EFFECTS OF FM100, A FRACTION OF LICORICE ROOT, ON SERUM GASTRIN CONCENTRATION IN RATS AND DOGS

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Abstract—The effects of FM100, a fraction of licorice root with antiulcer activity, on serum gastrin concentration were investigated in rats and dogs. The basal serum gastrin concentration in conscious rats was not affected by 400 mg/kg, p.o. of FM100, but was increased by 800 mg/kg, p.o. of FM100. The serum gastrin concentration of rats in which the antrum had been separated from the fundus was decreased by intraduodenal administration of FM100 in a dose-dependent manner. In conscious beagles, the increase in serum gastrin concentration induced by feeding was not affected by pretreatment with 400 mg/kg p.o. of FM100. However, in anesthetized dogs in which the antrum had been separated from the fundus, the increase in acid output and serum gastrin concentration after administration of peptone solution was prevented by the intraduodenal administration of 200 mg/kg of FM100. These results suggest that the gastric anti-secretory action of FM100 may be due to the inhibition of endogenous gastrin release.

FM100 is a fraction of licorice root with antiulcer activity which can be obtained from the methanol extract of licorice root by a fractional precipitation with sodium hydroxide and hydrochloric acid. FM100 has protective effects on acute ulcers in rats, healing effects on chronic ulcers in rats, and inhibiting effects on gastric secretion in rats and dogs (1–6). FM100 inhibited basal gastric secretion and the secretion induced by various stimulants except gastrin or a gastrin-like tetrapeptide. FM100 almost completely inhibited the secretion induced by the intragastric administration of alcohol or peptone and the mechanical distension of the stomach which stimulates the release of gastrin from the antral mucosa (4). These results suggest that FM100 inhibits gastric secretion by a mechanism involving the release of endogenous gastrin. In the present study, the effects of FM100 on serum gastrin concentration were investigated in rats and dogs.

MATERIALS AND METHODS
Male Sprague-Dawley rats (CRJ:CD) weighing 250–300 g, male and female mongrel dogs weighing 9–15 kg, and female beagles weighing 9–13 kg were used. They were deprived of food, except drinking water, overnight before the experiments. FM100 prepared in our laboratories was suspended in 0.5% carboxymethylcellulose solution for rats and anesthetized dogs and packed into capsules for conscious dogs. Atropine sulfate (Iwaki Seiyaku) and cimetidine (Kindly supplied by Smith Klein & French Laboratories Ltd.) were dissolved...
in 0.9% saline solution. Serum gastrin concentration was determined using a radioimmunoassay kit (Gastrin-Riakit, Dainabot Radioisotope Laboratory, Tokyo).

**Conscious rats:** One hour after p.o. administration of F\textsubscript{N}100 or i.m. injection of atropine sulfate or cimetidine, the rats were sacrificed by exsanguination and the blood was collected.

**Rats in which the antrum was separated from the fundus:** In rats under ether anesthesia, a small hairpin was used to separate the antrum from the fundus to prevent the entry of gastric juice into the antrum, and F\textsubscript{N}100 was given intraduodenally. Two hours after the operation, the rats were sacrificed by exsanguination and the blood was collected.

**Conscious beagles:** Capsules containing F\textsubscript{N}100 was given p.o. 60 min before feeding (150 g of boiled cow liver). Blood samples were drawn from the brachial vein 10 min before and at intervals after feeding.

**Anesthetized dogs in which the antrum was separated from the fundus:** Mongrel dogs were anesthetized with sodium pentobarbital and the antrum was separated from the fundus with stomach forceps. A metal fistula to collect gastric juice was fixed at the fundus and a polyethylene tube was inserted into the antrum through an incision in the duodenum. The stomach was rinsed with saline through a gastric tube until the rinsing solution became clean. Every 30 min, 200 ml of saline warmed to 37°C was allowed to flow into the stomach through the gastric tube and the acidity of the outflow saline from the gastric fistula was titrated with 0.1 N NaOH using phenolphthalein as an indicator. Ten milliliter of 40% peptone solution was administered through the antral tube into the antrum three times at an interval of 90 min. F\textsubscript{N}100 was given intraduodenally 30 min before the third administration of the peptone solution. The blood was collected from the femoral vein through a previously cannulated polyethylene tube.

**Statistics:** Statistical significance was evaluated by the Student’s t-test.

### RESULTS

**Conscious rats:** The basal serum gastrin concentration of rats that were fasted overnight was not affected by 400 mg/kg, p.o. of F\textsubscript{N}100 but was significantly increased by 800 mg/kg, p.o. of F\textsubscript{N}100 (Fig. 1). The serum gastrin concentration was also increased by the potent gastric antisecretory agents, atropine sulfate (3 mg/kg, i.m.) and cimetidine (50 mg/kg, i.m.).

**Rats in which the antrum was separated from the fundus:** The serum gastrin concentration was decreased by intraduodenal administration of F\textsubscript{N}100 in a dose-dependent manner, and the concentration in the group treated with 800 mg/kg of F\textsubscript{N}100 was significantly lower than that in the control group (Fig. 2).

**Conscious beagles:** The serum gastrin concentration of beagles that were fasted overnight was markedly increased upon feeding, but this increase was not significantly affected by pretreatment with 400 mg/kg p.o. of F\textsubscript{N}100 (Fig. 3).

**Anesthetized dogs in which the antrum was separated from the fundus:** Rat blood was collected from the femoral vein and the effect of F\textsubscript{N}100 on serum gastrin concentration was determined. The results are shown in Fig. 1. Each column represents the mean±SEM for 7–8 animals. *P<0.05, **P<0.01 vs. Control.
Fig. 2. Effect of Fx100 on serum gastrin concentration in rats in which the antrum was separated from the fundus. Rats were sacrificed 2 hr after intraduodenal administration of Fx100 or its vehicle. Each column represents the mean ±SEM for 8 animals in the Fx100 treated groups and 13 animals in the control group. **P<0.01 vs. Control.

Fig. 3. Effect of Fx100 on serum gastrin response to feeding in conscious beagles. Fx100 (400 mg/kg) was given p.o. 1 hr before feeding (150 g of boiled cow liver). Each value is the mean±SEM for 11 animals.

Fig. 4. Effects of Fx100 on increase in acid output (upper panel) and increase in serum gastrin concentration (lower panel) induced by intra-antral administration of peptone solution in dogs in which the antrum had been separated from the fundus. Fx100 (200 mg/kg) was given intraduodenally 30 min before the administration of 10 ml of 40% peptone solution. Each point represents the mean±SEM for 5 animals. *P<0.05 vs. pretreatment level

was separated from the fundus: The serum gastrin concentration and acid output were significantly increased by intra-antral administration of peptone solution in comparison with the pretreatment levels and they recovered back to pretreatment levels within 1 hr. The intraduodenal administration of 200 mg/kg of Fx100 prior to peptone solution prevented the significant increase in serum gastrin concentration and acid output (Fig. 4).

DISCUSSION

There have been many reports concerning the effects of various gastric antisecretory drugs on plasma or serum gastrin concentration; and it is well known that these drugs often increase, rather than decrease, the gastrin release response to some stimuli. Atropine enhanced gastrin release in man in response to feeding, insulin, sham feeding and distension (7-11); gastrin release in response to food in dogs (12); and the basal serum gastrin concentration in fasted rats (13). It was reported that cimetidine (14, 15) and secretin (16) increased serum gastrin response to a meal in man. Håkanson et al.
reported that deglycyrrhizinized licorice reduced the acid output in rats but significantly increased serum gastrin level. In the present study, atropine and cimetidine markedly increased basal serum gastrin concentration in fasted rats, and F_{x}100 (800 mg/kg) caused a significant increase in serum gastrin concentration. The mechanism for the increase in serum gastrin concentration by inhibitors of gastric secretion is controversial. Becker et al. (18) reported that neutralization of the antrum of the dog resulted in a significant increase of antral venous gastrin concentration and suggested the presence of a very sensitive feedback mechanism between antral pH and gastrin release. Several authors (13, 15, 17) explained the enhancement of gastrin release by an elevation of antral pH caused by inhibition of gastric secretion. However, Farooq et al. (9) explained the above results by postulating the existence of a cholinergic mechanism which inhibits, rather than stimulates, gastrin release. Feldman et al. (10) also suggested the existence of an inhibitory cholinergic neuron connecting a mucosal acid receptor to a gastrin cell, but they stated that the mechanism by which secretin increases serum gastrin concentration is unknown.

F_{x}100 lacks the anticholinergic action in spite of marked gastric antisecretory action, and the increase in serum gastrin concentration after F_{x}100 can not be explained by the blockage of inhibitory cholinergic neurons to gastrin cells. The results that F_{x}100 decreased the basal gastrin concentration or prevented the enhancement of serum gastrin responses to feeding in animals in which the antrum had been separated from the fundus suggest that low antral pH inhibits the effect of F_{x}100 on release of endogenous gastrin in intact animals. We (4) reported that F_{x}100 abolished the gastric secretory response to intragastric administration of alcohol or peptone and mechanical distension of the stomach which stimulate gastric secretion through release of endogenous gastrin, but F_{x}100 scarcely affected the secretion stimulated by exogenous gastrin or a gastrin-like tetrapeptide. From these results, we speculated that the inhibition of gastric secretion by F_{x}100 may be due to the inhibition of gastrin release from the antrum. As reported in a previous paper (19), we found that F_{x}100 caused a marked increase in exocrine pancreatic secretion, not by a cholinergic mechanism, but probably by a humoral mechanism. These results suggest that F_{x}100 affects secretion of gastric and pancreatic juice by a mechanism modulating release of gastrointestinal hormones, although further investigation is required in order to clarify the precise mechanism.

REFERENCES

1) Takagi, K. and Ishii, Y.: Peptic ulcer inhibiting properties of a new fraction of licorice root (F_{x}100). I. Experimental peptic ulcer and general pharmacology. Arzneim.-Forsch. 17, 1544–1547 (1967)

2) Ishii, Y.: Peptic ulcer inhibiting properties of a new fraction of licorice root (F_{x}100). II. Gastric antisecretory action. Arzneim.-Forsch. 18, 33–56 (1968)

3) Takagi, K., Okabe, S., Kawashima, K. and Hirai, T.: The therapeutic effect of F_{x}100, a fraction of licorice root, on acetic acid ulcer in rats. Japan. J. Pharmacol. 21, 832–833 (1971)

4) Ishii, Y.: Mechanism of gastric antisecretory activity of a new fraction of licorice root (F_{x}100). Japan. J. Pharmacol. 20, 71–79 (1970)

5) Okabe, S., Kunimi, H., Nosaka, A., Ishii, Y., Fuji, Y. and Nakamura, K.: Effects of F_{x}100, F_{x}100-DeG and glycyrrhizin on gastric secretion and experimental gastric ulcer in rats. Pharmacometrics 18, 469–474 (1979) (Abs. in English)

6) Ishii, Y., Niihima, K., Fuji, Y., Nakamura, K. and Yamashita, T.: Effects of F_{x}100, deglycyrrhizinized F_{x}100 and glycyrrhizin on clamping-cortisone induced ulcer in rats. Pharmacometrics 22, 581–585 (1981) (Abs. in English)

7) Walsh, J.H., Yalow, R.S. and Berson, S.A.: The effect of atropine on plasma gastrin response to feeding. Gastroenterology 60, 16–21 (1971)
8) Hansky, J. and King, R.W.F.: Effect of atropine on food-stimulated gastrin release after truncal vagotomy in man. Gastroenterology 73, 205-206 (1977)

9) Farooq, O. and Walsh, J.H.: Atropine enhances serum gastrin response to insulin in man. Gastroenterology 68, 662-666 (1975)

10) Feldman, M. and Walsh, J.H.: Acid inhibition of sham feeding-stimulated gastrin release and gastric acid secretion: Effect of atropine. Gastroenterology 78, 772-776 (1980)

11) Schiller, L.R., Walsh, J.H. and Feldman, M.: Distension-induced gastrin release. Effects of luminal acidification and intravenous atropine. Gastroenterology 78, 912-917 (1980)

12) Impicciatore, M., Walsh, J.H. and Grossman, M.I.: Low doses of atropine enhance serum gastrin response to food in dogs. Gastroenterology 72, 995-996 (1977)

13) Hakanson, R., Kroesen, J.H., Liedberg, G., Oscarson, J., Rehfeld, J.F. and Stadil, F.: Correlation between serum gastrin concentration and rat stomach histidine decarboxylase activity. J. Physiol. 243, 483-498 (1974)

14) Sewing, K.F., Hagie, L., Ippoliti, A.F., Isenber, J.I., Samloff, I.M. and Sturdevant, R.A.L.: Effect of one-month treatment with cimetidine on gastric secretion and serum gastrin and pepsinogen levels. Gastroenterology 74, 376-379 (1978)

15) Richardson, C.T.: Effect of H2-receptor antagonists on gastric acid secretion and serum gastrin concentration. Gastroenterology 74, 366-370 (1978)

16) Feldman, M., Walsh, J.H. and Richardson, C.T.: Serum gastrin response to secretin after vagotomy. Dig. Dis. Sci. 25, 921-923 (1980)

17) Hakanson, R., Leidberg, G., Oscarson, J., Rehfeld, J.F. and Stadil, F.: Effect of deglycyrrhizinized liquorice on gastric acid secretion, histidine decarboxylase activity and serum gastrin level in the rats. Experientia 29, 570-571 (1973)

18) Becker, H.D., Reeder, D.D. and Thompson, J.C.: The effect of changes in antral pH on the basal release of gastrin. Proc. Soc. exp. Biol. Med. 143, 238-240 (1973)

19) Ishii, Y. and Terada, M.: Effect of Fx100, a fraction of licorice root, on exocrine secretion from the rat pancreas. Japan. J. Pharmacol. 29, 664-666 (1979)