

Effects of the Gastric Juice Inhibitory Substance from *Streptomyces bottropensis* on Gastric Secretion and Experimental Ulcerations in Rats

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Effects of the gastric juice inhibitory substance (GIS) from the culture filtrate of *Streptomyces bottropensis* F4708 to several kinds of experimental gastric ulcers and gastric juice secretion in rats were studied. GIS inhibited ulceration in pylorus-ligated rats, and also markedly reduced gastric juice secretion. Moreover, it reduced aspirin-induced lesions in pylorus-ligated rats by lowering the gastric acidity. It showed a little effect on the healing of acetic acid-induced gastric ulcer. However, it isn't effective against stress-induced gastric lesions or histamine-induced ulceration. GIS reduced gastric acid secretion in vagotomized rats, and also, though with a little lowered activity, reduced gastric juice secretion in reserpine-treated rats. The protein part of GIS was proved to be important in the action of GIS but its dark brown pigment part alone could make effect. GIS lowered the body temperatures of rats.

Keywords—*Streptomyces bottropensis*; gastric juice inhibitory substance; glycoprotein; pylorus-ligated rats; aspirin-induced gastric lesions; acetic acid-induced ulceration; vagotomy; pronase digestion; hydrochloric acid hydrolyzate

In the previous paper,²⁾ we have found that *Streptomyces bottropensis* F4708 produced a new substance inhibiting gastric juice secretion and reported it as the gastric juice inhibitory substance (GIS). This substance is a dark brown glycoprotein-like substance and markedly inhibited gastric juice secretion in rats. Generally, peptic ulcers are considered to result from unbalance between the digestion of gastric juice and the resistance of gastrointestinal mucosa,³⁾ so the substances inhibiting gastric juice secretion exhibit antiulcerogenic activity. GIS also inhibited ulceration in pylorus-ligated rats.

In this report, antiulcerogenic activity of GIS to several experimental models of gastric ulcers were studied. Some investigations were also made about the factors concerned to its inhibition mechanism on gastric juice secretion.

Experimental

The preparation of GIS was described in the previous paper.²⁾ GIS was dissolved in saline and administered.

Male Wistar rats were used as experimental animals.

Experimental Gastric Ulcers in Rats—Gastric Ulceration in Pylorus-ligated Rats: The rats weighing 150 to 200 g were deprived of food but allowed free access to water for 24 hours prior to the experiments. Under ether anesthesia, the pylorus was ligated according to the method described by Shay, *et al.*³⁾ After 18 hours, the animals were sacrificed and the stomachs were removed. The gastric mucosa was exposed by opening the stomach along the greater curvature and the gastric ulcers developed in the forestomach were observed. The degree of gastric ulceration was estimated by the method of Narumi, *et al.*⁴⁾ Ulceration was given an ulcer index from 0 to 5 according to its severity. After the gastric contents were centrifuged, the gastric volume was measured. Total acid output and total peptic activity were determined according to the previous paper.²⁾ GIS was administered twice immediately and 9 hours after the ligation.

1) Location: 133-1, Yamadakami, Suita-shi, Osaka.

2) T. Mimura, N. Muto, E. Deguchi, K. Oyabu, S. Kondo, and S. Aonuma, *Yakugaku Zasshi*, **96**, 621 (1976).

3) H. Shay, S.A. Komarov, S.S. Fels, D. Merance, M. Gruenstein, and H. Siple, *Gastroenterology*, **5**, 43 (1945).

4) S. Narumi, T. Hirata, K. Gomaibashi, and M. Kanno, *J. Takeda Res. Lab.*, **29**, 85 (1970).

Aspirin-induced Gastric Lesions: Aspirin-induced gastric lesions in pylorus-ligated rats were produced according to the method of Okabe, *et al.*⁵⁾ Briefly, the rats weighing 150 to 200 g, fasted previously for 24 hours, were used and the pylorus was ligated as described above. Immediately after the ligation, 100 mg/kg of aspirin suspended in carboxymethylcellulose solution was given orally. After 5 hours, the rats were sacrificed by an overdose of ether. The stomach was removed, filled with 1% formalin and immersed in 1% formalin for 10 minutes. Then it was opened and examined for lesions in the glandular portion. The ulcer index was calculated as the sum of the length of each lesion in the stomach. GIS was intraperitoneally injected immediately after the pylorus ligation. In order to determine the effect of GIS on gastric secretion, the pylorus ligation preparation was also employed by applying the same time schedule. Five hours later, the animals were sacrificed, and the gastric contents were collected. After centrifugation, the gastric juice volume and acidity were measured. As control, a corresponding volume of saline was given intraperitoneally.

Stress-induced Gastric Lesions: As described by Takagi and Okabe,⁶⁾ the rats weighing 200 to 250 g were placed in a stress cage and immersed to the level of xiphoid process in a water bath (23°) for 20 hours. The animals were then sacrificed by a blow on the head and the stomachs were removed. After the stomach was treated with 1% formalin as described above, gastric lesions in the glandular portion were examined. The ulcer index was estimated as the sum of the length of each lesion. GIS was administered intraperitoneally 15 minutes prior to and 10 hours after the stress exposure.

Histamine-induced Ulceration: Histamine-induced ulceration was produced by the method of Buchner, *et al.*⁷⁾ The rats weighing 180 to 200 g, fasted for 48 hours, were given 300 mg/kg of histamine dihydrochloride intraperitoneally. After 4 hours, the rats were killed and the stomachs were removed. The stomach was cut open along the greater curvature and the degree of lesions found in the glandular portion was expressed as the ulcer index which was estimated by the method of Adami, *et al.*⁸⁾

Acetic Acid-induced Ulceration: The rats weighing about 200 g were used. Surgical procedure was performed according to the method of Takagi, *et al.*⁹⁾ 0.05 ml of 10% acetic acid was injected into subserosal layer in the glandular portion of anterior wall. The animals were fed normally and received GIS intraperitoneally twice a day for 10 days from the second day after the operation. The animals were sacrificed on the 12th day after the operation and the stomachs were removed. The length and width of the ulcer were measured and the product was expressed in terms of the ulcer index.

Gastric Secretion in Rats—Effect of GIS in Vagotomized Rats: The rats weighing 150 to 200 g, fasted overnight, were anesthetized with the subcutaneous injection of urethane. The gastric fistula rats were produced by the method described in the previous paper,⁹⁾ and bilateral subdiaphragmatic vagotomy was performed. The stomach was perfused with 0.0005N NaOH at a constant rate of 0.4 ml/min and 15 min collections of perfusate titrated for H⁺ content with 0.02N NaOH. Tetragastrin used as a stimulant of gastric acid secretion was injected into the femoral vein and GIS was administered intraperitoneally.

Effect of GIS in Reserpine-treated Rats: The rats weighing 150 to 200 g were fasted previously for 48 hours and injected reserpine subcutaneously in the dose of 4.0 mg/kg 24 hours prior to the experiments. Effect of GIS on gastric secretion in normal or reserpine-treated rats was studied using pylorus-ligated rats as described previously.

Biological Activity of Pronase-digested GIS: GIS was dissolved in 0.1M tris-HCl buffer (pH 7.8) containing 0.5 mM CaCl₂ and treated at 100° for 10 minutes. Pronase digestion (enzyme/substrate ratio, 1:50) was carried out at 50° for 48 hours in the same buffer. Then, the same quantity of pronase was added again 24 hours later. Inhibitory activity of this pronase-digested GIS on gastric secretion was determined in pylorus-ligated rats.

Biological Activity of Hydrolyzed-GIS Residue: GIS was hydrolyzed in 6N HCl at 100° for 24 hours. After centrifugation of hydrolyzate, nonhydrolyzed residues were lyophilized. Antisecretory activity of this sample was assayed using pylorus-ligated rats.

Effect on Body Temperature of Rats—The rats weighing 180 to 200 g, whose rectal temperatures were ranging from 37.5 to 39.9°, were used. After the intraperitoneal administration of GIS, the rectal temperatures were measured every one hour during 4 hours.

Results

1) Gastric Ulceration in Pylorus-ligated Rats

Effect of GIS on gastric ulceration in pylorus-ligated rats is shown in Table I. GIS in the intraperitoneal dose of 5.0 mg/kg, when administered twice immediately and 9 hours

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after the pylorus ligation, significantly lessened gastric ulcers and in rats treated with 10 mg/kg of GIS twice, gastric ulcers were almost completely prevented. Perforation was not found in GIS-treated groups. Effect of GIS on gastric secretion is summarized in Table II. In

TABLE I. Effect of GIS on Gastric Ulceration in Pylorus-ligated Rats (18 hr)

Treatment	Dose (mg/kg)	No. of rats	Incidence of ulceration (%)	Ulcer index (mean \pm s.e.)	% inhibition
Control	—	8	100	4.0 \pm 0.3	—
GIS	5.0 \times 2 ^{a)}	8	87.5	2.1 \pm 0.4 ^{b)}	47.5
	10.0 \times 2	8	25.0	0.5 \pm 0.4 ^{b)}	87.5

a) GIS was intraperitoneally administered twice immediately and 9 hours after the ligation. significantly different from control group: b) $p < 0.01$

account of perforation, gastric samples from control animals could be obtained from 6 animals out of 8. GIS significantly reduced the gastric juice volume, total acid output and total peptic activity in 18 hr pylorus-ligated rats.

TABLE II. Effect of GIS on Gastric Secretion in Pylorus-ligated Rats (18 hr)

Treatment	Dose (mg/kg)	No. of rats	Gastric volume (ml/100 g b.w.)	Total acid output (μ Eq/100 g b.w.)	Total peptic activity (mg as tyrosine/100 g b.w.)
Control	—	6	7.92 \pm 0.74	532.8 \pm 45.0	634.1 \pm 48.7
GIS	5.0 \times 2 ^{a)}	8	4.86 \pm 0.52 ^{b)}	365.8 \pm 31.4 ^{b)}	398.5 \pm 39.8 ^{b)}
	10.0 \times 2	8	2.21 \pm 0.16 ^{b)}	172.4 \pm 29.4 ^{b)}	179.2 \pm 12.4 ^{b)}

All values represent mean \pm s.e.

a) GIS was intraperitoneally administered twice immediately and 9 hours after the ligation. significantly different from control group: b) $p < 0.01$

2) Aspirin-induced Gastric Lesions

As shown in Table III, GIS in a dose of 10 mg/kg significantly reduced the severity of aspirin-induced lesions in pylorus-ligated rats. The inhibition percentages of GIS in doses

TABLE III. Effect of GIS on Aspirin-induced Gastric Ulceration in Pylorus-ligated Rats (5 hr)

Treatment	Dose (mg/kg, <i>i.p.</i>)	No. of rats	Ulcer index (mean \pm s.e.)	% inhibition
Control	—	8	35.2 \pm 5.1	—
GIS	5.0	8	28.2 \pm 3.9	19.8
	10.0	8	17.0 \pm 4.0 ^{a)}	51.7

Aspirin (100 mg/kg) was administered orally immediately after the ligation. significantly different from control group: a) $p < 0.01$

TABLE IV. Effect of GIS on Gastric Secretion in Pylorus-ligated Rats (5 hr)

Treatment	Dose (mg/kg, <i>i.p.</i>)	No. of rats	Gastric volume (ml/100 g b.w.)	Acidity (mEq/liter)
Control	—	8	3.93 \pm 0.44	107.3 \pm 4.6
GIS	5.0	8	1.28 \pm 0.10 ^{a)}	90.3 \pm 4.9 ^{b)}
	10.0	8	1.08 \pm 0.33 ^{a)}	60.0 \pm 7.8 ^{a)}

All values represent mean \pm s.e.

significantly different from control group: a) $p < 0.01$, b) $p < 0.05$

of 5.0 and 10 mg/kg were 19.8 and 51.7% respectively. GIS at 5.0 and 10 mg/kg produced a significant reduction of the gastric juice volume and acidity in 5 hr pylorus-ligated rats compared with those of control (Table IV).

3) Stress-induced Gastric Lesions

The result is summarized in Table V. Twenty hours after the stress exposure, evident glandular lesions were present in most animals. In rats treated with 10 mg/kg of GIS twice, the reduction of the ulcer index was not recognized.

4) Histamine-induced Ulceration

Intraperitoneal administration of 300 mg/kg of histamine produced gastric lesions in the glandular portion of rat stomach after 4 hours. GIS at 10 mg/kg was not effective in preventing gastric lesions (Table V).

TABLE V. Effect of GIS on Stress or Histamine-induced Ulceration in Rats

Ulceration	Treatment	Dose (mg/kg, <i>i.p.</i>)	No. of rats	Ulcer index (mean \pm s.e.)	% inhibition
Stress-induced ulceration	Control	—	8	26.9 \pm 4.0	—
	GIS	10.0 \times 2 ^{a)}	8	25.8 \pm 2.5	4.1
Histamine-induced ulceration	Control	—	8	2.5 \pm 0.3	—
	GIS	10.0 ^{b)}	8	2.3 \pm 0.3	8.0

a) GIS was administered twice 15 minutes prior to the stress exposure and 10 hours after it.

b) GIS was administered 10 minutes prior to the injection of histamine.

TABLE VI. Effect of GIS on Acetic Acid-induced Ulceration

Treatment	Dose (mg/kg)	No. of rats	Ulcer index (mean \pm s.e.)	Curative ratio (%)
Control	—	10	13.3 \pm 2.6	—
GIS	5.0 ^{a)}	10	9.4 \pm 1.5	29.3

a) GIS (5.0 mg/kg) was administered intraperitoneally twice a day for 10 days from the second day after the operation.

5) Acetic Acid-induced Ulceration

Effect of GIS on acetic acid-induced ulceration is presented in Table VI. GIS exhibited a little effect on the healing of acetic acid-induced gastric ulcer but it was not significant. The body weight of the animals treated with GIS showed the same increase compared with the control group.

6) Effect in the Perfused Stomach Preparation of Vagotomized Rats

The result is shown in Fig. 1. GIS at 4.0 mg/kg, when administered intraperitoneally, depressed remarkably the acid output stimulated by tetragastrin in the perfused stomach preparation of normal rats. In vagotomized rats, although

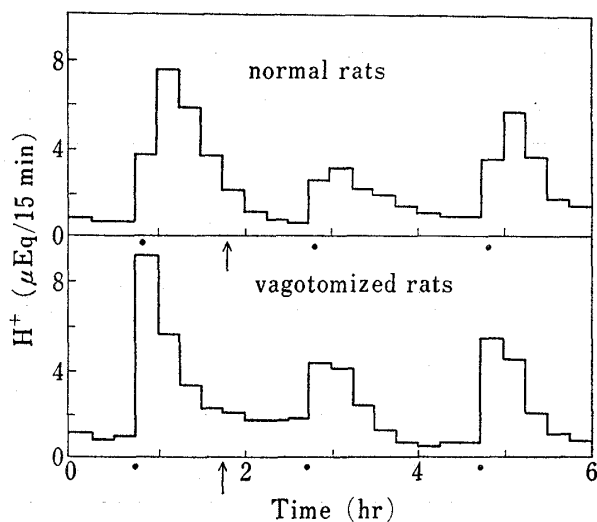


Fig. 1. Effect of GIS on Gastric Acid Secretion stimulated by Tetragastrin in the Perfused Stomach Preparation of Rats

mean value of 3 rats per group

↑ GIS: 4.0 mg/kg, *i.p.*, • tetragastrin: 10 μ g/kg, *i.v.*

the acid output induced by tetragastrin was slightly reduced, the inhibitory effect of GIS in a dose of 4.0 mg/kg was recognized similarly to that in normal rats.

7) Effect on Gastric Secretion in Reserpine-treated Rats

Effect of GIS on gastric secretion in pylorus-ligated rats, which were subcutaneously injected reserpine 24 hours prior to the pylorus ligation, is presented in Table VII. The gastric juice volume in reserpine-treated rats was almost the same as that in normal rats. While the inhibition percentage in the gastric juice volume by the injection of GIS was 81.4% in normal rats, the same dose of GIS reduced the gastric juice volume by 64.4% in reserpine-treated rats. Reserpine treatment showed a propensity to decrease the inhibitory activity of GIS on gastric secretion.

TABLE VII. Effect of GIS on Gastric Secretion in Reserpine-treated Rats

Pretreatment	Treatment	Dose (mg/kg, <i>i.p.</i>)	No. of rats	Gastric volume (ml/100 g b.w.)	% inhibition
—	Control	—	8	2.63 ± 0.20	—
	GIS	5.0	8	0.49 ± 0.09 ^{b)}	81.4
Reserpine ^{a)}	Control	—	8	2.75 ± 0.45	—
	GIS	5.0	8	0.98 ± 0.24 ^{b)}	64.4

Gastric secretion in pylorus-ligated rats (4 hr) was measured.

a) Twenty-four hours prior to the pylorus ligation, reserpine (4 mg/kg) was subcutaneously administered. significantly different from control group respectively: b) $p < 0.01$

8) Influence of Pronase Digestion and Hydrolysis on GIS

Effect of pronase-digested GIS and hydrolyzed-GIS residue on gastric secretion are shown in Fig. 2. The administration of pronase-digested GIS in a dose of 5.0 mg/kg significantly decreased all the parameters of gastric secretion compared with those of control group, but the inhibitory activity was clearly reduced compared to that of GIS-administered group. Moreover, the pigment component obtained after the acid hydrolysis of GIS resulted in a significant inhibition of gastric secretion in a dose of 5.0 mg/kg and revealed almost the same inhibitory activity as pronase-digested GIS at the same dose (Fig. 2).

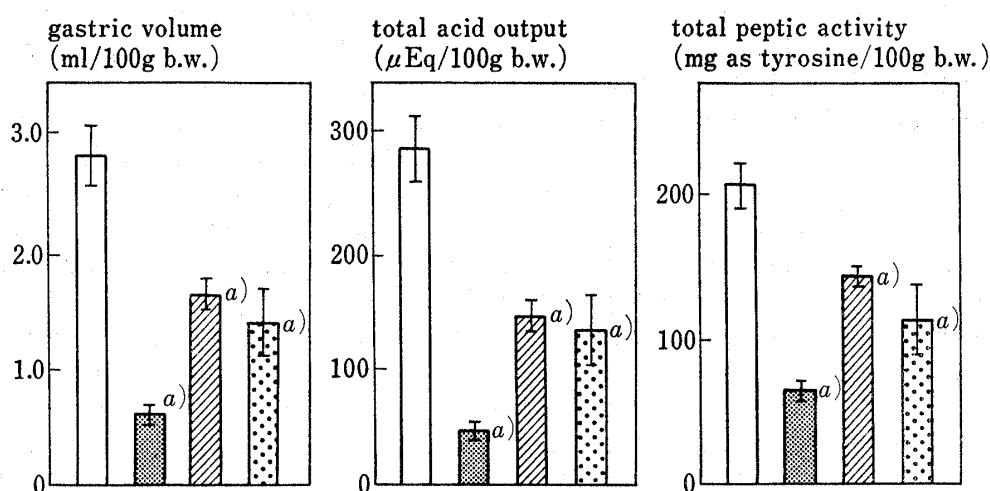


Fig. 2. Effects of Pronase-digested GIS and Hydrolyzed-GIS Residue on Gastric Secretion in Pylorus-ligated Rats (4 hr)

□: control (10)

▨: pronase-digested GIS (10)

■: GIS (10)

▤: hydrolyzed-GIS residue (10)

Numbers in parenthesis indicate the number of rats.

Each sample was intraperitoneally administered at the dose of 5.0 mg/kg.

Vertical bars are standard errors of the means.

significantly different from control group: a) $p < 0.01$

9) Effect on Body Temperature of Rats

As shown in Table VIII, GIS at 1.0 and 5.0 mg/kg clearly lowered the body temperature of rats. However, in rats treated with GIS in a dose of 0.5 mg/kg, any effect couldn't be observed.

TABLE VIII. Effect of GIS on Body Temperature of Rats

Treatment	Dose (mg/kg, <i>i.p.</i>)	No. of rats	Time after administration (hr)				
			0	1	2	3	4
			Rectal temperature ($^{\circ}\pm$ s.e.)				
Control	—	5	37.91 \pm 0.13	37.76 \pm 0.12	37.75 \pm 0.13	37.95 \pm 0.11	37.94 \pm 0.10
GIS	0.5	5	38.11 \pm 0.14	37.52 \pm 0.27	37.85 \pm 0.13	38.21 \pm 0.16	38.03 \pm 0.09
	1.0	5	37.91 \pm 0.07	36.56 \pm 0.33 ^{a)}	37.41 \pm 0.11	37.75 \pm 0.07	37.84 \pm 0.11
	5.0	5	38.20 \pm 0.13	36.04 \pm 0.22 ^{a)}	36.94 \pm 0.17 ^{a)}	37.69 \pm 0.10	37.99 \pm 0.16

significantly different from control group: a) $p < 0.01$

Discussion

In the previous paper,²⁾ we have reported that GIS decreased the ulcer index in pylorus-ligated rats—one of experimental peptic ulcer models—with a single dose of 10 mg/kg (*i.p.*), but it didn't significantly reduce the gastric juice volume at 18 hours. In this paper, we further investigated on the effects of GIS to ulceration in pylorus-ligated rats, and found it more effective to ulceration in pylorus-ligated rats with double dosage of 5.0 mg/kg (*i.p.*) than a single dosage of 10 mg/kg (*i.p.*). As this effect correlates with inhibitory action of GIS on gastric secretion at 18 hours, it appears to be based on lowering of gastric digestion. Moreover, GIS clearly exhibited protective effect to aspirin-induced gastric lesions. Gastric disorders induced by aspirin is considered to result from its induction of back diffusion of gastric acid,¹⁰⁾ and it has been proved that there must be acid in the stomach for that to occur.¹¹⁾ Furthermore, aspirin-induced gastric lesions have also been proved to depend mostly on gastric acidity, and little on pepsin.⁵⁾ From these facts, it can be said that the inhibitory activity of GIS to aspirin-induced gastric lesions in pylorus-ligated rats is due to its significant lowering of gastric acidity. However, it failed to inhibit ulceration induced by the stress exposure or histamine injection. Both ulcer models set animals in gastric oversecretion. Stress-induced ulceration is said to be caused by alteration in gastric blood flow¹²⁾ or inhibition to regeneration of mucous epithelium,¹³⁾ and histamine-induced ulceration is said to be caused by permeation of oversecreted gastric juice to mucous tissue.¹⁴⁾ Inhibitory activity of GIS on these functions seem to be not so strong as that on gastric juice secretion. On the other hand, consecutive administration of GIS for 10 days showed a tendency to accelerate the healing process of acetic acid-induced ulceration.

GIS reduced gastric acid secretion induced by tetragastrin both in normal and in vagotomized rats with similar patterns. This indicates that GIS doesn't act through vagi, but acts at the local site. GIS also reduced gastric juice secretion in reserpine-treated rats, but the activity was weaker than in nontreated rats. Reserpine releases bioamines like catecholamines and serotonin, and catecholamines are recognized to reduce gastric juice secretion.¹⁵⁾

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As GIS retained much of its inhibitory activity in reserpine-treated rats, bioamines seem to take little part in its action.

GIS is a glycoprotein with dark brown pigment. It is biologically stable after heating at 100° for 10 minutes or treatment with 0.5 N NaOH, but its activity decreased to about a half at the equivalent dose after whole digestion by pronase. This shows out that the protein part composing GIS evidently takes part in its inhibition of gastric juice secretion. Moreover, the dark brown pigment part, which was obtained after acid hydrolysis of GIS, was similarly active as pronase-digested GIS at 5.0 mg/kg. In these experiments, the pigment part was found to be hard to dissolve while GIS is soluble in water. Therefore, the protein and sugar parts can be considered to make much effect on the solubility of the pigment part. We consider that both protein and pigment components are concerned to revealing the biological activity of GIS and particularly the pigment part displays stronger activity when combined with the protein part and solubilized. Further investigations are being made to clarify the active site and the mechanism of action of GIS.