Effect of an Anti-Ulcer Agent, 2′-Carboxymethoxy-4, 4′-Bis (3-Methyl-2-Butenyl)oxy) Chalcone (SU-88), on the Biosynthesis of Gastric Sulfated Mucosubstances in Restrained and Water-Immersed Rats

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Abstract—2′-Carboxymethoxy-4,4′-bis(3-methyl-2-butenyloxy) chalcone (SU-88) has an anti-ulcer effect which is considered to increase the resistant factors of the gastric mucosa. In order to clarify the mechanism of the action of SU-88, the biosynthesis of gastric sulfated mucosubstances (SMS) in vivo and in vitro was investigated in rats with gastric erosion induced by restraint and water-immersion. The incorporation of $^{35}$S-sulfate into gastric SMS was significantly reduced 18 hr after the onset of stress. Pretreatment with SU-88 (500 mg/kg, p.o., x 10 days) prevented a reduction in the incorporation of $^{35}$S-sulfate due to stress when $^{35}$S sulfate was administered in vivo. On the contrary, the incorporating activity of $^{35}$S-sulfate into the SMS in the isolated rat gastric mucosa with erosion was significantly increased 12 hr after the onset of stress, as compared with that of the control group in vitro. The incorporation of $^{35}$S-sulfate into the SMS was still further increased by the oral administration of SU-88. The mode of action of SU-88 on the biosynthesis of SMS was discussed.
anti-ulcer effect of SU-88 and investigated the effect of this drug on the incorporation of 35S-sulfate into gastric SMS in rats subjected to restraint and water-immersion stress in vivo and in vitro.

Materials and Methods

In vivo experiments: Male Wistar strain rats weighing approx. 180 g were used in all experiments. SU-88, suspended in 0.4% (W/V) carboxymethyl cellulose, was given orally daily x10. Control animals were given the vehicle only. All animals were deprived of food, but had access to water ad libitum for 16 hr prior to the final dosing. One hr after the final dosing, 100 μCi of 35S labeled sodium sulfate (The Radiochemical Centre, Amersham) in 1 ml of distilled water was injected intraperitoneally. Animals were killed by decapitation, and the stomachs were removed. In the experiments on stress ulcer, immediately after the injection of 35S-sulfate, rats were placed in a stress cage and immersed in a water bath at 25°C for 18 hr. After the stress, the rats were killed, and the stomachs were removed. The stomach of each animal was incised along the greater curvature. The stomach was washed gently with chilled saline, and the forestomach was cut off. The rate of incorporation of 35S-sulfate into the glandular portion was determined by the methods of Lambert et al. (14) with some modifications. Tissue of the glandular portion was weighed, cut into small pieces and homogenized for a few minutes in 10 ml of distilled water in a chilled water bath. A 0.1 ml aliquot of the homogenate was taken for the determination of total radioactivity in the glandular portion. Subsequently, the homogenate was digested with the addition of 0.1 ml of papain (2x crystallized, 1 mg/ml, Sigma) in 0.2 M acetate buffer, pH 5.6, containing 1 mM EDTA and 5 mM cysteine at 65°C for 24 hr. The digest was centrifuged to remove a small amount of debris, and then three volumes of ice-cold ethanol were mixed with the supernatant solution. This mixture was centrifuged, and the resultant precipitate was dissolved in 10 ml of 0.02 M NaCl. SMS were precipitated from this solution with the addition of 1/10 volume of 1% cetyl-pyridinium chloride (CPC) at 4°C for 24 hr. After centrifugation, the precipitate was suspended in 1 ml of water. Ten ml of PCS scintillator (Amersham) was added prior to the determination of radioactivity in a Packard Tricarb Model 3255 liquid scintillation spectrometer, corrected for decay.

In vitro experiments: Male Donryu rats weighing approx. 250 g deprived of food for 24 hr were given a single oral dose of SU-88, 300 mg/kg, prepared as a CMC suspension. Thirty min after dosing, the rats were subjected to restraint and water-immersion stress. The animals were killed 6, 12 and 24 hr after the onset of stress, and the stomachs were excised immediately. Each stomach was treated as described above. The wet weight of the glandular portion was determined, and the tissue was pre-incubated at 37°C for 30 min in 20 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, before the addition of 100 μCi of 35S-sulfate; and incubation was carried out for 6 hr, gassing with 95% O2 and 5% CO2. Incubation was terminated by cooling, and then the tissue and the medium were separated by centrifugation at 1,000 g for 10 min. The tissue obtained was washed three times with 5 ml of chilled bicarbonate buffer and homogenized in 2 ml of 50 mM Tris-HCl buffer, pH 7.2, containing 2% Triton X-100 after incubation at 100°C for 3 min in a boiling water bath. The homogenate was centrifuged at 10,000 g for 20 min, and the supernatant was dialyzed against running tap water for three days and subsequently against distilled water for two days. This dialyzate was applied to a Bio-Gel A-1.5 m (1310 Bad) column, 0.9x60 cm, equilibrated with 50 mM Tris-HCl buffer, pH 7.2, containing 2% Triton X-100, pH 7.4; and the column was eluted with the same buffer as described above. The macromolecular fractions eluted in the void volume, designated as fraction I by Azumi et al. (10), were pooled, dialyzed and concentrated to 3 ml. The solution was applied to a DEAE-cellulose column, 0.4x90 cm, and eluted with a linear gradient of 0–0.4 M NaCl, according to the method of Mikuni-Takagaki and Hotta (13). Hexose was determined by the phenol-sulfuric acid method (16) using galactose as a standard. The radioactivity was determined in the
same manner as described before. Results were expressed as the mean ± S.E. of the mean. The significant difference of the data was evaluated using Student’s t-test.

**Results**

Changes in incorporation of $^{35}$S-sulfate into gastric tissue in vivo: The time course of radioactive concentrations in the stomach following intraperitoneal injection of $^{35}$S-sulfate is shown in Fig. 1. The concentrations in the homogenate of the glandular stomach showed a maximum within 30 min after the injection, and thereafter the level dropped quickly with time until 12 hr and then decreased more slowly. On the other hand, the level of $^{35}$S-sulfate in the CPC precipitated fraction which showed the rate of incorporation of $^{35}$S-sulfate into the SMS rose with time after injection and showed a peak at 8 hr. The level thereafter dropped gradually.

As shown in Table 1, pretreatment of the normal rat with SU-88 at a single oral dose of 500 mg/kg or successive oral doses for 10 days increased the concentration of $^{35}$S-sulfate in the gastric tissue, but this was not significantly different from the controls. In the SMS fraction, no significant increase was observed by the pretreatment with SU-88.

Subjection to restraint and water-immersion stress for 18 hr caused marked inhibition of the rate of $^{35}$S-sulfate incorporation into both the gastric tissue and the SMS fraction (Table 2). When SU-88 was given at 500 mg/kg/day for 10 consecutive days, a reduction in the rate of $^{35}$S-sulfate incorporation was significantly prevented. Especially, in the SMS fraction, the rate of incorporation was restored to over the normal level.

Changes in incorporation of $^{35}$S-sulfate into SMS in vitro: The effect of SU-88 on the incorporating activity of $^{35}$S-sulfate into SMS in vitro is shown in Table 3. The incorporating activity of $^{35}$S-sulfate into gastric SMS in vitro in the isolated rat gastric mucosa with erosion was significantly increased 12 hr

| Treatment          | Incorporation of $^{35}$S-sulfate |
|--------------------|----------------------------------|
|                    | Total gastric tissue | Isolated SMS |
|                    | dpm/100 mg tissue | % Change | dpm/100 mg tissue | % Change |
| Control            | 18290±1009 | - | 1951±355 |
| SU-88, 500 mg/kg×1 | 19902±2587 | +9 | 2068±448 | +6 |
| SU-88, 500 mg/kg×10| 22249±2373 | -22 | 2072±270 | +6 |

Rats were given an oral dose of SU-88 (500 mg/kg) once or daily for 10 days followed by an intraperitoneal injection of 100 μCi (approx.) Na$_2$ $^{35}$SO$_4$. The animals were killed 18 hr later. Each value represents the mean ± S.E. of 7 rats.
after the onset of stress, as compared with that in the control group. At that time, the incorporating activity by the oral administration of SU-88 was slowly increased in the later half of the subjection of stress, and a significant increase was observed at 24 hr.

The DEAE-cellulose column chromatography of fraction I (gastric high molecular glycoproteins) isolated by gel chromatography gave a peak of a charged fraction (tubes 17-40) and a peak of an unabsorbed fraction (Fig. 2). Most of the hexose was not retained on the DEAE cellulose column. The radioactivity in the charged fraction was increased by the administration of SU-88, and the degree of sulfation of SMS was also increased, as compared with that in the control group.

Discussion

The present investigation was undertaken in order to clarify the mechanism of action of SU-88 on the biosynthesis of gastric SMS.

It has been reported that biosynthesis of gastric mucosubstances was reduced in the development of gastric lesion and ulceration induced by some anti-inflammatory agents or stress. Lambert et al. (14) demonstrated that incorporation of 35S-sulfate into gastric SMS was decreased markedly in rats with erosion after subjection to restraint stress. A similar relationship between ulcerogenesis and the reduction in the synthesis of gastric mucosubstances has been reported by other investigators (9-11, 14, 15). Dekanski et al. (9) have shown that the impaired glycoprotein synthesis was a causative biochemical phenomenon prior to the development of gastric mucosal damage rather than a mere result of it.

On the other hand, it has already been reported that SU-88, an anti-ulcer drug, has
SU-88 on Gastric SMS Biosynthesis

In the present experiment, a significant decrease in the incorporation of $^{35}$S-sulfate into gastric SMS was found 18 hr after the onset of stress when $^{35}$S-sulfate was administered in vivo, similar to the report by Lambert et al. (14). Pretreatment with SU-88 in stressed rats significantly prevented the reduction in the incorporation of $^{35}$S-sulfate into SMS, although the incorporation of $^{35}$S-sulfate into SMS and the total gastric tissue in normal rats was not significantly increased by the treatment with this drug.

From these findings, there seems to be a possibility that the preventive effect of SU-88 on the reduction in the incorporation of $^{35}$S-sulfate by the stress may be merely caused through the increase in the mucosal blood supply. Therefore, we further investigated the effect of SU-88 on the incorporation of $^{35}$S-sulfate into SMS in vitro using the isolated gastric tissue after the administration of the drug to rats since under the experimental conditions in vitro, the incorporating activity of $^{35}$S-sulfate supplied by the medium reflects the synthetic activity of SMS in the gastric cells themselves, regardless of the quantity of gastric circulation.

The incorporating activity of $^{35}$S-sulfate into SMS in vitro was not changed 6 and 24 hr...

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**Fig. 2.** Typical elution profiles of DEAE-cellulose column chromatography (DE-52) of fraction I (gastric high molecular glycoproteins) isolated by gel chromatography. Rats were given a single oral dose of vehicle (A) or SU-88, 200 mg/kg (B). The animals were killed 12 hr after the onset of stress. Fraction I isolated by a Bio-Gel A-1.5 m column from 3 rats were pooled and applied to the DEAE-cellulose column (0.5x9 cm) and eluted with a linear gradient of 0-0.4 M NaCl. Hexose was detected by the phenol/H$_2$SO$_4$ method (absorbance 490 nm, O-O) and radioactivity of $^{35}$S was counted by a liquid scintillation counter ( ).

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**Note:** The figure shows typical elution profiles of DEAE-cellulose column chromatography (DE-52) of fraction I (gastric high molecular glycoproteins) isolated by gel chromatography. The elution profiles are presented in two sections, (A) and (B), each depicting the incorporation of $^{35}$S-sulfate into SMS under different conditions.
after the onset of stress, but was maintained over the normal level at 12 hr. These changes in biosynthetic activity of SMS seem to be a non-specific response of the gastric cells to a variety of adverse conditions. On the other hand, pretreatment with SU-88 did not show a significant increase in the incorporating activity of 35S-sulfate at 6 and 12 hr, but showed a significant increase at 24 hr, suggesting that the drug may act on the healing process of erosion. Furthermore, SMS obtained by the treatment with SU-88 appeared to be more sulfated than that from non-treated tissue, although the SMS of gastric tissue would seem to comprise a continuous spectrum of variation in the sulfate content. This observation is interesting in relation to the fact that SMS containing more sulfate show stronger inhibitory activity to peptic proteolysis.

From the results of this experiment, it was suggested that an increase in the incorporation of 35S-sulfate by the administration of SU-88 to restrained and water-immersed rats was not only a consequence of a sufficient supply of 35S-sulfate through the improvement in mucosal blood flow, but also a result of a stimulatory effect on the SMS synthetic activity of the gastric mucosa. However, whether SU-88 has a direct effect on the biosynthesis of SMS cannot be decided from the present experiments. Further investigations on the mechanism of action of SU-88 are under way.

References

1 Kyogoku, K., Hatayama, K., Yokomori, S., Sasazki, R., Sasajima, M., Sawada, J., Ohzeki, M., and Tanaka, I.: Anti-ulcer effect of isoprenyl flavonoids. II. Synthesis and anti-ulcer activity of new chalcones related to sophoradin. Chem. Pharm. Bull. (Tokyo) 27, 2943-2953 (1979)

2 Sasazki, R., Arai, I., Kobayashi, N., Mutoh, K., Sasajima, M. and Ohzeki, M.: Effects of 2'-carboxymethoxy-4,4'-bis(3-methyl-2-butenyloxy) chalcone (SU-88) on experimental ulcer models and gastric secretions. Pharmacometrics 18, 579-586 (1979) (Abs. in English)

3 Matsuo, Y., Seki, A., Yakabi, K., Sasazki, R., Arai, I., Isobe, Y. and Aihara, H.: Effect of 2'-carboxymethoxy-4,4'-bis(3-methyl-2-butenyloxy) chalcone (SU-88) on gastric local blood flow. Arzneimittelforsch. 33, 242-243 (1983)

4 Sasazki, R., Arai, I., Isobe, Y. and Aihara, H.: Effects of the anti-ulcer drugs SU-88 on the gastric blood flow and the cardiovascular system. Folia Pharmacol. Japon. 79, 193-202 (1982) (Abs. in English)

5 Suguro, N., Kagoshima, M., Makita, R. and Yaguchi, H.: Effects of 2'-carboxymethoxy-4,4'-bis(3-methyl-2-butenyloxy) chalcone (SU-88) on the gastric mucosal vessels. Pharmacometrics 23, 63-68 (1982) (Abs. in English)

6 Robert, A., Bayer, R.B. and Nezamis, J.E.: Gastric mucous content during development of ulcers in fasting rats. Gastroenterology 45, 740-751 (1963)

7 Hakkinen, I., Hartiala, K. and Lang, H.: The effect of restraint on the content of acid polysaccharides of glandular gastric wall in rat. Acta Physiol. Scand. 66, 333-336 (1966)

8 Takagi, K. and Yano, S.: Effect of anti-ulcer drugs on gastric mucous hexosamine in rats subjected to several ulcerogenic conditions. Chem. Pharm. Bull. (Tokyo) 20, 1170-1174 (1974)

9 Dekanski, J.B., Macdonald, A. and Sacra, P.: Effect of fasting, stress and drugs on gastric glycoprotein synthesis in the rat. Br. J. Pharmacol. 55, 387-392 (1975)

10 Azumi, Y., Ohara, S., Ishihara, K., Okabe, H. and Hotta, K.: Correlation of quantitative changes of gastric mucosal glycoproteins with aspirin-induced gastric damage in rats. Gut 21, 533-536 (1980)

11 Sander, L.D., Chandler, A.M. and Johnson, J.R.: Changes in liver and gastric mucosal hexosamine synthesis after restraint. Gastroenterology 68, 285-293 (1975)

12 Prino, G., Lietti, A. and Paglialunga, S.: Inhibition of gastric peptic activity by a new sulfate glycopeptide (GLPS). Arzneimittelforsch. 21, 918 (1971)

13 Mikuni-Takagaki, Y. and Hotta, K.: Characterization of peptic inhibitory activity associated with sulfated glycoprotein isolated from gastric mucosa. Biochim. Biophys. Acta 584, 288-297 (1979)

14 Lambert, R., Andre, C. and Martin, F.: Incorporation of radiosulfate in the rat subjected to restraint. Gastroenterology 56, 200-205 (1969)

15 Rainsford, K.D.: The effects of aspirin and other nonsteroidal antiinflammatory/analgesic drugs on biosynthesis in vitro.: Relationship to ulcerogenic actions. Biochem. Pharmacol. 27, 877-885 (1978)

16 Dubois, M., Gilles, A., Hamilton, J.K., Rebers, P.A. and Smith, F.: Colorimetric methods for determination of sugar and related substances. Anal. Chem. 28, 350-356 (1956)