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Studies on Biological Activities of Melanin from Marine Animals. III. Inhibitory Effect of SM II (Low Molecular Weight Melanoprotein from Squid) on Phenylbutazone-Induced Ulceration in Gastric Mucosa in Rats, and Its Mechanism of Action

TSUTOMU MIMURA,* KAZUHIRO MAEDA, TAICHIROU TERADA, YASUO ODA, KANAE MORISHITA and SHIGERU AONUMA

Faculty of Pharmaceutical Sciences, Osaka University, Yamadaoka 1–6, Suita, Osaka 565, Japan

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A low molecular weight melanoprotein from *Ommastrephes bartrami* LESUEL (Fr. SM II) has been reported to have a gastric secretion inhibitory activity in rats. The effect of Fr. SM II on the gastric mucosal defensive capability was studied in this investigation. It was demonstrated that Fr. SM II: i) counteracted ulceration induced by phenylbutazone and aspirin; ii) increased the contents of gastric glycoproteins in the ulcerated stomach; iii) lowered the activities of glycoprotein-degrading enzymes; and iv) increased the $^{35}SO_4^{2-}$ uptake into the gastric mucosa in intact rats concomitantly with an increase of glycoprotein content.

It is suggested that the anti-ulcerogenic activity of Fr. SM II is based on its glycoprotein-increasing activity in the gastric mucosa.

Keywords——squid melanin; gastric mucosa; phenylbutazone ulcer; hexosamine; sialic acid; uronic acid; [^{35}S]sulfate incorporation; histology; mucopolysaccharide

The stomach is believed to have a defensive capability which protects its gastric mucosa from proteolysis by gastric juice. Recently, Takagi and Yano[1] reported that an increase and decrease of the mucus glycoproteins were observed during the healing and induction processes, respectively, of ulceration. It was also reported that several substances which showed anti-ulcerogenic activities prevented the loss of glycoproteins from the gastric surface mucosal cells of the ulcerated stomach.[2] Hence, the glycoproteins, which are produced by underlying columnar epithelium and are abundant in the mucous layer, have attracted the attention of many investigators as possible gastric defensive factors.

The authors reported an anti-ulcerogenic activity of a low molecular weight melanoprotein (Fr. SM II) derived from ink bags of *Ommastrephes bartrami* LESUEL.[3] Since Fr. SM II showed gastric secretion inhibitory activity, we assumed that this might account for the anti-ulcerogenic activity of Fr. SM II. However, some anti-ulcer drugs (*e.g.* cimetidine) are reported to have mucus glycoprotein-increasing activity together with the gastric secretion inhibitory activity.[4] Thus, we were interested in studying the effect of Fr. SM II on the mucus glycoproteins.

In the present paper, the effect of Fr. SM II on the gastric defensive capability was investigated by examining the amount of glycoproteins in the gastric mucosa and mucous layers (these two layers will be considered jointly, and are designated as the gastric glandular portion hereafter). Phenylbutazone- and aspirin-induced ulcer models were chosen for the experiments because, in these ulcer models, the decrease of glycoproteins in the gastric glandular portion has been clearly demonstrated.[5,6] The amount of glycoproteins in gastric juice and/or the gastric glandular portion was estimated by measuring hexosamine, sialic acid

and uronic acid separately. The effect of Fr. SM II on the biosynthesis and degradation of the glycoproteins is discussed, based on the results of $^{35}SO_4^{2-}$ uptake and the mucopolysaccharase activity.

Experimental

Materials——Fr. SM II was obtained from the ink bags of *Ommastrephes bartrami* LESUEL as described in our previous paper.[3]

Experimental Ulcer Models in Rats——i) Phenylbutazone-Induced Gastric Ulcer: A phenylbutazone-induced ulceration model was induced according to the method of Suzuki *et al.*[7] Male Wistar strain rats weighing about 200 g were fasted for 24 h, but were provided with water *ad libitum*. Each rat received 200 mg/kg of phenylbutazone (Sigma Chemical Co.) suspended in 5% Gum Arabic solution perorally. Rats were sacrificed at 5 h after the administration of phenylbutazone and their stomachs were removed to examine the lesion in the glandular stomach area. The sum of the diameter of each lesion was used as an ulcer index. Each test sample was administered intraperitoneally or intravenously 30 min before the administration of phenylbutazone. Cimetidine (Smith Klein and Fujisawa) was used as a positive control.

ii) Aspirin-Induced Gastric Ulcer: Male Wistar rats weighing about 200 g were deprived of food for 24 h. According to the method of Okabe *et al.*,[8] rats orally received 100 mg/kg of aspirin suspended in 5% Gum Arabic solution immediately after pylorus ligation. Each test sample was administered intraperitoneally concurrently with the pylorus ligation. Cimetidine was used as a positive control reagent. Seven hours after the pylorus ligation, the rats were sacrificed and their stomachs were removed to examine the lesion in the glandular area. The sum of the length of all lesions was used as an ulcer index.

Determination of Glycoprotein Contents——The stomach specimens removed from the rats were incised at the greater curvature and rinsed with saline. The mucous layer and the gastric mucosa were scraped off from the glandular stomach. In the case of aspirin-induced gastric ulcer, gastric juice was collected from the pylorus-ligated rats.

i) Determination of Hexosamine: According to the method reported by Hiroi *et al.*,[4] the scraped substance from each stomach was homogenized individually with 3 ml of papain solution (Difco: 50 units of papain in 100 ml of 0.2 M acetate buffer at pH 5.6 containing 2 mM ethylenediaminetetraacetic acid (EDTA), 4 mM cysteine and 0.88% NaCl) and digested for 20 h at 37 °C. Undigested components were eliminated by centrifugation (3000 rpm × 10 min). A portion of the supernatant was hydrolyzed in 2 N HCl in a sealed ampoule at 110 °C for 14 h. Hexosamine was determined by the colorimetric method described by Gunner.[9] Glucosamine hydrochloride (Nakarai Chemical Co.) was used as a standard.

Hexosamine content in the gastric juice was measured by the method described by Glass.[10] Proteins were precipitated from the gastric juice by tricarboxylic acid (TCA, 5%) and distilled acetone (1.5 volumes) was added to the TCA-soluble fraction. The mixtures were then incubated for 1 h at 40 °C. After the incubation, the mixtures were centrifuged. The hexosamine content in the precipitates was measured by Gunner's method.[9]

ii) Determination of Sialic Acid: After a 1 h hydrolysis of the homogenate of the scraped substance in 0.1 N H_2SO_4 at 80 °C, the sialic acid content was determined by using thiobarbituric acid,[11] with *N*-acetylneuraminic acid (Sigma Chemical Co.) as a standard.

iii) Determination of Uronic Acid: The homogenate of the scraped substance was digested for 48 h at 37 °C in papain solution and allowed to stand overnight at 4 °C after the addition of 1% cetylpyridinium chloride solution. After centrifugation, the precipitates were redissolved in 0.01 N NaOH and reacted with carbazole according to the method of Bitter and Muir.[12] Glucuronic acid (Wako Pure Chemical Ind., Ltd.) was used as a standard.

Histochemical Study of the Stomach——Stomachs were excised from variously treated rats. They were fixed with Lillie's solution (formalin 100 ml, NaH_2PO_4 4.52 g, and $Na_2HPO_4 \cdot 12H_2O$ 16.38 g were added to 1.0 l of distilled water) by being immersed in the solution for 3 d at 4 °C (Lillie's solution was poured into the stomachs at the beginning of the fixation). After fixation, the glandular portion was excised and washed with cold PBS (phosphate-buffered saline). The tissue was mounted in paraffin and sectioned (5 μm thickness). Histochemical observations were done after staining of the specimens with hematoxylin–eosin (H–E), periodic acid–Schiff (PAS) and alcian blue.

Incorporation of $^{35}SO_4^{2-}$ into the Gastric Glandular Portion——This experiment was carried out according to the method of Ezer and Szporny.[13] Male Wistar rats weighing about 150 g intraperitoneally received Fr. SM II or cimetidine, followed by an injection of $Na_2{}^{35}SO_4$ solution (48.2 mCi/mmol, RCC Amersham: 100 μCi/kg) through the femoral vein. They were sacrificed at 2, 4, 6, 8, 12 and 24 h after the injection of $Na_2{}^{35}SO_4$. The gastric glandular portions were scraped off and weighed before being transferred into a scintillation vial.

After adding 1 ml of Soluene-350 (Packard Instrument Company, Inc.) to each container, the vials were left overnight at room temperature in order to dissolve the sample completely. Each sample was then neutralized with 17 N acetic acid and decolorized with a few drops of 30% hydrogen peroxide. Finally, 10 ml of ACS II (RCC Amersham) was added to each vial as a scintillator and the radioactivity was counted with a Beckman LS-150 liquid scintillation spectrometer.

Mucopolysaccharase Activity in Gastric Glandular Portion——The gastric glandular portion was scraped off and homogenized in a Teflon homogenizer in 0.25 M sucrose containing 0.04 M Tris–acetate buffer (pH 7.4). The supernatant (20000 *g* × 20 min) of the homogenate was subjected to the following enzyme assays.

β-Glucuronidase and *N*-acetyl-β-glucosaminidase activities were measured using the methods reported by Hasebe[14] and Ito,[15] respectively. Substrates used for these enzymes were 0.5 mM *p*-nitrophenyl-β-D-glucuronide (Nakarai Chemical Co.) in 0.1 M acetate buffer (pH 4.5), and 2.5 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide (Seikagaku Kogyo Co., Ltd.) in 0.05 M citrate buffer (pH 4.5), respectively. After incubation at 37 °C (16 h for β-glucuronidase and 30 min for *N*-acetyl-β-glucosaminidase), the reactions were stopped by adding 0.2 M borate buffer (pH 9.0) and *p*-nitrophenol liberated from the substrates was determined by measuring the absorbance at 400 nm. The enzyme activities were expressed as mg of *p*-nitrophenol liberated by 100 mg of protein during the incubation.

Measurement of Gastric Emptying Rate (GER)——GER was measured by the method described by Kato *et al.*[16] Phenol red suspended in water was administered orally to conscious rats at a dose of 2 mg/animal and the stomachs were removed 30 min after the administration. For the measurement of phenol red content in the stomach, the organ was minced and mixed with 100 ml of 0.1 N NaOH to dissolve the phenol red. The mixture was allowed to stand for 1 h at room temperature, then 2 ml of the supernatant fluid was diluted by adding 8 ml of 0.1 N NaOH. The amount of phenol red was quantitatively analyzed by measuring the optical density at 560 nm. GER was calculated according to the following formula.

$$\mathrm{GER} = \left\{1 - \frac{\text{amount of phenol red which remained in the stomach}}{\text{amount of phenol red administered}}\right\} \times 100$$

A test sample was administered intraperitoneally 30 min before the administration of phenylbutazone (or Gum Arabic solution).

Statistical Analysis——Student's *t*-test was applied to assess the significance of differences between the mean values for the control group and the sample-administered groups.

Results

Effects of Fr. SM II on Phenylbutazone- or Aspirin-Induced Ulceration

The anti-ulcerogenic activity of Fr. SM II on phenylbutazone-induced gastric ulcer is shown in Table IA, B. Both intraperitoneally and intravenously administered Fr. SM II (30 min before the administration of phenylbutazone) counteracted the ulcer-inducing activity of phenylbutazone.

The contents of glycoproteins were measured in the same stomachs as used in the above experiment. As shown in Table II, decreases of hexosamine, sialic acid and uronic acid (indicators of total mucopolysaccharides, glycoproteins and acidic polysaccharides, respectively) were observed in the rats which had received phenylbutazone. However, the previous administration of Fr. SM II (either intraperitoneally or intravenously) inhibited the

TABLE I. Effect of Fr. SM II on Phenylbutazone (PB)-Induced Ulceration in Rats (5 h)

Administration route	Treatment	Dose (mg/kg)	No. of rats	Ulcer index (mean ± S.E.)
A: *i.p.*	PB-control[a]	—	8	9.6 ± 3.0
	PB + Fr. SM II[b]	25	8	1.3 ± 0.4[c]
		10	8	1.7 ± 1.2[c]
	PB-control[a]	—	8	13.6 ± 3.5
	PB + cimetidine[b]	100	8	3.6 ± 0.5[e]
B: *i.v.*	PB-control[a]	—	8	13.6 ± 1.8
	PB + Fr. SM II[b]	25	8	3.2 ± 1.1[e]
		10	8	6.2 ± 2.2[d]

a) Saline. *b*) Sample was administered 30 min prior to the oral administration of phenylbutazone. Significantly different from the PB-control group: *c*) $p < 0.05$, *d*) $p < 0.01$, *e*) $p < 0.001$.

TABLE II. Effect of Fr. SM II on Hexosamine, Sialic Acid and Uronic Acid Contents in the Gastric Glandular Portion of Rats with Phenylbutazone (PB)-Induced Ulceration (5 h)

Treatment	Dose (mg/kg)	Hexosamine (μg glucosamine HCl/100 mg tissue wet wt.)	Sialic acid (μg *N*-acetylneuraminic acid/100 mg tissue wet wt.)	Uronic acid (μg glucuronic acid/100 mg tissue wet wt.)
A: *i.p.*				
Intact[a]	—	156.0± 8.9	35.5±1.6	56.7±1.8
PB-control[a]	—	103.4± 6.7	30.5±1.4	43.8±1.6
PB+Fr. SM II[b]	25	224.1±24.0[e]	46.8±2.8[e]	65.8±1.9[e]
	10	188.3±11.5[e]	42.2±2.2[c]	56.3±2.6[d]
PB+cimetidine[b]	100	210.5±30.1[d]	40.5±2.0[d]	56.1±3.9[c]
B: *i.v.*				
Intact[a]	—	212.1± 9.0	39.4±2.9	50.9±3.8
PB-control[a]	—	171.8±12.2	35.7±4.2	41.0±2.8
PB+Fr. SM II[b]	25	229.6±10.9[d]	47.6±3.5[c]	57.1±3.6[d]
	10	201.5± 8.8	37.1±2.1	53.8±2.6[d]

All values represent the means±S.E. ($n=8$). *a*) Saline. *b*) Sample administered 30 min prior to the oral administration of phenylbutazone. Significantly different from the PB control group: *c*) $p<0.05$, *d*) $p<0.01$, *e*) $p<0.001$.

TABLE III. Effect of Fr. SM II on Hexosamine, Sialic Acid and Uronic Acid Contents in the Gastric Glandular Portion of Intact Rats (5.5 h)

Treatment	Dose (mg/kg)	Hexosamine (μg glucosamine HCl/100 mg tissue wet wt.)	Sialic acid (μg *N*-acetylneuraminic acid/100 mg tissue wet wt.)	Uronic acid (μg glucuronic acid/100 mg tissue wet wt.)
Intact[a]	—	221.9±13.3	26.1±1.4	31.6±1.2
+Fr. SM II[b]	25	283.0±49.9	39.5±2.0[e]	43.6±3.4[d]
	10	229.8±19.1	36.6±0.9[e]	36.4±2.3
+cimetidine[b]	100	260.1±22.3	33.1±2.1[c]	38.6±3.1

All values represent the means±S.E. ($n=8$). *a*) Saline. *b*) Sample was administered intraperitoneally 5.5 h before the rats were sacrificed. Significantly different from the intact group: *c*) $p<0.05$, *d*) $p<0.01$, *e*) $p<0.001$.

TABLE IV. Effects of Fr. SM II on Hexosamine Contents in Gastric Juice and Gastric Glandular Portion of Rats with Aspirin (Asp)-Induced Ulceration (7 h)

Treatment	Dose (mg/kg)	Ulcer index	Gastric volume (ml/100 g b.w.)	Total hexosamine (μg in gastric juice/100 g b.w.)	Hexosamine (μg/ml gastric juice)	Tissue hexosamine (μg/100 mg tissue wet wt.)
Intact[a]	—	—	—	—	—	246.3±11.3
Asp-control[a]	—	29.3±2.1	6.23±0.27	95.4±10.9	27.9±3.9	187.4±18.2
Asp+Fr. SM II[b]	25	4.8±1.8[e]	3.12±0.18[e]	82.3± 8.2	46.9±4.1[e]	259.3±15.1[d]
	10	8.9±3.2[e]	4.06±0.31[e]	81.7± 5.5	36.8±3.1	242.3±17.1
Asp+cimetidine[b]	100	3.2±1.1[e]	3.54±0.26[e]	78.5± 8.7	39.9±4.0[c]	243.1±24.3

All values represent the means±S.E. ($n=8$). *a*) Saline. *b*) Sample was administered intraperitoneally immediately after pylorus ligation. Significantly different from the Asp-control group: *c*) $p<0.05$, *d*) $p<0.01$, *e*) $p<0.001$.

decrease of these parameters. The contents of glycoproteins in these groups were at the same level as or even higher than in intact (without treatment) rats.

The administration of cimetidine (used as a positive control) had a similar effect on these

three parameters.

The effect of Fr. SM II on the contents of glycoproteins in the glandular portion of intact rats is shown in Table III. The contents of each component, especially sialic acid, increased as the dose of Fr. SM II was increased.

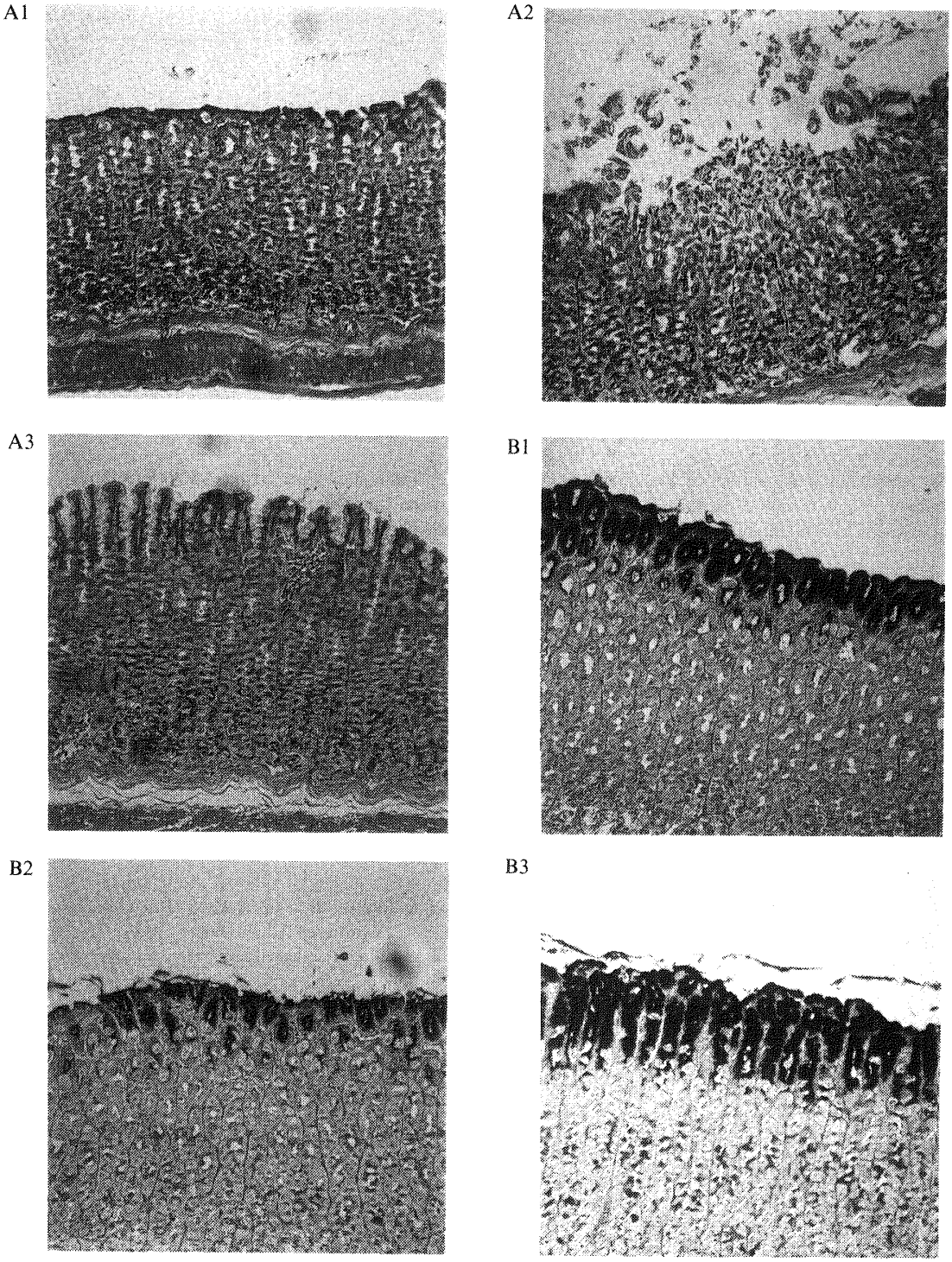

Fig. 1. Histochemical Improvement Caused by the Administration of Fr. SM II in the Gastric Glandular Portion of Rats with Phenylbutazone-Induced Ulceration

A1, intact (H–E staining, ×150); A2, phenylbutazone-induced ulcer (H–E staining, ×150); A3, phenylbutazone-induced ulcer + Fr. SM II 25 mg/kg, *i.p.* (H–E staining, ×150); B1, intact (PAS staining, ×150); B2, phenylbutazone-induced ulcer (PAS staining, ×150); B3, phenylbutazone-induced ulcer + Fr. SM II 25 mg/kg, *i.p.* (PAS staining, ×150).

Since it was impossible to collect gastric juice from the phenylbutazone ulcer model, an aspirin ulcer model was chosen to clarify the effect of Fr. SM II on the dynamic properties of the glycoproteins (Table IV).

The amount of glycoproteins was estimated by measuring hexosamine in the gastric glandular portion. The hexosamine found in the gastric glandular portion was decreased by the administration of aspirin and restored by concomitant administration of Fr. SM II. Fr. SM II reduced the volume of gastric juice considerably. However, in such a case, a high concentration of hexosamine was observed. Therefore, the net contents of the hexosamine in the gastric juice were not significantly different in the two groups (aspirin only, aspirin plus Fr. SM II). It was clear that the gastric juice-decreasing activity of Fr. SM II has no effect on the release of hexosamine into the gastric juice. Cimetidine showed a similar effect on the hexosamine contents in the gastric glandular portion and gastric juice.

Histochemical Observation of Rat Stomach

The effect of Fr. SM II on the gastric glandular portion of rat stomach was studied from a histochemical viewpoint. As shown in Fig. 1, A2, the epithelial cell layer of the stomach disintegrated and partial destruction of the lamia propria layer was caused by the administration of phenylbutazone. However, concomitant administration of Fr. SM II (25 mg/kg, *i.p.*) clearly prevented the loss of these layers (Fig. 1, A3), and the neutral polysaccharides, shown as PAS staining positive area, were increased in the surface epithelial cells of the stomach (Fig. 1, B3). Similar changes were also observed in the alcian blue staining specimens (not shown).

Effect of Fr. SM II on the Incorporation of $^{35}SO_4^{2-}$ into Gastric Glandular Portion of Rat Stomach

As shown in Fig. 2, in the control group (saline was administered), the radioactivity found in the glandular portion of the stomach decreased with the passage of time. In the Fr. SM II-administered group, accumulation of the radioactivity was observed at 4 to 6 h after the $^{35}SO_4^{2-}$ injection. A similar accumulation of radioactivity was observed in the cimetidine-administered group.

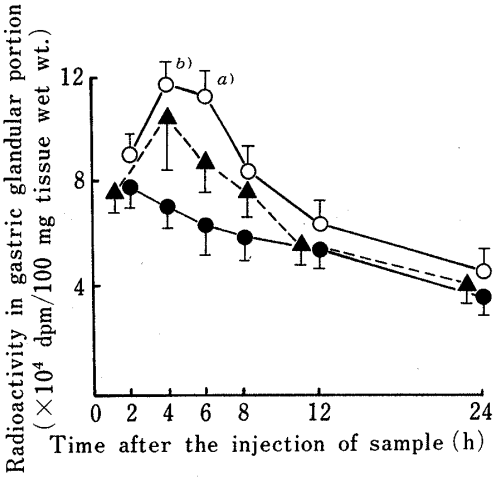

Fig. 2. Effect of Fr. SM II on the $^{35}SO_4^{2-}$ Incorporation into Glycoproteins in the Gastric Glandular Portion of Intact Rats

—●—, saline; —○—, Fr. SM II 25 mg/kg, *i.p.*; —▲—, cimetidine 100 mg/kg, *i.p.*

Each point represents the mean ± S.E. of four experiments.

Significantly different from the saline-treated group: *a*) $p<0.05$, *b*) $p<0.01$.

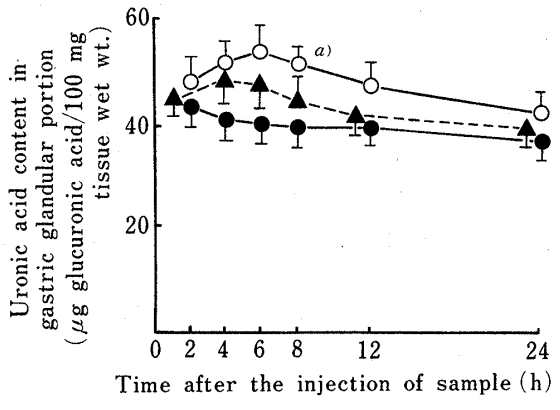

Fig. 3. Changes of Uronic Acid Content in the Gastric Glandular Portion of Intact Rat Following Intraperitoneal Injection of Fr. SM II

—●—, saline; —○—, Fr. SM II 25 mg/kg, *i.p.*; —▲—, cimetidine 100 mg/kg, *i.p.*

Each point represents the mean ± S.E. of four experiments.

Significantly different from the saline-treated group: *a*) $p<0.05$.

Table V. Effect of Fr. SM II on Changes of Mucopolysaccharase Activities in the Gastric Glandular Portion of Rats with Phenylbutazone (PB)-Induced Ulceration (5 h)

Treatment	Dose (mg/kg)	β-Glucuronidase (mg *p*-nitrophenol/100 mg protein/16 h)	*N*-Ac-β-glucosaminidase (mg *p*-nitrophenol/100 mg protein/h)
Intact[a]	—	29.2±3.6	16.5±0.8
PB-control[a]	—	39.5±3.4	29.5±2.7
PB+Fr. SM II[b]	25	33.1±4.4	17.5±0.3[d]
	10	34.9±2.3	19.3±1.4
PB+cimetidine[b]	100	33.2±0.8	19.1±1.5
Intact[a]	—	27.3±4.1	15.8±0.2
+Fr. SM II[c]	25	26.7±2.2	14.6±0.3
+cimetidine[c]	100	27.0±3.2	15.1±0.8

All values represent the means±S.E. ($n=5$). *a*) Saline. *b*) Sample was administered intraperitoneally 30 min prior to the oral administration of phenylbutazone. *c*) Sample was administered intraperitoneally 5.5 h before rats were sacrificed. Significantly different from the PB-control: *d*) $p<0.05$.

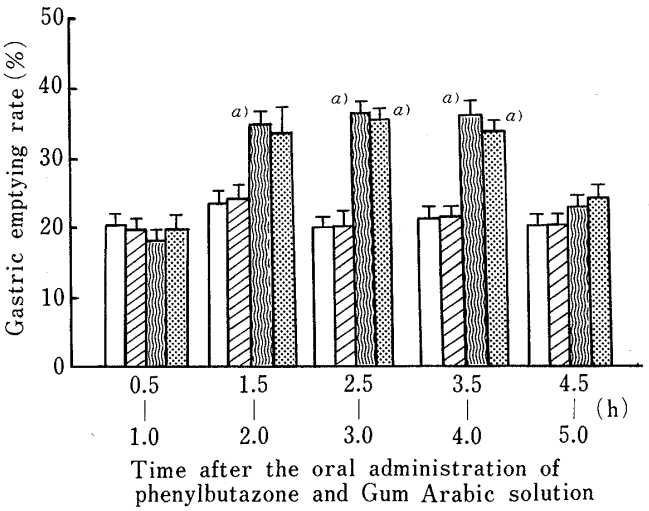

Fig. 4. Effect of Fr. SM II on Gastric Emptying Rate (GER) Enhanced by Phenylbutazone

□, control (Gum Arabic solution *p.o.*+saline *i.p.*); ▨, Gum Arabic solution *p.o.*+Fr. SM II 25 mg/kg *i.p.*; ▥, PB-control (phenylbutazone *p.o.*+saline *i.p.*); ▩, phenylbutazone *p.o.*+Fr. SM II 25 mg/kg *i.p.*
Each column and vertical bar represent the mean ±S.E. of five experiments.
Significantly different from the control group: *a*) $p<0.05$.

As shown in Fig. 3, the change of uronic acid content coincided with that of the radioactivity in all cases.

Effect of Fr. SM II on the Mucopolysaccharase (MPase) Activity in Gastric Glandular Portion of Rat

Increases of β-glucuronidase and *N*-acetyl-β-glucosaminidase activities were noted in the phenylbutazone-induced ulcerated stomach. These enzyme activities were suppressed by the concomitant administration of Fr. SM II. However, the enzyme activities were not lowered to the levels of an intact rat. Cimetidine, used as a positive control, kept the MPase activity in the stomach at a very low level.

Interestingly, the MPase activity in the intact stomach was not influenced by the administration of either Fr. SM II or cimetidine (Table V).

Effect of Fr. SM II on GER in Rats with Phenylbutazone-Induced Ulceration

Phenol red was administered orally 0.5, 1.5, 2.5, 3.5 and 4.5 h after the oral injection of phenylbutazone for the measurement of changes in GER.

As shown in Fig. 4, in the control group, which received 5% Gum Arabic solution, the GER was about 20—24% throughout the experiment. In the phenylbutazone-administered group, a significant increase of the GER (35—37%) was observed during 1—4 h after the administration. The GER returned to normal at 4.5—5 h. In both cases, the concomitant administration of Fr. SM II caused no change in the GER throughout the experiment.

Discussion

It has been suggested that HCO_3^- (which neutralizes the gastric acid)[17] and mucus (which resists penetration of the gastric acid)[18] protect the gastric mucosa from self-digestion. It was also reported that a certain mucus glycoprotein has anti-peptic activity.[19] These observations led us to assume that various factors combine to form a defensive capability against gastric acid.

Takagi and Yano[1] and Ito *et al.*[20] reported a decrease of glycoproteins in the gastric mucosa during ulceration, and the restoration of glycoproteins by the administration of several anti-ulcer drugs. These results indicate an important role of mucus glycoproteins in the gastric defensive capability. In the present paper, the authors have dealt mainly with the dynamic properties of glycoproteins in the gastric glandular portion.

Fr. SM II showed a potent anti-ulcer action in the phenylbutazone ulcer model. The anti-ulcerogenic effect was confirmed by microscopic observations. In the phenylbutazone-administered group, PAS-positive substances apparently disappeared from the epithelial cells, as described by Iida and Sato.[21] However, a concomitant administration of Fr. SM II prevented the loss of the PAS-positive substances. These results suggested that Fr. SM II influences the gastric defensive capability.

In the experiment described in Tables II and III, the contents of hexosamine, sialic acid and uronic acid in the gastric mucus glycoproteins were measured separately. There was no marked specific increase in any of the three components.

The increase of mucus glycoproteins might be a result of a decreased release of glycoproteins into the gastric cavity. In order to clarify this point, we chose the aspirin ulcer model and examined the effect of Fr. SM II on the release of glycoproteins into gastric juice (since it was impossible to collect the gastric juice from the phenylbutazone ulcer model). As shown in Table IV, it was found that the increase of mucus glycoproteins in the gastric glandular portion was not due to a change in the release of glycoproteins into gastric juice.

A decrease of glycoproteins was observed in the phenylbutazone and/or aspirin ulcer model (Tables II and IV). The concomitant administration of Fr. SM II significantly prevented the induction of ulcers. Although ulcer induction was not completely prevented by the concomitant amdinistration of Fr. SM II, the contents of glycoproteins in this group were no less than that in the intact group. This indicated that Fr. SM II has a gastric glycoprotein-increasing activity through an increase of the biosynthesis and/or a decrease of the degradation.

We measured the MPase activity to estimate the degradation characteristics and it was found the increased MPase activity after the administration of phenylbutazone was lowered by the concomitant administration of Fr. SM II (Table V). This clearly suggested that the glycoprotein-increasing effect of Fr. SM II in the phenylbutazone ulcer stomach (Table II) was based on an inhibition of the degradation system.

Although Fr. SM II failed to bring the MPase activity back to the level in intact rats, the amount of glycoproteins was at a higher level than in the intact rat stomach. Moreover, it was also demonstrated that Fr. SM II increased the glycoproteins without influencing the MPase activity in intact rat stomach (Table III). Therefore, we assumed that Fr. SM II influences the biosynthesis of the glycoproteins. The increase of $^{35}SO_4^{2-}$ uptake into the gastric glandular

portion (most obviously seen at 4—6 h after the administration of Fr. SM II) also supported the assumption that Fr. SM II increased the biosynthesis of the glycoproteins.

It is clear that the anti-ulcerogenic activity of Fr. SM II is based not only on the inhibition of gastric juice secretion but also on the increase of gastric glycoproteins which may be involved in the gastric defensive capability.

The nature of the macromolecular glycoproteins which were increased by Fr. SM II and shown to have gastric mucosal defensive activity will be described in our next paper.

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