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Biological Properties of the Gastric Juice Inhibitory Substance produced by *Streptomyces bottropensis* F4708[1]

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Gastric juice inhibitory substance (GIS) produced by *Streptomyces bottropensis* F4708 suppressed carrageenin-induced edema but did not show analgesic effect or influence the circulation system. Effect of adrenergic antagonists on the gastric juice inhibition and lowering of body temperature by GIS was examined. The gastric juice inhibitory activity of GIS was not affected by premedication with Dibenamine or Propranolol. However, the lowering of body temperature by GIS was markedly reinforced by premedication with the antagonists. The dark brown pigment component from GIS was presumed to be a melanin-like substance by comparing its chemical properties with those of DOPA-melanin which exhibited a significant reduction of gastric secretion in rats. Therefore, the gastric juice inhibitory activity of GIS was found to depend on this pigment component in its molecule. Lowering of body temperature of rats was not observed by the administration of DOPA-melanin.

Keywords——*Streptomyces bottropensis*; gastric juice inhibitory substance; pigment component; melanin like pigment; DOPA-melanin; body temperature; circulation system; carrageenin-induced edema

We have already reported that the gastric juice inhibitory substance (GIS) obtained from the culture filtrate of *Streptomyces bottropensis* F4708 markedly reduced gastric juice secretion in rats and that it had an anti-ulcerogenic activity in several experimental models of gastric ulcer.[3,4] This substance was composed of dark brown pigment and a glycoprotein, and was a typical pigment protein such as melanoprotein. Melanin is a common name for black or brown pigment produced by organisms and it is well known that it is bound to protein after its production to form a water-soluble pigment, melanoprotein.[5]

This paper deals with the biological activity of GIS, and the chemical and biological properties of its pigmentary component.

Experimental

Preparation of GIS——GIS was prepared as described in the previous paper.[3] GIS was dissolved in saline before use.

Effect on Carrageenin-induced Paw Edema——According to the method of Winter, *et al.*,[6] 0.1 ml of 1% carrageenin solution was injected subcutaneously into the foot pad of the hind paw of male Sprague-Dawley rats weighing about 150 g. Swelling rate of the edema was determined just after the injection and at 1 hr intervals after 2 hr. The sample of GIS was administered intraperitoneally 30 min before carrageenin injection.

1) A part of this work was presented at the 97th Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April, 1977.
2) Location: *133-1, Yamadakami, Suita-shi, Osaka 565, Japan.*
3) T. Mimura, N. Muto, E. Deguchi, K. Oyabu, S. Kondo, and S. Aonuma, *Yakugaku Zasshi*, **96**, 621 (1976).
4) T. Mimura, N. Muto, J. Tanaka, H. Oshita, N. Onishi, and S. Aonuma, *Chem. Pharm. Bull.* (Tokyo), **25**, 897 (1977).
5) J. Oikawa, *Seikagaku*, **48**, 872 (1976).
6) C.A. Winter, E.A. Risley, and G.W. Nuss, *Proc. Soc. Exp. Biol. Med.*, **111**, 544 (1962).

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Analgesic Effect——The method of Randall-Selitto[7] was adapted. Male Wistar rats weighing about 100 g were injected with 0.1 ml of 1% carrageenin solution subcutaneously in the left hind paw. The right paw was left without treatment as a control. The sample of GIS was administered intraperitoneally at the same time and the threshold was measured at 1, 2, and 3 hr later. Analgesic ratio was calculated as the ratio of the sum of the threshold values at 1, 2, and 3 hr to that of the control.

Effects on Squirming and Capillary Permeability——According to the method of Whittle,[8] male mice (ddy strain), weighing 21±1 g, were used. GIS was administered intraperitoneally to each animal 10 min after intravenous injection of 0.1 ml of a solution of Pontamine Sky Blue 6 BX. Each dose group consisted of five mice.

Effect on the Circulation System——Male Beagle dogs weighing about 11 kg were anesthetized with sodium pentobarbital before the experiment. Arterial blood flow was measured with probes of electromagnetic flowmeter attached to the left common carotid and femoral arteries. Blood pressure was determined with pressure transducer by a cannula inserted into the right femoral artery, and heart rate was recorded with a cardiotachometer operated by pulsation of blood. The sample of GIS was administered intravenously through a cannula inserted into the femoral vein or intra-arterially through a needle inserted into the left femoral artery.

Effect on Gastric Secretion——Gastric secretion was measured by the rat pylorus ligation method with 4 hr ligation period as described in the previous papers.[3,4] As adrenergic antagonists, Dibenamine hydrochloride or Propranolol hydrochloride dissolved in saline was injected subcutaneously 30 min before the ligation.

Effect on Body Temperature——Rectal temperature of rats was measured by the method described in the previous paper.[4] Dibenamine or Propranolol was administered subcutaneously 30 min before administration of GIS.

Preparation of Dark Brown Pigment Component from GIS——GIS was hydrolyzed in 6 N HCl for 24 hr at 100°. After centrifugation, the dark brown precipitate was dialyzed at pH 7.0 and lyophilized.

Synthesis of DOPA-melanin——According to the method of Mencher and Heim,[9] DOPA-melanin was synthesized by bubbling air into 0.01 N NaOH solution of DL-DOPA. Synthesized DOPA-melanin was dialyzed against distilled water (pH 7.0) and lyophilized.

Thin-Layer Gel Filtration——According to the method of Johansson and Rymo,[10] Sephadex G-200 (superfine) was prepared into a layer 0.6 mm thick and equilibrated with 0.1 M NaCl–0.05 M phosphate buffer (pH 7.6).

SDS-Polyacrylamide Gel Electrophoresis——Electrophoresis was performed according to the method of Fairbanks, *et al.*,[11] using 7.5% gel.

Results

Inhibitory Effect of GIS on Carrageenin-induced Paw Edema

Effect of GIS on edema of the rat hind paw induced by carrageenin is shown in Fig. 1. Significant inhibition was observed in the groups administered 0.5, 1.0, or 5.0 mg/kg (*i.p.*) of GIS.

Analgesic Effect of GIS

Analgesic ratios of edematous and normal paws of rats administered 5.0 mg/kg (*i.p.*) of GIS were not different from those of the control group.

Effects of GIS on Squirming and Capillary Permeability

Intraperitoneal injection of GIS at 1.0 or 5.0 mg/kg did not induce squirmings and had no effect on peritoneal capillary permeability in mice.

Effect of GIS on the Circulation System

Effect of GIS was observed after intravenous administration of 0.1, 0.3, or 1.0 mg/kg or intra-arterial administration of 0.1 or 0.3 mg/kg. Blood flow in the femoral artery tended to decrease after dosing of 1.0 mg/kg (*i.v.*), but the blood pressure and blood flow in the carotid

7) L.O. Randall and J.J. Selitto, *Arch. Intern. Pharmacodyn.*, **111**, 409 (1957).
8) B.A. Whittle, *Brit. J. Pharmacol.*, **22**, 246 (1964).
9) J.R. Mencher and A.H. Heim, *J. Gen. Microbiol.*, **28**, 665 (1962).
10) B.J. Johansson and L. Rymo, *Acta Chem. Scand.*, **16**, 2069 (1962).
11) G. Fairbanks, T.L. Stech, and D.F.H. Wallach, *Biochemistry*, **10**, 2606 (1971).

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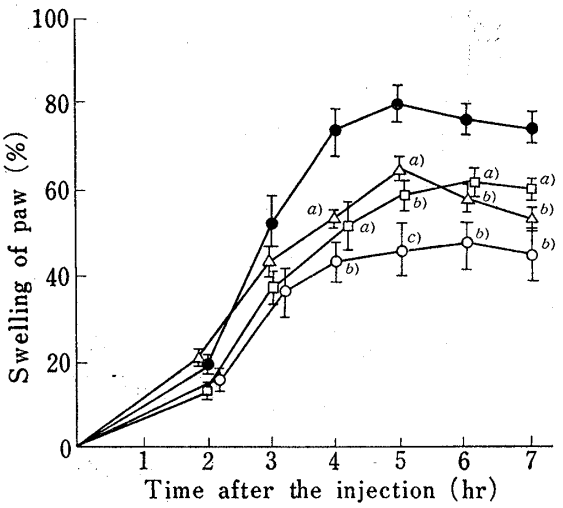

Fig. 1. Effect of GIS on Carrageenin Edema of the Rat Hind Paw

●—●: control (n=8).
△—△: GIS 0.5 mg/kg, *i.p.* (n=6).
□—□: GIS 1.0 mg/kg, *i.p.* (n=6).
○—○: GIS 5.0 mg/kg, *i.p.* (n=6).
Vertical line: s.e.
Significantly different from control group:
a) $p<0.05$, *b*) $p<0.01$, *c*) $p<0.001$.

artery did not change. Dosing of 0.3 mg/kg (*i.a.*) had no effect.

Effect of Adrenergic Antagonist on Biological Activity of GIS

i) Effect of GIS on Gastric Secretion——As shown in Table I, single dose of Dibenamine hydrochloride (37 mg/kg, *s.c.*) or Propranolol hydrochloride (20 mg/kg, *s.c.*) reduced gastric juice secretion. However, GIS (1.0 mg/kg, *i.p.*) retained its activity under the influence of these drugs, and moreover its gastric juice inhibitory activity was equal to that of the control.

ii) Effect of GIS on Body Temperature of Rats——Results are shown in Fig. 2. Administration of Dibenamine hydrochloride (37 mg/kg, *s.c.*) alone had no effect on body temperature, but the temperature lowered 30 min after administration of Propranolol hydrochloride (20 mg/kg, *s.c.*). GIS (1.0 mg/kg, *i.p.*) lowered body temperature 1 and 2 hr after its administration, and this effect was markedly reinforced by pretreatment with the two drugs, especially with Propranolol.

Table I. Effect of GIS on Gastric Secretion in Dibenamine or Propranolol-treated Rats

Pretreatment[a]	Treatment[b]	Gastric volume[c] (ml/100 g b.w.)	Inhibition (%)
—	—	2.86±0.39	—
	GIS	1.41±0.18	50.7
Dibenamine hydrochloride	—	1.53±0.45	—
	GIS	0.68±0.09	55.6
Propranolol hydrochloride	—	1.30±0.11	—
	GIS	0.66±0.18	49.2

Four rats were used for each group.
Values represent mean±s.e.
a) Dibenamine hydrochloride (37 mg/kg) or Propranolol hydrochloride (20 mg/kg) was administered subcutaneously 30 min before the pylorus ligation.
b) GIS (1.0 mg/kg) was administered intraperitoneally immediately after the pylorus ligation.
c) Gastric volume was measured using 4 hr pylorus-ligated rats.

Comparison of Properties of the Pigment Component from GIS and DOPA-melanin

i) Absorption Spectrum——As shown in Fig. 3, the dark brown pigment from GIS resembles DOPA-melanin very closely in ultraviolet and visible spectra.

ii) Solubility——Both the pigment from GIS and DOPA-melanin were insoluble in organic solvents such as chloroform, acetone, ethanol, benzene, toluene, and ethyl acetate. The pigment was slightly soluble in water (pH 7.0) although DOPA-melanin was soluble. Both of them dissolved in dilute alkali.

iii) Thin-Layer Gel Filtration——The dark brown pigment remained at the origin, unlike to GIS. DOPA-melanin was also adsorbed on the gel and was not developed.

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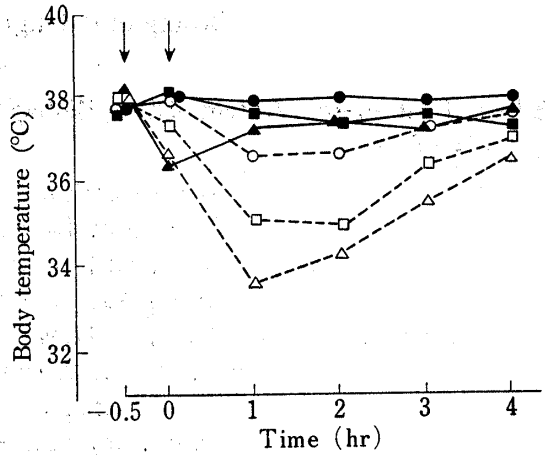

Fig. 2. Effect of GIS on Body Temperature of Dibenamine- or Propranolol-treated Rats

The first arrow indicates subcutaneous injection of Dibenamine hydrochloride (37 mg/kg) or Propranolol hydrochloride (20 mg/kg). The second arrow indicates intraperitoneal injection of GIS (1.0 mg/kg).
●—●: control. ○---○: GIS alone.
▲—▲: Dibenamine alone. □---□: Dibenamine and GIS.
■—■: Propranolol alone. △---△: Propranolol and GIS.
Three rats were used for each group.

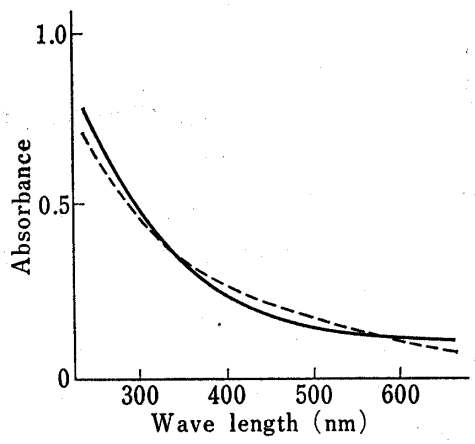

Fig. 3. Absorption Spectra of Dopa-melanin and Pigment obtained from GIS

Each sample was dissolved in 1.0 N NaOH at a concentration of 20 μg/ml.
------: DOPA-melanin.
——: pigment obtained from GIS.

iv) SDS-Polyacrylamide Gel Electrophoresis——Both the pigment from GIS and DOPA-melanin were different to GIS in electrophoretic pattern. They were both strong by adsorbed to the gel, remaining almost at the origin.

Biological Activity of DOPA-melanin

i) Inhibitory Effect on Gastric Secretion——Effect of DOPA-melanin on gastric secretion is shown in Table II. Intraperitoneal administration of DOPA-melanin (0.2 mg/kg) caused a significant reduction of gastric secretion, but DL-DOPA, which was used to synthesize DOPA-melanin, has no inhibitory activity at 200 mg/kg (*s.c.*).

TABLE II. Effect of DOPA-melanin on Gastric Secretion in Pylorus-ligated Rats (4 hr)

Treatment	Dose (mg/kg, *i.p.*)	Gastric volume (ml/100 g b.w.)	Total acid output (μEq/100 g b.w.)	Total peptic activity (mg as tyrosine/ 100 g b.w.)
Control	—	2.75±0.28	298.3±44.3	205.2±21.6
DOPA-melanin	0.2	1.50±0.42[a]	158.5±53.1	111.7±26.4[a]
	1.0	0.96±0.10[b]	101.1±17.9[b]	97.2± 9.8[b]
	5.0	0.52±0.14[b]	45.9± 9.7[b]	62.0± 7.1[b]

Five rats were used for each group.
Values represent mean±s.e.
Significantly different from control group: *a*) $p<0.05$, *b*) $p<0.01$.

ii) Effect on Body Temperature of Rats——DOPA-melanin (0.2 or 1.0 mg/kg, *i.p.*, at which significant reduction of gastric secretion was observed) has no effect on body temperature of rats.

Discussion

In the preceding paper,[4] we reported that GIS, the gastric juice secretion inhibitor effective to several gastric ulcers, also had the effect of lowering body temperature in rats.

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It is known that drugs that lower body temperature inhibit the formation of edemas in experimental animals. Similarly, GIS inhibited edema induced by carrageenin. It was clarified that GIS had neither analgesic nor irritative effect.

The inhibitory effect of GIS on gastric secretion was not considered to result from the change of blood flow, because GIS did not have any effect on blood flow.

At present, role of the splanchnic nerve on gastric secretion has not been elucidated as yet. Bass and Patterson[12] reported that exogeneous adrenaline inhibited gastric juice secretion, and that adrenergic antagonists also reduced it. In the present work, Dibenamine and Propranolol were used as adrenergic antagonists in order to determine whether gastric juice inhibitory effect of GIS depended on adrenergic action. Both antagonists inhibited gastric secretion by a single administration, but the gastric juice inhibitory activity of GIS was not affected at all and was almost equal to that in normal group. The lowering of normal body temperature by GIS was markedly reinforced by premedication with the two antagonists, especially Propranolol. From these results, it can be said that the gastric juice inhibition and lowering of body temperature induced by GIS show different actions under the influence of adrenergic antagonists. Therefore, the action mechanism of each effect by GIS is apparently different.

The dark brown pigment component from GIS, which was reported to have a gastric juice inhibitory activity in the previous paper,[4] had chemical properties similar to those of DOPA-melanin, which has a strong gastric juice inhibitory activity, though it did not lower normal body temperature of rats. Therefore, effect of lowering body temperature by GIS is considered to depend on the protein part of its molecule. Thus, the gastric juice inhibition and lowering of body temperature induced by GIS differed in its active component. It can be considered that the pigment component, which has a melanoid structure, contributes mainly to the inhibition of gastric secretion and this action is not inhibited by adrenergic antagonists.

12) P. Bass and M.A. Patterson, *J. Pharmacol. Exp. Ther.*, **156**, 142 (1967).

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