Cytoprotective Effect of SU-88, an Anti-Ulcer Agent, in the Rat

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Abstract—The gastric cytoprotective action of SU-88, an anti-ulcer agent, was studied in rats. SU-88 dose-dependently prevented the formation of gastric lesions induced by absolute ethanol as observed by PGE₂. The efficacy of SU-88 when given i.p. was more potent than the p.o. administration. Indomethacin (5 mg/kg, p.o.) given 30 min prior to SU-88 dosing blocked this protective effect, whereas it was not affected when indomethacin was given 30 min after the SU-88 dosing. Cimetidine, on the other hand, failed to exert a protective effect against the ethanol-induced lesions and caused a significant increase in the lesions induced by 0.6N HCl. Pretreatment with SU-88 prior to cimetidine resulted in a marked reduction in the lesions. SU-88 was found to increase the synthesis of gastric glycoproteins and to prevent the reduction of glycoprotein synthesis caused by the administration of absolute ethanol. However, no increase in the synthesis was observed 5 min after the SU-88 dosing, although the lesion was significantly suppressed at that time. These findings indicate that SU-88 possesses a cytoprotective effect and that this effect seems to be mediated by the increase in endogenous PG.

2'-Carboxymethoxy-4,4'-bis(3-methyl-2-butenyloxy)chalcone (SU-88) is a synthetic derivative of sophoradin (1), isolated from the root of a Chinese medical plant, Sophora subprostrata CHUN et T. CHEN. SU-88 was shown to prevent the formation of acute gastric ulcers and accelerate the healing of a chronic ulcer produced by acetic acid (2), although it showed only a slight inhibitory effect regarding gastric secretion and acid output in rats. It has recently been reported that the mechanism for the anti-ulcer effect of SU-88 might be due to a strengthened gastric mucosal barrier through an increase in the mucosal blood flow (3–5) and the stimulation of biosynthesis of sulfated mucosubstances (6).

Recently, prostaglandins (PG) and PG derivatives have been shown to prevent the formation of an ulcer by a mechanism independent of their anti-secretory properties (7–9) and to protect the gastric mucosa against lesions induced by various necrotizing agents (10). The exact mechanism for this property of PG called “cytoprotection” is unknown. In addition, mild irritants such as 20% ethanol also exhibit cytoprotective effects against damaging agents (adaptive cytoprotection) which is considered to result from the endogenous formation of PG (11–13), suggesting that endogenous PG may play an important role in maintaining the cellular integrity of the gastric mucosa.

These findings prompted us to investigate whether SU-88 also displays some cytoprotective effects. In the present study, we examined the effects of SU-88 on acute gastric lesions induced by necrotizing agents. In addition, we also examined the effects of this drug regarding the synthesis of gastric mucus which was postulated as one of the possible mechanisms for cytoprotection.

Materials and Methods

Induction of gastric lesions: Gastric lesions were produced according to the method of
Robert et al. (10). Male Wistar rats weighing approx. 180 g were fasted for 24 hr and deprived of water for 19 hr prior to the experiments. All rats were housed in wire-mesh bottom cages throughout the study to prevent coprophagy. One milliliter of absolute ethanol or 0.6N HCl, employed as necrotizing agents, were administered orally, and 1 hr later, the animals were sacrificed by decapitation. The stomachs were removed, opened along the greater curvature and washed gently with ice-cold saline. The gastric lesions that occurred in the glandular portion were determined. In this study, the length of lesions was measured in millimeters, and lesion severity was expressed as the total length of lesions per stomach.

Drug administration: The drugs tested in this study were freshly prepared just before the experiment as follows: SU-88 and indomethacin was suspended in 0.4% carboxymethyl cellulose (CMC); PGE2 (Sigma) was dissolved in a small volume of ethanol and diluted with water; cimetidine (SK & Fujisawa) was diluted with water. The control rats were given 0.4% CMC. Each drug was given 30 or 60 min before the oral administration of the necrotizing agents.

Determination of rate of mucus synthesis: The method employed to determine the rate of mucus synthesis in the stomach was similar to that described by Dekanski et al. (14). After determination of gastric lesions, each stomach was separated into the corpus and antrum. Portions of the corpus were punched out in circles, 14 mm diam., to make similar tissue sizes. Each tissue was preincubated at 37°C for 10 min in 2 ml of Krebs-Medium I, pH 7.4, before the addition of 1 μCi of N-acetyl-D-(1-3H) glucosamine (The Radiochemical Centre, Amersham); and incubation was carried out for 2.5 hr, gassing with 95% O2 and 5% CO2 every 20 min. The incubation was stopped by cooling with ice, draining the medium and washing the tissue twice with 5 ml of ice-cold saline. The tissue was then homogenized in 20 ml of 5 mM EDTA, pH 7.4, under cooling. Glycoproteins were precipitated overnight at 4°C with 10% trichloroacetic acid -1% phosphotungstic acid. The precipitable glycoproteins from this were washed twice with ice-cold saline and were extracted twice with chloroform/ethanol (1:1, v/v) for defatting. The resultant glycoproteins were dried, weighed accurately and solubilized with an addition of 0.5N NaOH. One half ml of the solution was neutralized with HCl and mixed with 10 ml of Insta-gel (Packard) prior to the determination of the radioactivity in a Packard Tricarb Model 3255 liquid scintillation spectrometer.

Statistical analysis: Results obtained were expressed as the mean±S.E.M. The significant difference of the data was evaluated using Student’s t-test.

Results

Cytoprotective action of SU-88, PGE2 and cimetidine: When absolute ethanol was orally administered, elongated hemorrhagic lesions that occurred mostly in the corpus were observed. As shown in Table 1, the control rats given the vehicle only, p.o. and i.p., had mean total lesion lengths of 56.29±8.73 and 47.43±5.26 mm, respectively. Histological observations showed that the gastric damage induced by ethanol was a complete loss of surface epithelium and in some cases loss of the upper part of the gastric gland, accompanied by intramucosal hemorrhage and necrosis, staining with hematoxylin and eosin. SU-88 given both p.o. and i.p. prevented the ethanol-induced gastric lesion as observed by PGE2 (Table 1), in a dose-dependent manner (Fig. 1). The damage was, in the most part, limited only to the luminal epithelium cells and not extended into the gland. The effect of SU-88 when given i.p. was more potent than the p.o. administration. On the other hand, cimetidine given 50 mg/kg i.p. failed to display the preventive effects. SU-88 also significantly prevented the formation of gastric lesions induced by a subsequent administration of 0.6N HCl. The lesion length was significantly increased by cimetidine (100 mg/kg, i.p.), and the lesion was reduced markedly when SU-88 (100 mg/kg, i.p.) was administered prior to cimetidine, as observed in rats receiving SU-88 only (Table 2).

Effect of indomethacin before and after the administration of SU-88 on ethanol-induced gastric lesions: As shown in Table 3,
the treatment with indomethacin (5 mg/kg, p.o.) 30 min later after the administration of SU-88 (100 mg/kg, i.p.) resulted in a significant reduction in the gastric lesion induced by a subsequent administration of ethanol, whereas the cytoprotective action by SU-88

![Graph showing the relationship between dose and lesion length.](image)

**Fig. 1.** Effect of various doses of SU-88 on ethanol-induced gastric lesions. All rats were given 1 ml of absolute ethanol and killed 1 hr later. Various doses of SU-88 were administered p.o. (dotted line) or i.p. (solid line) 30 min prior to ethanol. Control rats were given 0.4% CMC. Each point represents the mean of 9 rats. Significantly different from the control (*P<0.05, **P<0.01 and ***P<0.001).

Table 1. Effect of SU-88, PGE\textsubscript{2} and cimetidine on ethanol-induced gastric lesions in rats

| Drug        | Dose (mg/kg) | Route | n  | Length of lesion per stomach (mm) | % Change |
|-------------|--------------|-------|----|-----------------------------------|----------|
| Control (CMC) |              |       |    | 47.43±5.26                        |          |
| PGE\textsubscript{2} | 0.1          | i.p.  | 7  | 11.00±7.80**                      | −76      |
| SU-88       | 50           | i.p.  | 7  | 13.86±5.24***                     | −71      |
|             | 100          | i.p.  | 7  | 4.14±1.65***                      | −91      |
| Control (CMC) |              |       |    | 56.29±8.73                        |          |
| PGE\textsubscript{2} | 0.1          | p.o.  | 7  | 1.00±0.38***                      | −98      |
| SU-88       | 100          | p.o.  | 7  | 14.43±7.20**                      | −74      |
|             | 300          | p.o.  | 7  | 5.57±2.45***                      | −90      |
| Control (H\textsubscript{2}O) |              |       |    | 65.20±6.21                        |          |
| Cimetidine  | 50           | i.p.  | 10 | 65.40±6.80                        |          |

All rats were given 1 ml of absolute ethanol, p.o., and killed 1 hr later. Drugs were administered p.o. or i.p. 30 min prior to ethanol. Control rats were given 0.4% CMC or distilled water. Each value represents the mean±S.E. Significantly different from the control (*P<0.01, **P<0.001).

Table 2. Effect of SU-88 and cimetidine on 0.6N HCl-induced gastric lesions in rats

| Drug        | Dose (mg/kg) | Route | n  | Length of lesion per stomach (mm) | % Change |
|-------------|--------------|-------|----|-----------------------------------|----------|
| Control (CMC) |              | i.p.  | 8  | 28.44±7.66                        |          |
| SU-88       | 100          | i.p.  | 8  | 9.38±2.74*                        | −67      |
| Cimetidine  | 100          | i.p.  | 8  | 55.63±8.33                        | +96      |
| SU-88\textsuperscript{a)} | 100          | i.p.  | 8  | 10.70±3.17*                        | −62      |
| Cimetidine  | 100          | i.p.  | 10 | 10.70±3.17*                        |          |

All rats were given 0.6N HCl and killed 1 hr later. Drugs were administered i.p. 30 min prior to HCl. Each value represents the mean±S.E. \textsuperscript{a)}: SU-88 was given 30 min prior to cimetidine. Significantly different from the control (*P<0.05).
was abolished by the pretreatment with indomethacin prior to SU-88 dosing.

Onset and duration of cytoprotective action by SU-88: SU-88 was administered at various times prior to ethanol to determine the onset and duration of preventive effects. As shown in Fig. 2, a significant reduction in the lesion was caused when SU-88 was administered from 5 to 180 min at 50 mg/kg and from 5 to 240 min at 100 mg/kg prior to ethanol.

Effect of SU-88 on synthesis of gastric glycoproteins: As shown in Table 4, the synthetic activity of glycoproteins in the antrum was about twice as potent as that in the corpus, as determined in terms of the N-acetyl-D-(1-3H) glucosamine incorporated. Pretreatment of rats with SU-88 (100 mg/kg, i.p.) 30 min before ethanol caused a significant increase in the glycoprotein synthetic activity, approximately twice in the corpus and four times in the antrum, compared with the value in the controls. However, no significant change was observed 5 min after the SU-88 dosing (data not shown). In the gastric tissue, which developed apparent lesions by ethanol, a striking decrease in the synthetic activity was noted both in the corpus and antrum. Pretreatment with SU-88 (100 mg/kg, i.p.) prior to ethanol showed a complete halting of the decrease.

Discussion

We demonstrate in the present experiments that SU-88, an anti-ulcer agent, showed cytoprotective action as observed by PGE$_2$ in rats. It has been reported that various types of PG given exogenously can prevent the formation of gastric lesions induced by necrotizing agents such as absolute ethanol (10). On the other hand, the endogenous production of PG caused by mild irritants such

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### Table 3. Effect of indomethacin treatment before and after the administration of SU-88 on ethanol-induced gastric lesions

| Drug          | Dose (mg/kg) | Route | n  | Length of lesion per stomach (mm) | % Change |
|---------------|--------------|-------|----|----------------------------------|----------|
| Control (CMC) |              | i.p.  | 9  | 54.22±7.61                       |          |
| SU-88         | 100          | i.p.  | 10 | 1.78±0.80***                    | -97      |
| SU-88         | 100          | i.p.  | 8  | 9.33±3.02***                   | -83      |
| SU-88         | 100          | p.o.  | 8  | 12.33±2.21***                  | -77      |
| Indomethacin  | 5            | i.p.  | 10 | 73.70±6.13                       | +36      |
| Indomethacin  | 5            | p.o.  | 10 | 43.10±7.64                       | -21      |

All rats were given 1 ml of absolute ethanol, p.o., and killed 1 hr later. Drugs were administered 30 or 60 min prior to ethanol. Each value represents the mean±S.E. Significantly different from the control (**P<0.01 and ***P<0.001).
as 20% ethanol also can prevent gastric necrosis (11-13). Recently, Robert (16) reported that sodium salicylate completely prevented the formation of gastric lesions produced by either absolute ethanol or 0.6N HCl. This protective effect was completely inhibited by indomethacin, the PG synthesis inhibitor, and considered to be due to the stimulation of formation of PG by its action as a mild irritant (17).

The protective action of SU-88 with parenteral administration was more potent than that with oral dosing, suggesting that this drug exerts mainly a systemic action rather than a local effect on the gastric mucosa. In order to examine the possible involvement of endogenous PG in the protective action of SU-88, the rats were treated with indomethacin before and after the administration of SU-88. The results indicate that indomethacin blocked the protective action by SU-88 when given 30 min prior to SU-88 dosing, but did not have this effect when given 30 min after the SU-88 dosing. These findings were supported by the observation that SU-88 inhibited the activity of the PG inactivating enzyme, 15-hydroxy-PG-dehydrogenase, in the gastric mucosa of hogs, but did not affect the cyclooxygenase activity in vitro (18). Therefore, it may be concluded that the mechanism of the protective action by SU-88 seems to be due to the increase in the endogenous PG content through its inhibitory effects on the PG inactivating enzyme. It has already been reported that SU-88 has significant preventive and curative effects on the various types of experimental gastric ulcers (2). Postulated mechanisms of the anti-ulcer effects involved the improvement of gastric blood flow and the enhancement of the synthesis of sulfated mucosubstances depressed by adverse conditions such as stress loading (3, 6). On the other hand, it is well known that several PG's increased in the gastric mucosal blood flow (19-22) and stimulated synthesis and release of gastric mucus (23-25). In addition, Basso et al. (26) reported that the PG generation rate was significantly decreased in the gastric mucosa of rats subjected to cold-restraint stress. Consequently, part of the anti-ulcer effects of SU-88 is explained by the increase in the levels of endogenous PG which provide an enhancement of the gastric mucosal resistance.

The mechanism of cytoprotection is unknown yet, but several hypotheses can be considered (27). In order to access the mechanism of the cytoprotective action caused by SU-88, we examined the effects of this drug on the synthesis of gastric mucus, which is one of the postulated mechanisms of cytoprotection. The rate of incorporation of N-acetyl-D-(1-3H)glucosamine into the gastric glycoproteins was significantly increased 30 min after SU-88 dosing. Moreover, the marked reduction of the rate of incorporation caused by the administration of absolute ethanol was completely halted by

Table 4. Effect of SU-88 on the incorporation of N-acetyl-D-(1-3H)glucosamine into rat gastric glycoproteins in ethanol-induced gastric lesions

| Treatment                          | Incorporation of N-acetyl-D-(1-3H)glucosamine (dpm/mg glycoproteins) |
|------------------------------------|-----------------------------------------------------------------------|
|                                    | Corpus                                                               | Antrum                                                               |
| Control (CMC, i.p.)                | 130.9±17.3                                                           | 410.4± 45.4                                                          |
| SU-88 (100 mg/kg, i.p.)            | 233.9±29.1*                                                          | 1209.1±327.3*                                                        |
| Control-Ethanol                    | 41.4± 4.4***                                                         | 102.2± 33.2**                                                        |
| SU-88 (100 mg/kg, i.p.)-Ethanol    | 92.9±10.8$$                                                          | 350.± 53.7$$                                                         |
| SU-88 (300 mg/kg, p.o.)-Ethanol    | 77.9± 8.3$$                                                          | 211.0± 48.4                                                          |

SU-88 or vehicle was given 30 min prior to ethanol. One hour later, animals were killed and the rate of N-acetyl-D-(1-3H)glucosamine was determined. Each value represents the mean±S.E. of 7-12 (Corpus) and 5-6 (antrum) specimens. Significantly different from the control (*P<0.05, **P<0.01 and ***P<0.001) and from control-ethanol ($$$P<0.05, $$P<0.01).
pretreatment with SU-88. However, it is less likely that the increase in the mucus synthesis is the major factor for cytoprotective action because no increase in the rate of mucus synthesis was observed 5 min after the SU-88 dosing, but the lesion was significantly suppressed at that time.

On the other hand, cimetidine, the H2-receptor antagonist, failed to provide any protective effect on ethanol-induced lesions; moreover, lesion severity was significantly increased as a result of 0.6N HCl. Arakawa et al. (28) reported that the increased lesion caused by cimetidine resulted from the deficiency of mucosal PG and the withdrawal of the defensive force against the mucosal damage by the necrotizing agents. Pretreatment with SU-88 prior to cimetidine was capable of preventing the development of 0.6N HCl-induced lesions as observed when SU-88 was given alone, suggesting that a combined treatment of SU-88 with cimetidine can be useful for therapeutic applications in ulcers.

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