Effect of Omeprazole on Delayed Healing of Acetic Acid-Induced Gastric Ulcers in Rats

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Abstract—Omeprazole, a gastric mucosal proton pump inhibitor, significantly and dose-dependently prevented the delayed healing of acetic acid-induced gastric ulcers in response to repeatedly administered indomethacin to rats. Both basal and histamine-stimulated gastric acid secretions in rats with acetic acid-induced ulcers that were given indomethacin were markedly and persistently (>24 hr) inhibited after 4 weeks treatment with omeprazole. The prevention of delayed ulcer healing by omeprazole appears to be due to its long-lasting antisecretory activity.

We reported that repeated administration of indomethacin for 2 or 4 weeks significantly delayed the healing of acetic acid-induced gastric ulcers in rats (1). The delayed ulcer healing with indomethacin was significantly prevented when prostaglandin E_2 (PGE_2) or NC-1300 (a gastric mucosal proton pump inhibitor) was given concomitantly at an antisecretory dose (1, 2). Omeprazole, another proton pump inhibitor, exhibits potential and long-lasting (>18 hr) antisecretory activities in intact rats (3, 4) and enhances the spontaneous healing of acetic acid-induced gastric ulcers in rats (5). The present study was performed to determine whether or not omeprazole also prevents the delay in ulcer healing in response to indomethacin and whether or not the agent inhibits gastric acid secretion in rats with ulcers.

Male Donryu rats (Nihon SLC Co.), weighing 240–260 g at the beginning of the experiments, were used. Gastric ulcers were induced by submucosal injection of 20% acetic acid (0.03 ml) into the glandular portion, as previously described (1, 6). Five days after the acid injection, there were well-defined deep ulcers in the rats' stomachs. Thus, the 5th day after the acid injection was designated as the day of ulceration. To delay the ulcer healing, indomethacin (Sigma),

suspended in a minimal volume of Tween 80 and saline, was given s.c. at 1 mg/kg once daily (9:00 a.m.) for 2 or 4 weeks after ulceration. Omeprazole (Hässle), suspended in 0.5% carboxymethylcellulose, or the vehicle alone was given p.o. at 10, 30 and 100 mg/kg once daily 0.5 hr after indomethacin treatment (9:30 a.m.) for 2 or 4 weeks. The animals were deprived of food but allowed free access to water for 24 hr after the final administration of omeprazole. The animals were then killed under ether anesthesia. Their stomachs were removed, inflated with 8 ml of 2% formalin, and placed in 2% formalin for 10 min. Then, the ulcerated area (mm^2) was determined under a dissecting microscope (Olympus, $\times 10$).

The effects of indomethacin and indomethacin plus omeprazole on both basal and histamine-stimulated gastric acid secretion in rats with acetic acid-induced gastric ulcers were determined. Basal acid secretion was studied by ligating the pylorus for 4 hr from 1.0 hr after the final administration of indomethacin, or from 0.5 or 20 hr after the final administration of omeprazole, given for 4 weeks. The animals were killed 4 hr after the pylorus ligation, and then the volume and acidity were determined after centrifugation at 3,000 rpm for 10 min. Acidity was determined by titrating the gastric juice

with 0.1 NaOH to pH 7.0 using an automatic titrator (Comtite, Hiranuma). Acid output is expressed as $\mu\text{Eq/hr}$. Histamine-stimulated gastric acid secretion was studied by injecting histamine $\cdot 2\text{HCl}$ (Nacalai Tesque), dissolved in saline, s.c., at 20 mg/kg twice at 2 hr intervals into pylorus-ligated rats. Four hours later, the animals were killed, and then the gastric contents were collected and analyzed as described above. Student's *t*-test was used to determine the statistical significance of the data, and $P < 0.05$ was regarded as being significant. All data are means $\pm$ one S.E.

Two and four weeks after ulceration, the ulcerated areas in the control groups that received the vehicle alone were 6.6 ± 1.1 and 3.8 ± 0.5 mm², respectively. When indomethacin was given for 2 and 4 weeks, the ulcerated areas were 16.0 ± 1.5 and 11.2 ± 0.8 mm², respectively (Fig. 1). The difference between the corresponding control and indomethacin-treated groups was statistically significant. Omeprazole, given concomitantly with indomethacin for 2 and 4 weeks, dose-dependently prevented the delayed healing of acetic acid-induced gastric ulcers. In 2-

week experiments, the prevention of delayed healing was significant when 10, 30 and 100 mg/kg of omeprazole was administered; the prevention being 27.5, 60.0 and 75.6%, respectively. After 4 weeks treatment with omeprazole, the prevention was significant only when 30 and 100 mg/kg of omeprazole were given, the prevention being 33.9 and 55.4%, respectively.

Neither the basal nor histamine-stimulated gastric secretions in rats with ulcers was affected by indomethacin treatment for 4 weeks (Fig. 2). When the secretion study was performed 0.5 hr after the final administration of omeprazole, the basal and histamine-stimulated acid secretions were found to be nearly completely inhibited by treatment with 30 and 100 mg/kg of the agent. At that time, the volume in the basal secretion was not significantly inhibited. However, the volume in histamine-stimulated secretion was significantly inhibited, the inhibition with 30 and 100 mg/kg being 51.7 and 44.8%, respectively. When the secretion study was begun from 20 hr after the final treatment with omeprazole, the basal acid secretion was still significantly inhibited, the inhibition

Fig. 1. Effect of omeprazole on delayed healing of acetic acid-induced gastric ulcers (A.A.) in rats. Healing of gastric ulcers was delayed by repeated administration of indomethacin (1 mg/kg, s.c., once daily) for 2 or 4 weeks after ulceration. Omeprazole, together with indomethacin, was given p.o. once daily for 2 or 4 weeks after ulceration. Data are means $\pm$ one S.E. *Significantly different from A.A. alone at $P < 0.05$. *Significantly different from A.A. + indomethacin at $P < 0.05$.

Fig. 2. Effects of indomethacin and indomethacin+omeprazole on basal and histamine-stimulated gastric acid secretions in rats with acetic acid ulcers (A.A.). Indomethacin (1 mg/kg, s.c.) and indomethacin+omeprazole (p.o.) were given once daily for 4 weeks after ulceration. Basal and stimulated secretions were determined by means of pylorus ligation from 0.5 and 20 hr after the final administration of omeprazole. Stimulated secretion was obtained by giving histamine·2HCl (20 mg/kg, s.c., twice, immediately and 2 hr after pylorus ligation). The animals were killed 4 hr after pylorus ligation. Data are means±one S.E. *Significantly different from A.A.+indomethacin at $P<0.05$.

with 30 and 100 mg/kg being 42.4 and 60.4%, respectively. The volume of basal secretion was unaffected by the agent, as observed in the study performed 0.5 hr after omeprazole treatment. Histamine-stimulated acid secretion was also significantly inhibited with 100 mg/kg of omeprazole, the inhibition being 47.3%. However, the volume of gastric juice was not affected by that time.

We reconfirmed that daily administration of indomethacin for 4 weeks significantly delayed the spontaneous healing of acetic acid-induced gastric ulcers in rats. Inter-

estingly, omeprazole significantly prevented the delayed ulcer healing in response to indomethacin in a dose-dependent manner. Similar to the intact animals, omeprazole, given repeatedly for 4 weeks, significantly inhibited both the basal and histamine-stimulated gastric secretions in rats with ulcers even 24 hr after the final administration. Therefore, it is most likely that the preventive effect of omeprazole on delayed ulcer healing is causally related to its long-lasting antisecretory effect. It should be noted that 4 weeks treatment with indome-

thacin had no effect on basal or histamine-stimulated secretion. These results suggest that the mechanism by which indomethacin delays gastric ulcer healing is unrelated to increased acid secretion. Non-antiinflammatory agents, including indomethacin, significantly reduce various prostanoids levels in the gastric mucosa of experimental animals, as reviewed by Whittle and Vane (7). We have reported that indomethacin given once or repeatedly for 4 weeks significantly reduced the endogenous PGE₂ level in the gastric mucosa around ulcers (1). Accordingly, we postulated that a reduction in endogenous PGE₂, as well as other PGs which were synthesized through the action of cyclooxygenase, weakened the mucosal defensive mechanism. Therefore, gastric acid which is secreted normally might attack the weakened ulcerated area, resulting in delayed ulcer healing. Recently, Ogiwara et al. (8) found that repeated administration of indomethacin for 2 or 4 weeks to rats with acetic acid-induced gastric ulcers significantly inhibited the decrease in connective tissue once formed in the ulcer base. The underlying mechanism remains unknown. It would be of interest to determine whether or not omeprazole, in addition to its antisecretory activity, can counteract the unfavorable effect of indomethacin on connective tissue. At present, we conclude that omeprazole significantly prevents the delayed gastric ulcer healing in response to indomethacin, most probably through its long-lasting antisecretory effect.

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