Effects of Orally Administered Human Epidermal Growth Factor on Natural and Delayed Healing of Acetic Acid-Induced Gastric Ulcers in Rats

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Abstract—We examined the effects of orally administered human epidermal growth factor (hEGF) on healing of acetic acid-induced gastric ulcers in rats. hEGF, given twice daily at 30 and 100 μg/kg for 2 weeks or at 100 μg/kg for 4 weeks to rats with ulcers, had no effect on natural healing or the gastric secretion, delayed one caused by indomethacin. Oral hEGF had no effect on basal histamine-stimulated gastric secretion, and stomach weight. These results indicate that oral hEGF has no biological activity on the pathophysiology of the stomach.

Recent studies demonstrated that exogenous epidermal growth factor (EGF), given orally or subcutaneously, significantly accelerated the healing of chronic gastric ulcers in rats (1-4). We also found that genetically produced human EGF (hEGF), given subcutaneously at 100 and 300 μg/kg (antisecretory doses) twice daily, significantly accelerated the natural and delayed healing of acetic acid-induced gastric ulcers in rats (5). In this study, we examined whether or not orally administered hEGF also accelerates natural and/or delayed healing of acetic acid-induced gastric ulcers and inhibits gastric acid secretion in rats.

Male Donryu rats, weighing 240-260 g, were used. Under ether anesthesia, gastric ulcers were produced by a submucosal injection of 20% acetic acid (0.03 ml/rat) into the antrum near the junction of the corpus (6). To delay healing of the ulcers, indomethacin (1 mg/kg, Sigma), suspended in saline containing a few drops of Tween 80, was given subcutaneously once daily (9:00 A.M.) for 4 weeks beginning 5 days after the acid injection (7). hEGF (30 and 100 μg/kg, genetically produced with Escherichia coli by Wakunaga Pharm. Co.), dissolved in saline containing 0.01% Tween 80 or the vehicle alone, was given orally twice daily (9:30 A.M., 6:30 P.M.) for 2 (natural healing) or 4 (delayed healing) weeks beginning 5 days after the acid injection. After the final administration, the animals were deprived of the food but allowed free access to water for 20 hr. After sacrificing the animals, the stomachs were removed, inflated by injecting 8 ml of 2% formalin and then put into 2% formalin for 10 min. Subsequently, the stomachs were incised along the greater curvature. The ulcerated area (mm²) was determined under a dissecting microscope (×10) by an observer who was unaware of the treatment given. The wet weight of each stomach was measured. The antisecretory activities of hEGF on basal and histamine-stimulated gastric acid secretion were determined using pylorus ligation and acute fistula preparations. Animals were deprived of food for 20 hr before the experiment and kept in raised mesh-bottomed cages to prevent coprophagy. In the former animals, the abdomen was incised under ether anesthesia and the pylorus ligated. Four hours later, the animals were killed, and the gastric contents were collected. In the latter animals, the abdomen was incised under ether anesthesia, and a gastric fistula with an esophageal cannula which removes the inflow of saliva into the gastric juice was prepared in the forestomach.
After closing the abdomen, the animals were placed in Bollman cages, and the gastric samples were collected at 1 hr intervals for 4 hr; and histamine·2HCl (20 mg/kg, Nacalai Tesque), dissolved in saline, was given subcutaneously, twice, at 2 hr intervals. The collection of gastric secretion and the administration of histamine started from 1 hr after operation. The samples were analyzed for volume and acidity: acidity was titrated to pH 7.0 against 0.1 N NaOH. hEGF was given orally 0.5 hr before the pylorus ligation or 1 hr before the acute fistula operation. Statistical analysis of data (N=8–38) was performed using Student's t-test, and P<0.05 was regarded as being significant.

Submucosal injection of acetic acid consistently produced well-defined deep ulcers in the stomachs (the ulcerated area was about 30–35 mm² on the 6th day after injection). In control animals, the gastric ulcers were spontaneously reduced in size and depth with time for 2 weeks. Oral hEGF, given at 30 and 100×2 μg/kg/day for 2 weeks, had no effect on natural healing of acetic acid-induced gastric ulcers (Table 1).

In addition, hEGF, given at 100×2 μg/kg/day together with indomethacin for 4 weeks, also had no effect on the ulcer healing. The weight of these stomachs in rats treated with hEGF did not differ from that in the control groups 2 and 4 weeks later. Oral hEGF (100 μg/kg) significantly affected neither the basal gastric secretion nor the histamine-stimulated one in rats (Table 2).

The present study shows that oral hEGF had no effect on ulcer healing, gastric acid secretion and stomach weight. Our results were different from the findings by others who showed the promoting effect of oral EGF on natural healing of experimental gastric ulcers (2–4). Olsen et al. (2) reported that oral EGF, given at 30 μg/kg/day in the drinking water for 25 or 50 days, promoted the natural healing of chronic gastric ulcers to the same extent as cimetidine did. Konturek et al. (3) also showed in rats that oral EGF, given at 30 μg/kg/day in the drinking water for 7 days, significantly accelerated the healing of acetic acid-induced gastric ulcers and increased the mucosal growth. More recently, Hase et al. (4) reported that hEGF,

| Table 1. Effects of oral hEGF on healing of acetic acid-induced gastric ulcers in rats |
|-----------------------------------------------|
| Ulcer healing (μg/kg/day) | Treatment | No. of rats | Ulcerated area (mm²) | Healing rate (%) | Weight of the stomach (g) |
| Natural (2W) |
| Control |
| 38 |
| 5.9±0.6 |
| 13.6 |
| 2.4±0.04 |
| hEGF (30×2) |
| 23 |
| 5.1±1.0 |
| 6.8 |
| 2.1±0.03 |
| hEGF (100×2) |
| 24 |
| 5.5±0.8 |
| 6.6 |
| 2.3±0.1 |
| Delayed (4W) |
| Control |
| 24 |
| 11.9±1.4 |
| 0.8 |
| 2.7±0.1 |
| hEGF (100×2) |
| 25 |
| 11.8±1.2 |
| 0.8 |
| 2.6±0.04 |

hEGF was given orally twice daily (9:30 A.M., 6:30 P.M.) for 2 or 4 weeks. Healing of gastric ulcers were delayed by giving indomethacin (1 mg/kg, subcutaneously) once daily (9:00 A.M.) for 4 weeks. These values are means±S.E. No statistical difference was observed between the individual groups.

| Table 2. Effects of oral hEGF on basal and histamine-stimulated gastric acid secretion in pylorus-ligated and acute fistula rats |
|-------------------------------------------------------------|
| Gastric secretion | Treatment (μg/kg) | No. of rats | Volume (ml/hr) | Acidity (mEq/l) | Acid output (μEq/hr) |
| Basal secretion |
| Control |
| 8 |
| 1.0±0.1 |
| 85.2±6.3 |
| 92.3±17.0 |
| hEGF (100) |
| 8 |
| 1.0±0.1 |
| 80.8±5.5 |
| 83.4±19.9 |
| Histamine-stimulated secretion |
| Control |
| 8 |
| 0.9±0.1 |
| 118.5±6.9 |
| 110.4±19.7 |
| hEGF (100) |
| 8 |
| 1.0±0.1 |
| 103.4±10.2 |
| 117.8±35.0 |

hEGF was given orally 0.5 hr before the pylorus ligation or 2 hr before the histamine-stimulation. Animals were killed 4 hr after the ligation and stimulation. These values are means±S.E. No statistical difference was observed between the individual groups.
given orally once daily at 100 μg/kg/day during the initial 9 days after acetic acid injection, significantly accelerated the healing of gastric ulcers when examined 30 days later. In addition, Olsen et al. (2) and Konturek et al. (3) reported that the agent at that dose had no influence on the gastric secretion. These results suggest that the effect of oral EGF on ulcer healing is not due to the antisecretory activity but due to stimulation of mitogenic action. Different experimental conditions such as the method of ulcer production, the severity of ulcers, the strain of animals and duration of drug administration might account for the difference. As we reported, a single subcutaneous administration of hEGF (≥30 μg/kg) significantly inhibited gastric acid secretion (both basal and histamine-stimulated secretions) and accelerated the ulcer healing (5). In contrast, oral hEGF even at 100 μg/kg had no effect on gastric acid secretion. We found that several antisecretory agents significantly accelerate the healing of acetic acid-induced gastric ulcers (both natural and delayed healing by indomethacin) (7–9). The failure of oral hEGF to accelerate the ulcer healing may be accounted for by the disappearance of antisecretory activity through the oral route. Oral hEGF had no effect on the weight of the stomach, thereby suggesting that hEGF has no appreciable biological effects as long as it is given by the oral route. hEGF might be largely destroyed in the stomach or intestine before absorption and become a biologically inactive form under the present conditions. We conclude that oral hEGF has no pathophysiological effect on the rat stomach.

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