of three different ages.

2. Materials and methods

2.1. Animals

Male Wistar strain SPF rats of three different age groups (8, 48, and 96 weeks old) (Nippon Clea, Japan) were used in the experiments. The animals were housed in an air-conditioned room at 23 ± 1 °C.

2.2. Drugs

The drugs used were cimetidine (Sigma, St. Louis, MO, USA) and omeprazole (Fujisawa-Astra, Osaka, Japan). Both drugs were suspended in 0.5% gum arabic.

2.3. Induction of gastric ulcers and ulcer healing efficacy of test drugs

The three different ages of rats were allowed daily access to commercial food pellets from 9:00 to 10:00 a.m. and 5:00 to 6:00 p.m. throughout the experimental period from 3 days prior to ulcer induction (Ito et al., 1994a). Tap water was always available ad libitum. Gastric ulcers were induced in the three different ages of rats by exposing the serosal surface of the corpus wall to 100 μl of 100% acetic acid for 60 s in accordance with the method of Okabe et al. (1977).Cimetidine was given orally, twice daily (10:30 a.m. and 6:30 p.m.) to each rat of the three different ages at 0.5 ml per 100 g of body weight for 14 consecutive days from the first day after serosal application of acetic acid. Omeprazole was given orally, once daily (10:30 a.m.) for the same period from the first day. Control animals were given just the vehicle (0.5% gum arabic). On the 15th day, the animals were sacrificed by rapid decapitation under ether anesthesia. The stomachs were removed, filled with 5 ml of 10% formalin, and allowed to stand for 5 min. The stomachs were cut open along the greater curvature. The longitudinal and abscissal lengths of the upper, opened part of the ulcer were measured with a micrometer, which was mounted 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 h. The formalin-fixed tissue was then cut so that a little of the normal tissue surrounding the ulcer remained. Thereafter, the central part of the ulcer was cut vertically against the serosa along the long diameters. These tissues, cut in half, were embedded in paraffin and cut into 2- and 3-μm-thick sections. The sections were stained with hematoxylin and eosin. Micrographic histological measurements of the stained preparations were performed as shown in Fig. 1. The healing effect of each test compound was evaluated by comparing the ulcer index, the defective area in the ulcerated region, the index for the decrease in the exposed floor, and the index for the mucosal regeneration of each test drug with the indexes of the respective control.

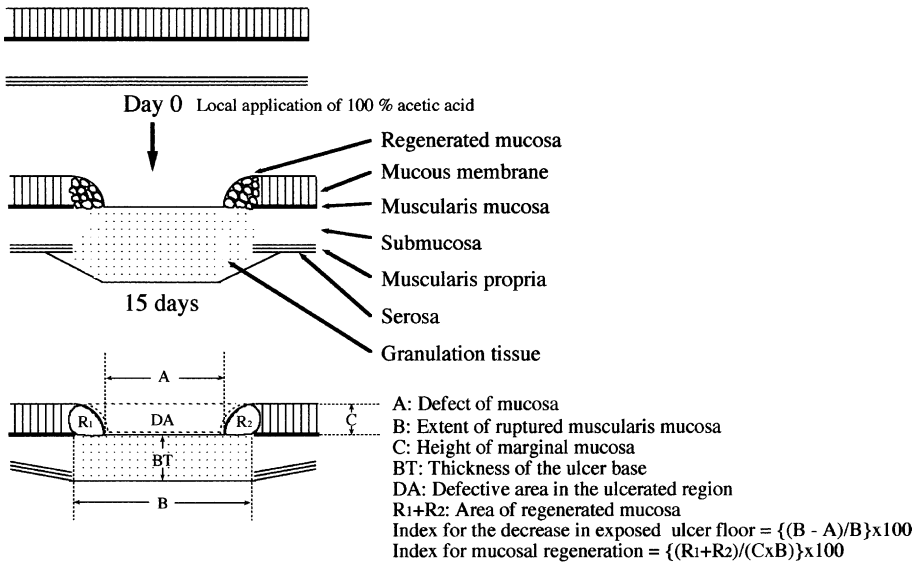

Fig. 1. Method used for histological measurements. Schematic drawings of the vertical section in the ulcerated region on the 15th day after serosal application of acetic acid.

2.4. Effects of test drugs on basal gastric acid secretion

Normal rats of three different ages were fasted for 24 h. Then, the effects of cimetidine and omeprazole on basal acid secretion were examined in the test group. The test drug (cimetidine or omeprazole) was given orally. Control animals were orally given the vehicle (0.5% gum arabic). At 1 h after administration of the test drug or just the vehicle, the pylorus of each rat was ligated under ether anesthesia. The gastric juice was collected for 5 h after ligation. The volume of gastric juice was measured, the acidity was determined by an automatic titrator (ABT-101, Tohadenpa, Tokyo, Japan), and the total acid output during the 1-h period was calculated.

2.5. Basal serum gastrin levels and effects of test drugs on them

In the first experiment, to determine basal serum gastrin levels in the rats of three different ages, blood samples were taken from the cavernous sinus of these animals with a capillary under ether anesthesia.

In the second experiment, to examine the serum gastrin-elevating activities of cimetidine and omeprazole in the rats of three different ages, the test drug or the vehicle (0.5% gum arabic) was given orally to these animals after fasting for 24 h. Blood samples were taken just before (0 h) and 1, 4, 7, 13, and 19 h after administration of the test drug or just the vehicle. The serum gastrin levels were determined by

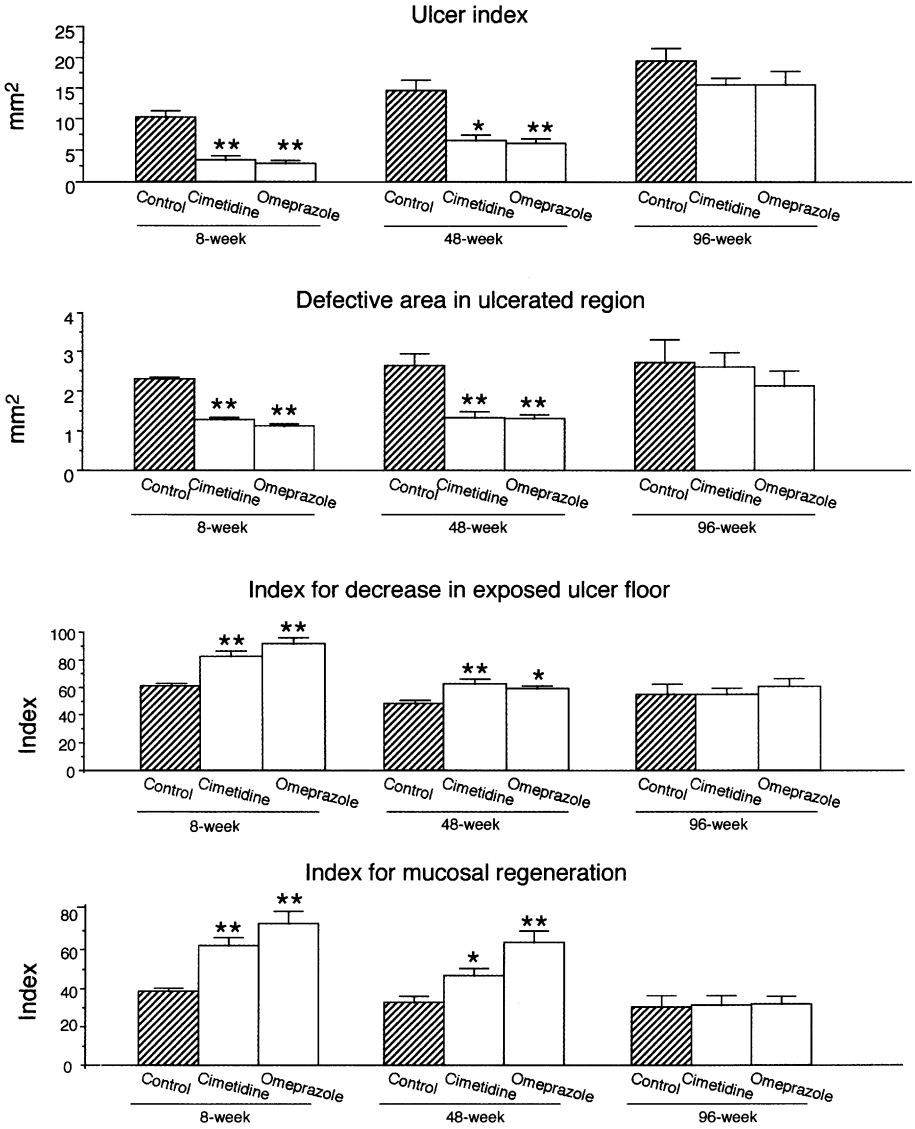

Fig. 2. Influence of aging on ulcer healing activities of cimetidine and omeprazole in acetic acid-induced gastic ulcers in rats. Cimetidine (100 mg/kg) was administered orally, twice daily for 14 consecutive days beginning on the first day after serosal application of acetic acid. Omeprazole (50 mg/kg) was administered orally, once daily for the same period. The ulcer healing activities of both drugs were evaluated on the 15th day. Each column shows the mean ± S.E. for 8 to 10 rats. Significantly different from respective control, * $P<0.05$, ** $P<0.01$.

means of radioimmunoassay using a GASTRIN RIA KIT (Dainabot, Tokyo, Japan).

2.6. Statistical analysis

The results obtained are expressed as the mean ± 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.

3. Results

3.1. Effects of test drugs on ulcer healing

Repeated oral administration of cimetidine or omeprazole for 14 consecutive days markedly accelerated the healing of gastric ulcers in 8- and 48-week-old rats (Fig. 2). Specifically, in 8- and 48-week-old rats, cimetidine (100 mg/kg twice daily) decreased the ulcer index by 67% and 55%, respectively, compared to respective control and decreased the defective area in the ulcerated region by 48% and 50%, respectively. In addition, in 8- and 48-week-old rats, cimetidine increased the index for the decrease in the exposed ulcer base by 35% and 30%, and increased the index for mucosal regeneration by 60% and 44%, respectively. In 8- and 48-week-old rats, omeprazole (50 mg/kg once daily) decreased the ulcer index by 72% and 58%, and decreased the defective area in ulcerated region by 52% and 50%, respectively. In addition, in 8- and 48-week-old rats, omeprazole increased the index for the decrease in the exposed ulcer base by 50% and 23%, and increased the index for mucosal regeneration by 91% and 96%, respectively. Thus, the healing effects of both drugs in 48-week-old rats were not significantly different from those in 8-week-old rats. However, in 96-week-old rats, cimetidine and omeprazole did not accelerate the ulcer healing.

3.2. Effects of test drugs on basal gastric acid secretion

The volumes of gastric juice collected by a 5-h pylorus ligation of 8-, 48-, and 96-week-old control rats were 1.2 ± 0.1, 0.8 ± 0.1, and 0.5 ± 0.0 ml/h, respectively (Fig. 3). On the other hand, the total acid outputs in 8-, 48-, and 96-week-old rats were 81.3 ± 7.2, 37.6 ± 6.1, and 24.3 ± 3.6 μEq/h, respectively. Thus, the volume of gastric juice and the total acid output in the control rats markedly decreased with age. A single oral administration of cimetidine (100 mg/kg) reduced the volume of gastric juice in 8-, 48-, and 96-week-old rats by 50%, 38%, and 60%, respectively, compared to the respective control. In addition, cimetidine inhibited the total acid output in 8-, 48-, and 96-week-old rats by 77%, 100%, and 95%, respectively. On the other hand, a single oral administration of omeprazole (50 mg/kg) reduced the volume of gastric juice in 8-, 48-, and 96-week-old rats inhibited by 42%, 38%, and 60%, respectively. In addition, omeprazole inhibited the total acid output of 8-, 48-, and 96-week-old rats by 100%, 100%, and 96%, respectively.

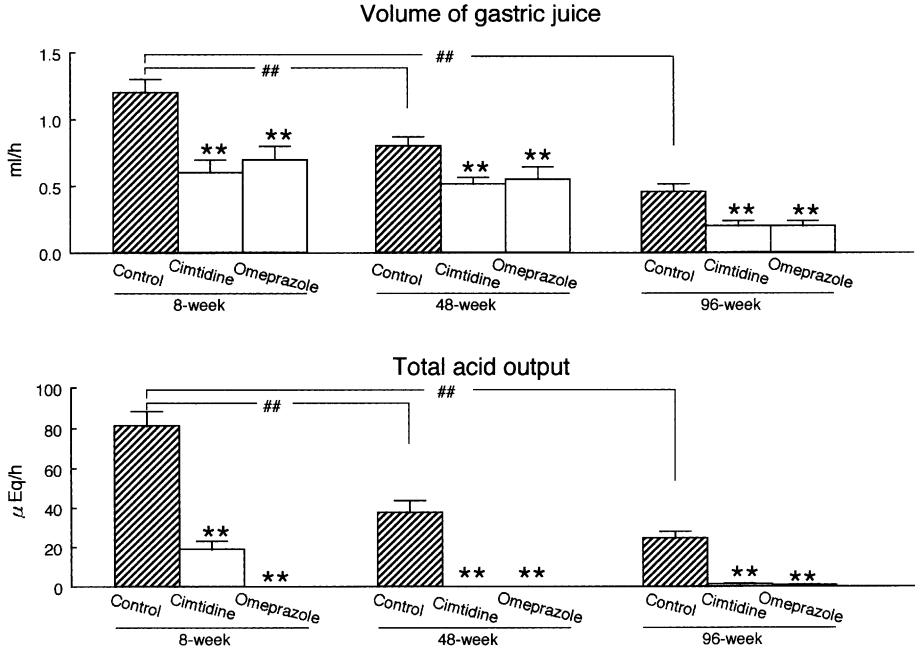

Fig. 3. Influence of aging on gastric acid secretion-inhibitory actions of cimetidine and omeprazole in rats. After fasting for 24 h, cimetidine (100 mg/kg) or omeprazole (50 mg/kg) was administered orally. At 1 h after the administration, the pylorus of each rat was ligated and then gastric juice was collected for 5 h to evaluate the effects of both drugs on basal gastric secretion. Each column shows the mean ± S.E. for eight rats. Significantly different from respective 8-week-old, ##$P < 0.01$. Significantly different from respective control, **$P < 0.01$.

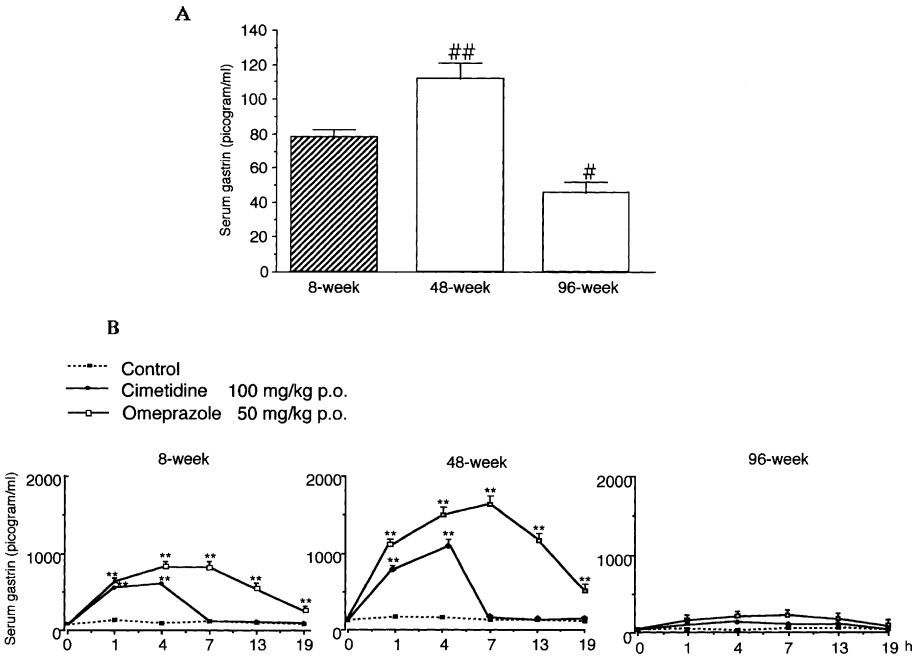

Fig. 4. Influence of aging on basal serum gastrin levels (A) and on serum gastrin-elevating activities of cimetidine and omeprazole (B) in rats. In experiment A, blood samples were taken from rats fasted for 24 h. In experiment B, cimetidine (100 mg/kg) or omeprazole (50 mg/kg) was administered orally to rats fasted for 24 h. Blood samples were taken at just before (0 h) and 1, 4, 7, 13, and 19 h after the administration. Each plot shows the mean ± S.E. for five rats. Significantly different from respective control, ** $P<0.01$.

3.3. Basal serum gastrin levels and effects of test drugs on them

The basal serum gastrin levels in 48-week-old rats were significantly higher than in 8-week-old rats (48-week: 112 ± 9 pg/ml vs. 8-week: 78 ± 4 pg/ml, $P<0.01$) (Fig. 4A). However, the basal gastrin levels in 96-week-old rats were markedly lower than in 8-week-old rats (96-week: 45 ± 0.3 pg/ml vs. 8-week: 78 ± 4 pg/ml, $P<0.05$). A single oral administration of cimetidine (100 mg/kg) or omeprazole (50 mg/kg) to 8- and 48-week-old rats caused a marked elevation in the serum gastrin levels (Fig. 4B). Specifically, the gastrin levels in 8- and 48-week-old rats reached the maximum at 4 and 7 h, respectively, after the administration (8- and 48-week: cimetidine, 555 ± 12 and 1086 ± 25 pg/ml, respectively; omeprazole, 980 ± 21 and 1678 ± 34 pg/ml, respectively). Thus, the elevation in gastrin levels by both drugs was markedly higher in 48-week-old rats than in 8-week-old rats.

However, the gastrin-elevating action of cimetidine and omeprazole in 96-week-old rats was extremely weak at the times of measurement (cimetidine at 4 h: 120 ± 5 pg/ml; omeprazole at 7 h: 205 ± 12 pg/ml).

4. Discussion

The present study indicated that in 8- (young) and 48-week-old (aged) rats, cimetidine, a histamine H_2 receptor antagonist, and omeprazole, a proton pump inhibitor, have potent gastric ulcer healing-promoting actions, mainly through the trophic action of gastrin. However, the study also indicated that neither antisecretory drug promoted ulcer healing in 96-week-old (extremely aged) rats. In these rats, neither drug caused hypergastrinemia.

As mentioned in Introduction, in general, the incidence of gastric ulcers in humans increases with aging. Gastric acid secretion (Khalil et al., 1988; Majumdar et al., 1988; Ohno et al., 1988) and pepsin activity (Ohno et al., 1988) are lower in elderly persons than in young persons. In the present experiment, aging was associated with a decrease in basal gastric acid secretion in 8-, 48-, and 96-week-old rats. Cimetidine and omeprazole strongly inhibited basal gastric acid secretion in these three ages of rats. Cimetidine and omeprazole markedly accelerated the healing of acetic acid-induced gastric ulcers in 8- and 48-week-old rats. However, both drugs failed to promote healing in the 96-week-old rats. Therefore, it is unlikely that the inhibition of acid secretion is the main factor in the healing of gastric ulcers in 8- and 48-weak-old rats.

As mentioned in Introduction, cimetidine and omeprazole are thought to cause hypergastrinemia through the elevation of intragastric pH due to antisecretory action (Peters et al., 1983; Larsson et al., 1986). In addition to stimulating gastric acid secretion, gastrin has been shown to possess trophic actions, for example, it stimulates the proliferation of gastric mucosal cells (Willems et al., 1972; Johnson and Guthrie, 1974; Johnson et al., 1975; Hansen et al., 1976). We have already demonstrated by using rats that there is a clear relationship between the serum gastrin-

increasing action and the ulcer healing-promoting action of cimetidine and omeprazole in acetic acid-induced gastric ulcers in rats (Ito et al., 1994a). In addition, we clarified that, under the condition of a decreased density of gastrin cells in the antrum induced by 6-hydroxydopamine treatment, the acceleration of gastric ulcer healing by daily repeated administrations of cimetidine or omeprazole was completely negated without affecting the inhibition of acid secretion by either drug (Ito et al., 1994b). In this experiment, we demonstrated that pentagastrin accelerated the healing of chronic gastric ulcers in rats. Konturek et al. (1988) have demonstrated that twice-daily s.c. injections of growth hormone-releasing factor (GRF) enhance the healing-rates of acetic acid-induced gastric ulcers in rats via an increase in serum gastrin and tissue epidermal growth factor and that somatostatin almost completely negated the ulcer healing effect of GRF. It has been shown that tetragastrin prevents ethanol-induced rat gastric mucosal injury via an increase in the mucin content (Komuro et al., 1992). The above findings suggest that gastrin may play an important role in both gastric mucosal protection and gastric ulcer healing. Therefore, in the next experiment, we examined the influence of aging on serum gastrin-elevating activities of cimetidine and omeprazole by using rats of three different ages. Cimetidine and omeprazole produced a marked elevation of serum gastrin levels in 8- and 48-week-old rats. In these rats, both drugs showed equal gastric ulcer healing-promoting actions. However, no hypergastrinemia was observed after the administration of cimetidine or omeprazole in 96-week-old rats. In these rats, neither drug was an effective accelerator of ulcer healing. As mentioned in Introduction, it has been hypothesized that cimetidine and omeprazole promote gastrin release through the elevation of intragastric pH due to their antisecretory actions (Peters et al., 1983; Larsson et al., 1986). Therefore, the results obtained in 96-week-old rats suggest that regulation via a negative feedback mechanism between intragastric pH and gastrin secretion may not operate in extremely aged rats. These results also suggest that the marked acceleration of gastric ulcer healing by cimetidine and omeprazole seen in 8- and 48-week-old rats may be due to the trophic effects of gastrin rather than the inhibition of acid secretion.

The degree of hypergastrinemia induced by the administration of cimetidine and omeprazole was greater in 48-week-old rats than in 8-week-old rats. The ulcer healing effects of both drugs in 48-week-old rats were not different from those in 8-week-old rats. Defensive factors such as gastric blood flow (Masuda et al., 1991), gastric mucosal PG content (Cryer et al., 1992; Goto et al., 1992; Lee and Feldman, 1994), and bicarbonate secretion (Kim et al., 1990) cause an age-related decrease in humans and animals. Therefore, the above results suggest that the decrease in these defensive factors may also affect healing in 48-week-old rats.

In summary, cimetidine and omeprazole, which are potent gastric acid inhibitors, mainly accelerate gastric ulcer healing by the trophic action of gastrin in aged rats as well as in young rats. However, both drugs were ineffective in extremely aged rats. In these rats, both drugs had a markedly weak gastrin-elevating action. However, aging causes changes in various physiological functions. Therefore, further studies need to clarify the reasons why neither of these potent anti-ulcer drugs is an effective ulcer healing agent in extremely aged rats.

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