Simultaneous Evaluation of Gastric and Duodenal Ulcers-Healing Activities of Anti-ulcer Agents in Rats

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We investigated the effects of cimetidine, omeprazole and atropine, antisecretory anti-ulcer agents, on the healing of gastric and duodenal ulcers simultaneously induced in the same rats. Furthermore, we examined the effect of histamine, an acid secretory agent, on the healing of both ulcers. When the effects of test drugs were assessed on the 15th day after local application of acetic acid, repeated oral administration of cimetidine (50 and 100 mg/kg twice daily) or omeprazole (25 and 50 mg/kg once daily) markedly accelerated the healing of both gastric and duodenal ulcers. Atropine (10 mg/kg twice daily, *p.o.*) showed a healing effect on duodenal ulcers only. The repeated subcutaneous administration of histamine (30 mg/kg 3 times daily) apparently delayed the healing of duodenal ulcers but not gastric ulcers. In conclusion, this experimental chronic ulcer model in rats is useful for directly comparing the effects of anti-ulcer drugs on the healing of gastric and duodenal ulcers. In addition, the increase in acid secretion appears to have a greater influence on the delay of ulcer healing in the duodenum than in the stomach.

Key words gastric ulcer; duodenal ulcer; gastric acid; anti-ulcer agent

Acetic acid-induced ulcers in rats have been widely used to evaluate the effects of anti-ulcer agents on the healing of chronic gastric or duodenal ulcers.^{1,2)} However, the effects of test drugs on the healing of chronic ulcers using this model have been evaluated more in the stomach than in the duodenum. The effects of test drugs on duodenal ulcers have been evaluated by using experimental models of acute duodenal ulcers induced in rats by the subcutaneous or oral administration of cysteamine³⁾ or mepirizole.⁴⁾ The preventive effects of test drugs on these acute duodenal ulcers are evaluated by giving the drugs once or twice before treatment with cysteamine or mepirizole. The effects of test drugs on the healing of chronic duodenal ulcers are not the same as their preventive effects on acute duodenal ulcers. Furthermore, drugs which accelerate the healing of chronic gastric ulcers are not always effective in healing chronic duodenal ulcers. Therefore, it is necessary to evaluate the drug effects on the healing of chronic duodenal ulcers as well as chronic gastric ulcers.

In the present study, in order to evaluate the effects of antisecretory anti-ulcer agents on the healing of chronic gastric and duodenal ulcers, we performed a direct comparison of the effects of cimetidine, a histamine H₂ receptor antagonist, omeprazole, a proton pump inhibitor, and atropine, an anti-cholinergic agent, on the healing of both ulcers simultaneously induced in the same rats by local application of acetic acid. Furthermore, we examined the effects of histamine, an acid secretory agent, on the healing of both ulcers.

MATERIALS AND METHODS

Animals Male Sprague Dawley strain SPF rats (Nippon SLC, Shizuoka, Japan), weighing 220–240 g, were used in the experiments. The animals were housed in an air-conditioned room at 23 ± 1 °C.

Drugs The drugs used were cimetidine (Sigma Chemical Co., St. Louis, U.S.A.), omeprazole (Fujisawa-Astra, Osaka, Japan), atropine sulfate (Sigma Chemical

Co., St. Louis, U.S.A.) and histamine dihydrochloride (Merck, Darmstadt, Germany). Cimetidine and omeprazole were suspended in 0.5% gum arabic. Atropine and histamine were dissolved in 0.5% gum arabic and 0.9% saline, respectively.

Induction of Gastric and Duodenal Ulcers The rats were allowed daily access to commercial food pellets between 9:00–10:00 a.m. and 5:00–6:00 p.m. throughout the experimental period from 3 d prior to the ulcer induction.⁵⁾ However, tap water was always supplied *ad libitum*. Gastric and duodenal ulcers were induced in the same rats by exposing the serosal surface of the corpus wall to 100 µl of 100% acetic acid for 1 min through a metal mold 8 mm in diameter, and by exposing the serosal surface of duodenal wall 10 mm distal to the pylorus to 50 µl of 80% acetic acid, respectively, by a modification of the method of Okabe *et al.*²⁾ An excess of acetic acid was gently swabbed and then the abdomen closed.

Effects of Cimetidine, Omeprazole and Atropine on Ulcer Healing Cimetidine and atropine were given orally, twice daily (10:30 a.m. and 6:30 p.m.) to each rat in both groups in a volume of 0.5 ml per 100 g of body weight for 14 consecutive days from the day (the 1st day) after local application of acetic acid. Omeprazole was given orally, once daily (10:30 p.m.) for the same period from the 1st day. Control animals were given 1% gum arabic instead of the respective test drug. On the 15th day, the animals were killed by rapid decapitation. The stomach, including part of the duodenum, was removed, filled with 8 ml of 10% formalin and allowed to stand for 10 min. The stomach and duodenum were open along the greater curvature and its duodenal extension. The longitudinal and abscissal lengths of the upper, opened part of the gastric and duodenal ulcers were measured with a micrometer which was set on a stereoscopic microscope, and the product of both lengths (mm²) was expressed in terms of an ulcer index.

Effects of Histamine on Gastric Acid Secretion and Ulcer Healing In order to examine the effect of histamine on gastric acid secretion in normal rats, the animals that had

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been fasted for 24 h were given histamine (30 mg/kg) subcutaneously in a volume of 0.5 ml per 100 g of body weight. The gastric juice was collected by 3 h pylorus ligation under ether anesthesia from 0, 3 and 6 h after the administration of histamine. Gastric juice from the control animals was collected by 3 h pylorus ligation immediately after the subcutaneous administration of saline. The volume of gastric juice was measured and the acidity was determined with an automatic titrator (ABT-101, Tohadempa, Tokyo, Japan), and total acid output during a 1 h period was calculated.

In order to evaluate the effect of gastric acid on ulcer healing, histamine (30 mg/kg) was given subcutaneously in a volume of 0.5 ml per 100 mg of body weight twice (10:30 a.m. and 6:30 p.m.) or three times daily (10:30 a.m., 1:30 p.m. and 6:30 p.m.) for 9 consecutive days from the 6th day after the application of acetic acid. On the 15th day, the size of both ulcers was measured as mentioned above.

Statistical Analysis The results obtained are expressed the mean $\pm$ 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 of Gastric Antisecretory Drugs on Ulcer Healing Figures 1, 2 and 3 show the effects of repeated oral administration of cimetidine, omeprazole or atropine for 14 consecutive days on the healing of gastric and duodenal ulcers simultaneously induced in the same rats.

Cimetidine given at doses of 50 and 100 mg/kg twice daily decreased the index for gastric ulcers by 62% and 65%, respectively, and the index for duodenal ulcers by 44% and 65%, respectively (Fig. 1).

Omeprazole at 25 and 50 mg/kg once daily decreased the index for gastric ulcers by 47% and 70%, respectively, and the index for duodenal ulcers by 69% and 72%, respectively (Fig. 2).

Atropine at doses of 5 and 10 mg/kg twice daily was ineffective in promoting the healing of gastric ulcers (Fig. 3). However, this drug at 10 mg/kg twice daily significantly decreased the index for duodenal ulcers, by 50%.

Effect of Gastric Secretagogue on Ulcer Healing A single subcutaneous injection of histamine at 30 mg/kg to normal rats significantly increased the volume of gastric juice by 59% and 31%, respectively, and total acid outputs by 165% and 53%, respectively, in rats with a ligated pylorus for 0—3 h and 3—6 h after the injection (Fig. 4).

Fig. 1. Effect of Cimetidine on the Healing of Acetic Acid-Induced Gastric (A) and Duodenal Ulcers (B) in Rats

Cimetidine was given twice daily for 14 consecutive days beginning the day after local application of acetic acid. Each column denotes the mean $\pm$ S.E. for 7 to 9 rats. Significantly different from respective control, * p < 0.05.

Fig. 2. Effect of Omeprazole on the Healing of Acetic Acid-Induced Gastric (A) and Duodenal Ulcers (B) in Rats

Omeprazole was given once daily for 14 consecutive days beginning the day after local application of acetic acid. Each column denotes the mean $\pm$ S.E. for 8 to 9 rats. Significantly different from respective control, * p < 0.05, ** p < 0.01.

Fig. 3. Effect of Atropine on the Healing of Acetic Acid-Induced Gastric (A) and Duodenal Ulcers (B) in Rats

Atropine was given orally, twice daily for 14 consecutive days beginning the day after local application of acetic acid. Each column denotes the mean $\pm$ S.E. for 7 to 8 rats. Significantly different from respective control, * p < 0.05.

Fig. 4. Effects of Histamine on Gastric Secretion in Normal Rats

To determine the gastric secretion, each rat received a 3 h pylorus ligation from 0, 3 and 6 h after the subcutaneous injection of histamine (30 mg/kg). Each column denotes the mean $\pm$ S.E. for 5 rats. Significantly different from respective control, * p < 0.05, ** p < 0.01.

Fig. 5. Effect of Histamine on the Healing of Acetic Acid-Induced Gastric (A) and Duodenal Ulcers (B) in Rats

Histamine (30 mg/kg) was given subcutaneously, twice or three times daily for 9 consecutive days beginning the 6th day after the local application of acetic acid. Each column denotes the mean $\pm$ S.E. for 8 to 9 rats. Significantly different from respective control, ** p < 0.01.

Repeated subcutaneous injection of histamine at 30 mg/kg 3 times daily for 9 consecutive days, from the 6th day after the application of acetic acid, significantly increased the index for duodenal ulcers by 74%, although the same treatment with histamine twice daily did not affect it (Fig. 5). The healing of gastric ulcers was unaffected by treatment with histamine twice or three times daily.

DISCUSSION

The present study indicates that an experimental model of gastric and duodenal ulcers simultaneously induced in the same rats by local application of acetic acid is useful for directly comparing the effects of anti-ulcer agents on the healing of both ulcers. Furthermore, the degree of acid secretion may influence to a greater degree the healing of duodenal ulcers than gastric ulcers.

Our previous studies indicated that cimetidine and omeprazole were 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.⁵⁾ Therefore, we limited their food intake time throughout this experimental period. In the present experiment, among three gastric antisecretory agents, cimetidine (50 and 100 mg/kg twice daily, *p.o.*) and omeprazole (25 and 50 mg/kg once daily, *p.o.*) exhibited rapid healing effects on both gastric and duodenal ulcers. On the other hand, atropine (10 mg/kg twice daily, *p.o.*) was as effective as cimetidine (100 mg/kg twice daily, *p.o.*) in healing duodenal ulcers, but atropine (5 and 10 mg/kg twice daily, *p.o.*) did not affect the healing of gastric ulcers, although this drug (10 mg/kg, *p.o.*) showed an antisecretory action equivalent to that of cimetidine (100 mg/kg, *p.o.*).⁵⁾ This result agrees with that of Okabe *et al.*²⁾ who evaluated the effects of atropine on the healing of both ulcers induced separately in different rats by local application of acetic acid. It is generally believed that the inhibition of acid secretion is the most important factor for the healing of both gastric ulcers and duodenal ulcers. However, we cannot conclude from the results of the effect of atropine on the healing of gastric ulcers that the inhibition of acid secretion is an essential factor for the healing of gastric ulcers. Our previous studies⁵⁾ indicated that cimetidine and omeprazole resulted in potent and long-lasting antisecretory and gastrin-releasing actions, while the elevation of serum gastrin level by atropine was weak and temporary. In addition, pretreatment with 6-hydroxydopamine, which reduces gastrin release through chemical sympathectomy, completely abolished the gastric ulcer-healing promoting actions of cimetidine or omeprazole without affecting their antisecretory action.⁶⁾ Furthermore, it has been indicated

that pentagastrin enhances the healing of experimental chronic gastric ulcers.^{6,7)} Therefore, it is postulated from these findings that gastrin is an important factor in the healing of gastric ulcers and that cimetidine and omeprazole mainly accelerate the healing of gastric ulcers through the trophic action of gastrin, such as the proliferation of gastric mucosal cells.⁸⁻¹⁰⁾ In addition, a weak action of atropine on gastrin release may be related to the fact that this agent had no effect on the healing of gastric ulcers. Anti-cholinergic agents, including atropine, are well-known to inhibit gastric motility, which may lead to the delayed healing of gastric ulcers by delaying gastric emptying.¹¹⁾ Therefore, the inhibitory action of atropine on gastric motility may partly affect the unfavorable effect of this agent on the healing of gastric ulcers.

In the present experiment, the increased acid secretion by repeated daily subcutaneous treatment of histamine caused the delayed healing of duodenal ulcers, although it did not affect the healing of gastric ulcers. This result indicates that the increase in acid secretion influences to a greater degree the delay of ulcer healing in the duodenum than in the stomach. In addition, the results obtained with atropine and histamine suggest that the duodenal wall may be more sensitive to acid than the gastric wall. However, further experiments are necessary to clarify the role of changes in acid secretion in the healing of gastric ulcers.

In summary, a model of experimental gastric and duodenal ulcers simultaneously induced in the same rats is not only suitable for directly comparing the effects of anti-ulcer agents on the healing of both ulcers, but also could elucidate factors and mechanisms for the healing of both ulcers.

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