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Original articles

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EFFECT AND MECHANISM OF SUCRALFATE ON HEALING OF ACETIC ACID-INDUCED GASTRIC ULCERS IN RATS

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We examined the effect of sucralfate on spontaneous and delayed healing of experimental gastric ulcers and the underlying mechanism of action. Gastric ulcers were produced 5 days after submucosal injection of 20% acetic acid (0.03 ml) into the antral-oxyntic border of rat stomachs. To delay the healing of ulcers, indomethacin was administered s.c. at 1 mg/kg once daily for 4 weeks from 5 days after the acid injection. Sucralfate, administered p.o. three times daily, significantly accelerated the spontaneous healing of ulcers, the healing rates being 13.7%, 43.7% and 47.1% with 100 mg/kg, 300 mg/kg and 600 mg/kg, respectively. In addition, the drug also significantly prevented the delay in ulcer healing caused by indomethacin, the preventive rates being 56.6% and 83.9% with 300 mg/kg and 600 mg/kg, respectively. Sucralfate, even at 1000 mg/kg, had no effect on the mucosal prostaglandin E_2 (PGE_2) level around the ulcers and did not affect the reduced PGE_2 content caused by indomethacin. A single dose of sucralfate significantly increased the volume and the pH of the gastric contents in a dose-dependent manner, the effects persisting for up to 8 hr. These results suggest that the mechanism by which sucralfate accelerates the healing of gastric ulcers is unrelated to endogenous PGs but related to the acid-neutralizing activity.

Key words: *sucralfate, acetic acid ulcer, endogenous prostaglandin, indomethacin, delayed healing*

INTRODUCTION

Sucralfate (the basic aluminum salt of sucrose sulfate), a potential antiulcer drug, apparently prevents the development of various gastric lesions and accelerates the spontaneous healing of gastric ulcers induced in animals (1-3). Concerning the mechanism of sucralfate protection, there exists controversy as

to the role of endogenous prostaglandins (PGs). Two groups contend that PGs might be involved in the protection because of an increased level of PGs after sucralfate treatment and the disappearance of the protective action after indomethacin treatment (4-6). On the other hand, another group could not find any increase in the PGs level with sucralfate and found protective activity even after indomethacin treatment (7). In the previous paper, we reported that the healing of gastric ulcers was apparently delayed by repeated administration of indomethacin at a dose which markedly reduced the PGE_2 level in the gastric mucosa (8). Thus, it was of interest to determine whether or not sucralfate could prevent the delay in ulcer healing in animals in which PGs were greatly reduced as well as the enhancement of spontaneous healing. To elucidate the mechanism of action of sucralfate on ulcer healing, we determined the effect of the drug on the mucosal PGE_2 level around ulcers and gastric secretion. Abstracts of a part of this study have been published (9, 10).

MATERIALS AND METHODS

Male Donryu rats (S.L.C., Shizuoka), weighing 260-280 g, were used in all experiments. The animals were not fasted prior to ulcer induction to facilitate acetic acid injection into the gastric wall, but in other studies, they were fasted for 15 h and deprived of water for 2 h before the experiments. These animals were kept in raised mesh-bottom cages to prevent coprophagy. Eight to 25 rats were used in each experiment.

Induction of Acetic Acid Ulcers

A detailed description of the method for ulcer induction was given previously (8, 11). Briefly, under ether anesthesia, the abdomen was incised and the stomach exposed.Then, 0.03 ml of 20% acetic acid (v/v) was injected into the submucosal layer of the antral-oxyntic border of the anterior wall of the stomach. After closure of the abdomen, the animals were caged and maintained in the usual manner. Since well-defined deep ulcers were observed 5 days after the acid injection, we defined the 5th day as the day of ulceration and all treatments were begun after the day of ulceration.

To delay the healing of gastric ulcers, indomethacin was administered s.c. at 1 mg/kg once daily (9:00 am) for 4 weeks (8). Sucralfate was administered p.o. at 100 ~ 600 mg/kg 3 times daily (9:30 am, 2:30 pm and 7:30 pm) for 2 or 4 weeks to assess its accelerating effect on the spontaneous healing or delayed healing caused by indomethacin, respectively. Control animals received the vehicle alone. These animals were killed with an overdose of ether 15 h after the final administration of sucralfate. Before autopsy, the animals were deprived of food for 15 h but had free access to tap water. The stomach was removed, inflated with 8 ml of 2% formalin, immersed in 2% formalin for 15 min, to lightly fix the gastric wall, and then opened along the greater curvature. The ulcerated area (mm^2) was determined under a dissecting microscope with a square grid ($\times 10$). The person who measured the ulcerated area was unaware of which treatment the animals had been given.

Gastric Secretory Study

The effect of sucralfate on gastric secretion was determined, using the pylorus ligation technique, in rats without ulceration. Briefly, under ether anesthesia, the abdomen was incised and the pylorus ligated. Four hours after the ligation, the animals were killed, their stomachs removed, and then the gastric contents collected. The samples were analyzed as to volume and pH. Sucralfate was administered p.o. at 100 ~ 600 mg/kg immediately after, or 1, 4 or 8 h before the pylorus ligation. In some cases, the animals were pretreated with indomethacin s.c. at 1 mg/kg 30 min before the sucralfate treatment and the pylorus was ligated 1.5 h later. Control animals received the vehicle alone.

Determination of the Gastric Mucosal PGE_2 Level around Ulcers

The effects of indomethacin and sucralfate, either alone or in combination, on the mucosal PGE_2 level around gastric ulcers were examined. Experiments were performed on the following 5 groups; sham-operated rats without ulceration, and ulcerated rats treated with the vehicle alone (control), indomethacin alone, sucralfate alone or indomethacin plus sucralfate, respectively. Indomethacin (1 mg/kg) was administered s.c. once daily for 1 week after the ulceration, while sucralfate (300 or 1000 mg/kg) was administered p.o. 3 times daily for the same period. The animals were killed 2 h after the final administration of indomethacin or the vehicle. In the control group, they were killed 0, 2, 6, 12, 18 and 24 h after the final administration of indomethacin. The stomachs were removed, opened along the greater curvature and then rinsed with ice-cold saline. The tissue samples were immediately put into liquid nitrogen and the frozen tissues lyophilized for 12 h using a freeze dryer (Eyela, Tokyo Rikagaku, Tokyo). The mucosa around the ulcer was then collected, weighed and put into 5 ml of 100% (v/v) methanol containing 3×10^{-4} M sodium mecrofenac (Warner-Lambert, Tokyo), to prevent new formation of PGs. After homogenization, the samples were processed for extraction and thin layer chromatography of PGE_2, as described previously (12, 13). The level of PGE_2 was determined by radioimmunoassay using rabbit antiserum to PGE_2 (Institute of Pasteur Production, Marnes, La Coquette). Each assay was performed in duplicate and the values were expressed in micrograms per gram of dry tissue weight.

Preparation of Drugs

Indomethacin (Sigma Chemical Co., St. Louis) was suspended in saline with a trace of Tween 80 (Nacarai Tesque, Kyoto). Sucralfate (Chugai Pharm. Co., Tokyo) was suspended in distilled water. These drugs were prepared immediately before use and administered in a volume of 0.5 ml per 100 g body weight.

Statistics

A two-tailed Dunnett's multiple comparison or Student's t-test was employed to determine the statistical significance of the data obtained in this study, at the level of $P < 0.05$. All data represent the means $\pm$ SEM for 7 to 25 rats per group.

RESULTS

Effect of Sucralfate on Healing of Gastric Ulcers

Most of the animals withstood the acid injection. The mortality rate due to free perforation of ulcers was less than 3%. Five days later, there were well-defined deep ulcers (about 35 ~ 40 mm^2), with a 100% incidence.

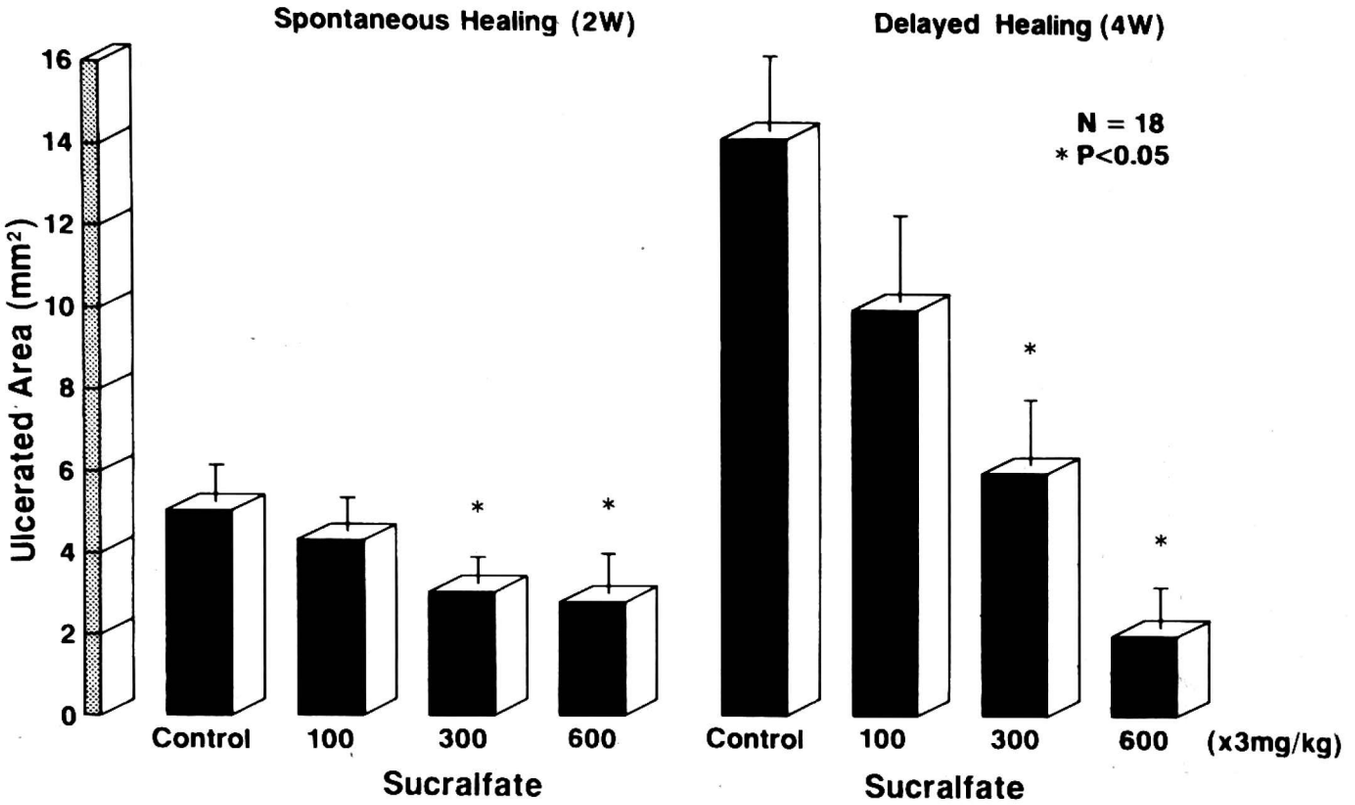

Fig. 1. Effects of sucralfate on spontaneous healing of gastric ulcers induced in rats and delayed healing caused by indomethacin. The drug was administered for 2 weeks in the case of spontaneous healing and for 4 weeks in the case of delayed healing. Data represent means $\pm$ 1 SEM. *Statistically significant difference from the corresponding control group, at $P < 0.05$.

Histologically, the ulcers penetrated the muscularis mucosae in all cases and the base of ulcers invariably adhered to the liver. These ulcers decreased in size quickly within 2 weeks (the area of ulcers being 5.1 ± 0.6 mm^2). Treatment with sucralfate for 2 weeks significantly enhanced the spontaneous healing, the healing rates being 43.7% and 47.1% with 300 and 600 mg/kg, respectively (*Fig. 1*). We confirmed our previous finding that the healing of gastric ulcers was significantly delayed by daily administration of indomethacin for 4 weeks (14.3 ± 1.7 mm^2 vs 5.1 ± 1.1 mm^2 in the control group). It should be noted that treatment with sucralfate prevented the delay in ulcer healing caused by indomethacin in a dose-dependent manner. The rates of prevention were 28.7%, 56.6% ($P < 0.05$) and 83.9% ($P < 0.05$) with 100 mg/kg, 300 mg/kg and 600 mg/kg (*Fig. 2*), respectively.

Effects of Sucralfate and Sucralfate plus Indomethacin on Gastric Secretion

Four hours after pylorus ligation, there was accumulation of gastric contents (3.6 ~ 5.4 ml; pH, about 1.4). Sucralfate (100 ~ 600 mg/kg), administered immediately after the ligation, significantly increased the volume of the gastric contents in a dose-dependent manner (*Fig. 3*). In addition, there was a significant increase in pH with 300 and 600 mg/kg. With 600 mg/kg, these

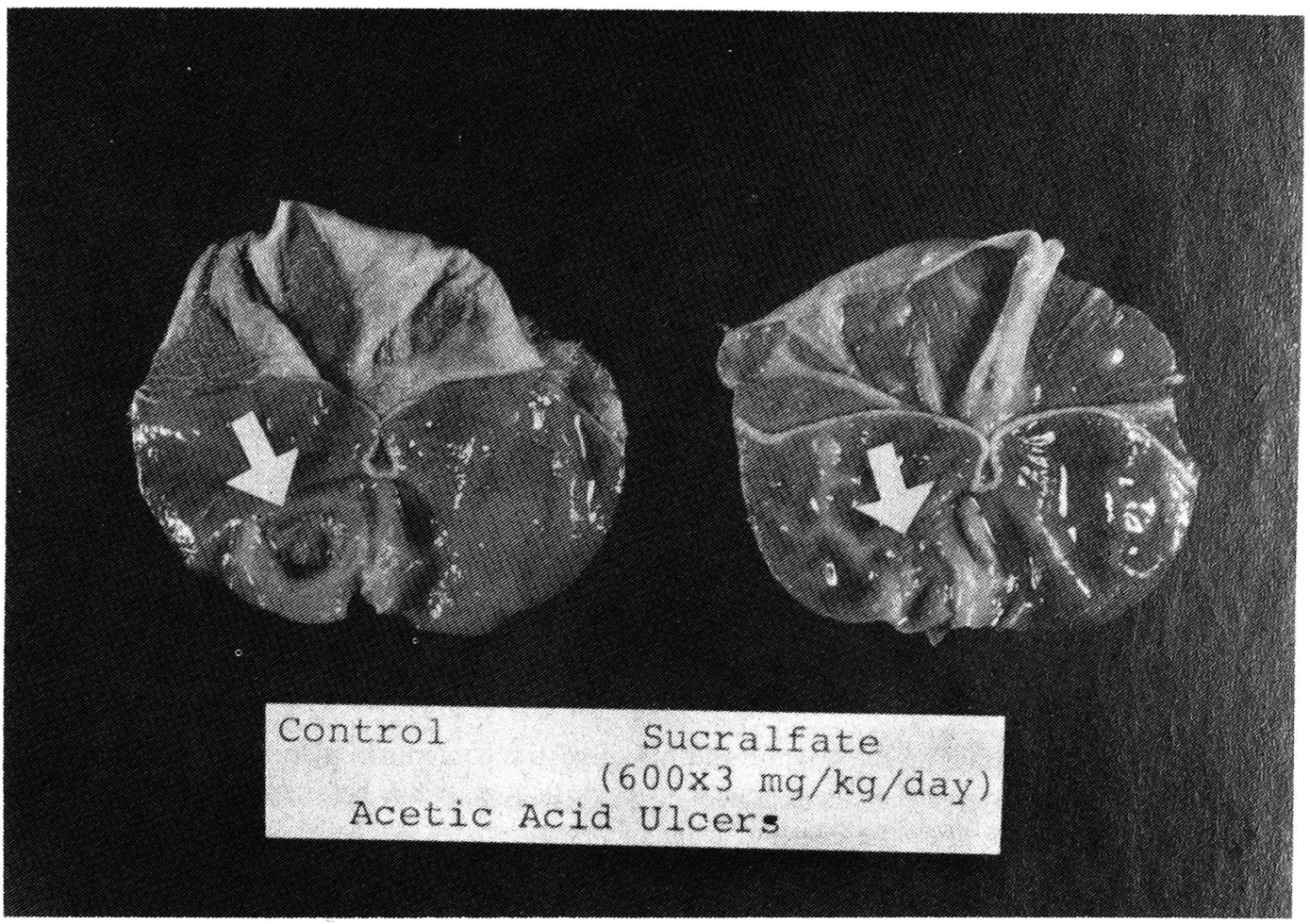

Fig. 2. Gross appearances of acetic acid-induced gastric ulcers in rats treated with indomethacin (1 mg/kg) alone (left) and indomethacin plus sucralfate (600 mg/kg × 3) (right) for 4 weeks. Note that sucralfate potently accelerated the delayed healing of the gastric ulcer induced by indomethacin.

values were 10.2 ± 0.7 ml and 3.1 ± 0.1, respectively. The stimulative activity of sucralfate toward the volume peristed for 4 h, but could not be observed 8 h later. However, the increase in pH value in response to sucralfate persisted for up to 8 h with over 300 mg/kg. Pretreatment with indomethacin did not affect the effect of sucralfate on the volume or pH (*Fig. 4*).

Effect of Sucralfate with or without Indomethacin on the Mucosal PGE_2 Level

In the sham-operated group, the PGE_2 level in the gastric mucosa was 3.3 ± 0.4 μg/g dry tissue wt. In rats with 1-week-old ulcers, there was a considerable, but not significant, increase in the PGE_2 level (4.2 ± 0.3 μg/g dry tissue wt) (*Fig. 5*). Sucralfate, administered at 300 and 1000 mg/kg × 3 for 1 week, had little or no effect on the PGs level. Repeated administration of indomethacin at 1 mg/kg to rats with ulcers for 1 week significantly reduced the PGE_2 level, by 77.8%, at 2 h after the final administration. The level remained significantly lowered for 6 h, but had returned to the control

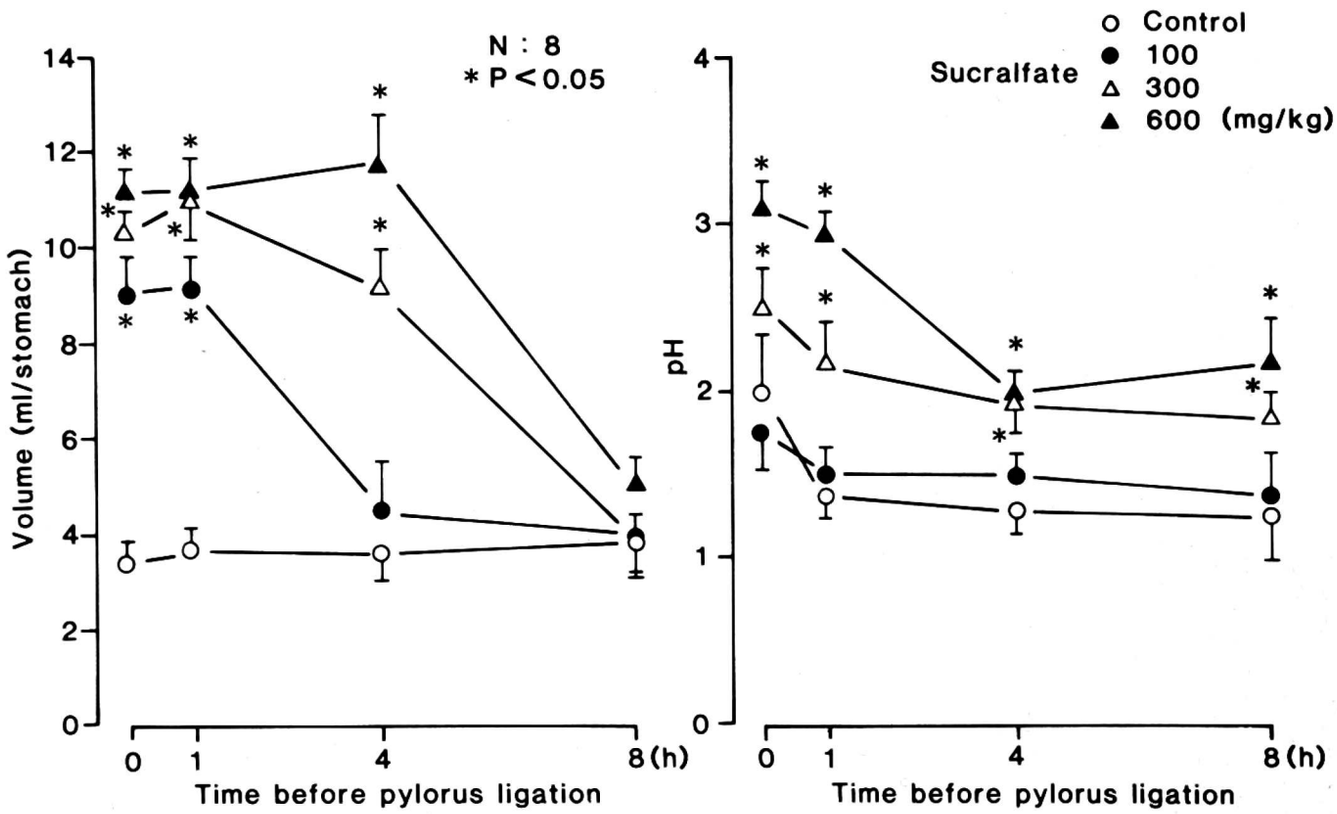

Fig. 3. Effects of sucralfate on the volume and pH of gastric contents in pylorus-ligated rats (4 h). Sucralfate was administered p.o. immediately after, or 0, 1, 4 or 8 h before the ligation, and the animals were killed 4 h after the ligation. Data represent means ±1 SEM for 8 rats per group. *Statistically significant difference from the control, at $P < 0.05$.

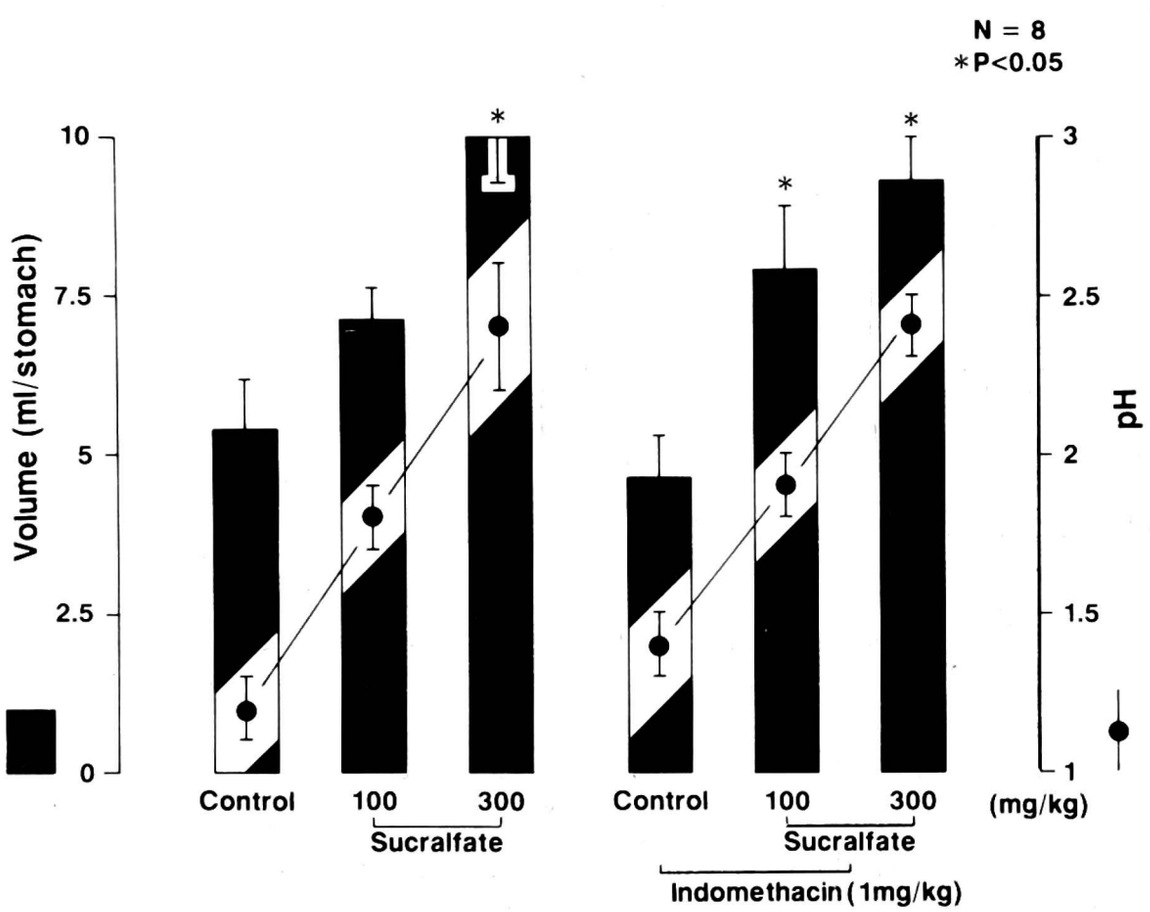

Fig. 4. Effects of pretreatment with indomethacin (1 mg/kg) on the increases in the volume and pH of the gastric contents caused by sucralfate in pylorus-ligated rats (4 h). Note that pretreatment with indomethacin did not exert any influence on the increases in the volume and pH caused by sucralfate. Data represent means ±1 SEM for 8 rats per group. *Statistically significant difference from the control, at $P < 0.05$.

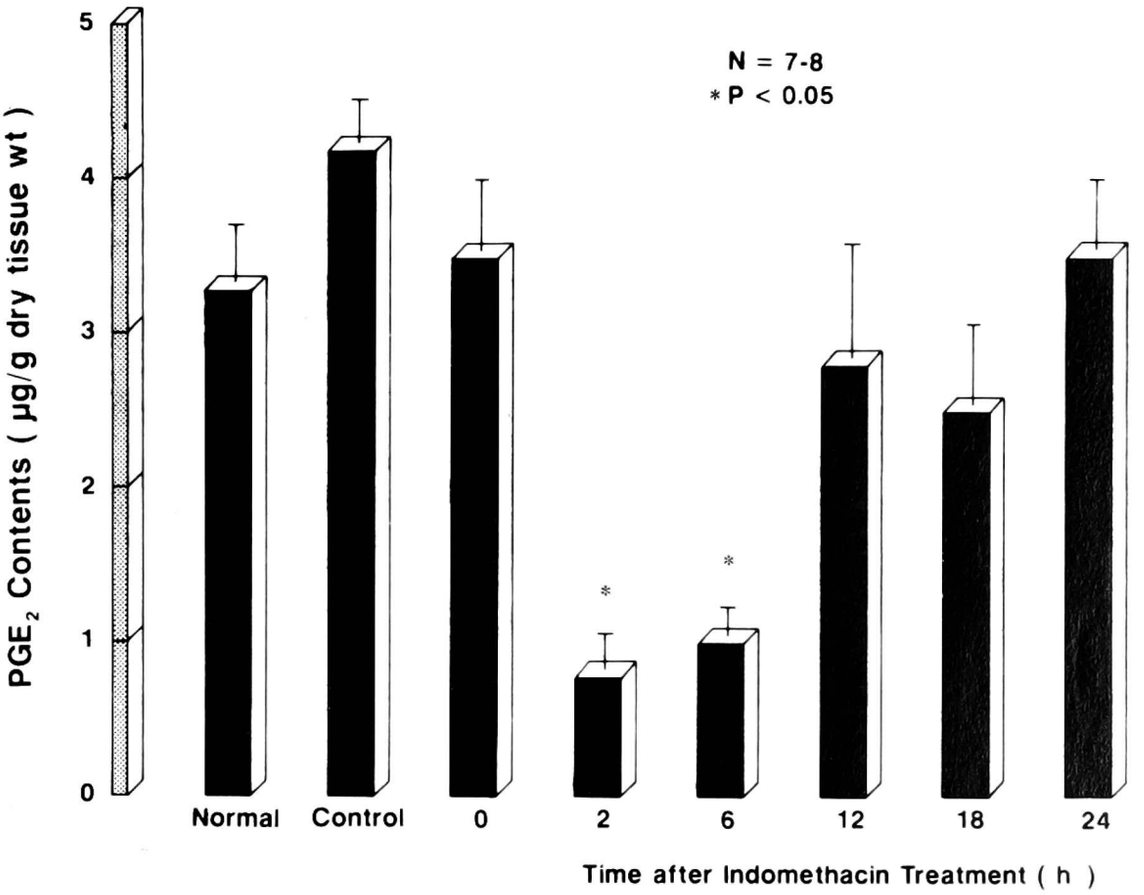

Fig. 5. Time course changes in the mucosal PGE_2 level around acetic acid-induced gastric ulcers in rats after repeated doses of indomethacin (1 mg/kg) for 1 week after the ulceration. Data represent means ± 1 SEM for 7 to 8 rats per group. *Statistically significant difference from the control level, at $P < 0.05$.

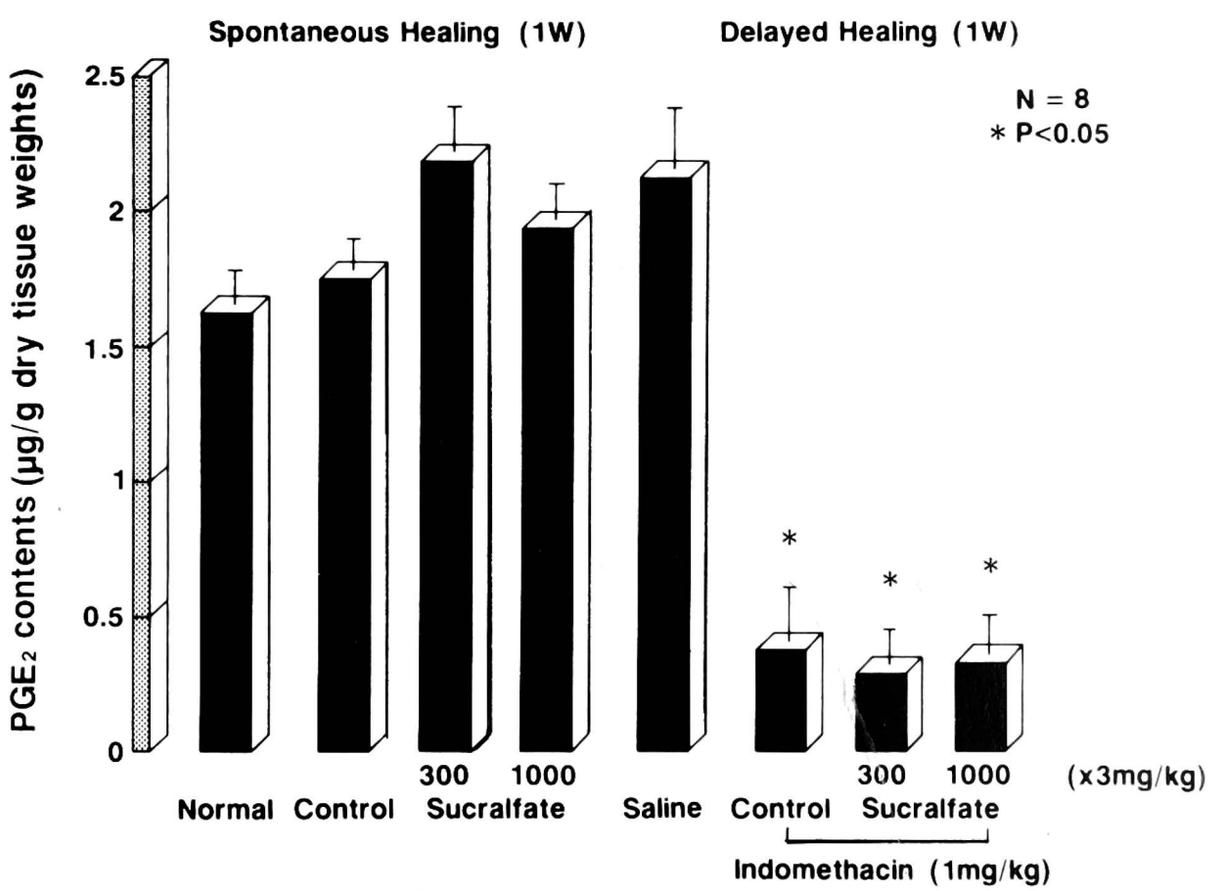

Fig. 6. Effect of sucralfate on the mucosal PGE_2 level around gastric ulcers in rats treated with or without indomethacin (1 mg/kg) for 1 week after the ulceration. The animals were killed 2 h after the final administration of indomethacin or the vehicle. Data represent means ± 1 SEM. for 8 rats per group. *Statistically significant difference from the control, at $p < 0.05$.

level 24 h later. On the other hand, treatment with sucralfate alone (300, 1000 mg/kg × 3) for 1 week had no effect on the mucosal PGE_2 level around the ulcers and did not affect the reduced PGE_2 level caused by indomethacin (*Fig. 6*).

DISCUSSION

The present study confirmed the beneficial effect of sucralfate on the spontaneous healing of acetic acid-induced gastric ulcers. In addition, we found that sucralfate has the ability to markedly prevent the delay in ulcer healing caused by indomethacin. Konturek et al. (14) also confirmed that sucralfate administerted at 400 mg/kg/day for 7 days significantly prevented the delay in healing of actic acid-induced gastric ulcers by subcutaneous indomethacin (2 mg/kg) in rats. These data provide important support for the clinical usage of sucralfate to provent the adverse effects of non-steroidal anti-inflammatory drugs (NSAIDs) on the gastrointestinal tract . In particular, it would be better to administer sucralfate together with NSAIDs when the latter have to be prescribed to patients with ulcers.

Szelenyi et al. (15, 16) and Tanaka et al. (17) reported that repeated administration of indomethacin or other NSAIDs significantly delayed the healing of acetic acid-induced gastric ulcers in rats. Since these agents significantly reduced the PG level in the ulcerated area of the stomach, they suggested that the mechanism underlying the delayed ulcer healing caused by these agents may be partly related to a PG deficiency. While our experimental protocol was slightly different to those of the above authors, we also confirmed the delayed healing of gastric ulcers and the reduced PGs level after indomethacin treatment (8). In the present study, we reconfirmed that indomethacin at 1 mg/kg significantly reduced the mucosal PGE_2 content in the ulcerated area. The PGE_2 level remained significantly lowered for 6 h and had returned to the basal value 24 h after the final administration of indomethacin. That the PGE_2 level in this study was much higher (about 10 times) than in other studies may be due to the different way of data expression; we expressed the data as μg/g dry tissue wt, while others expressed them as μg/g wet tissue wt. Wang et al (8) demonstrated that repeated doses of PGE_2, which could restore the deficient PGE_2 level in the gastric mucosa, significantly attenuated the delay in the healing of gastric ulcers in indomethacin-treated rats. These data supported the contention that a PGs deficiency is a factor involved in the mechanism by which indomethacin delays the healing of ulcers.

This study showed that sucralfate exerted a significant accelerating effect on the delayed healing of acetic acid-induced gastric ulcers caused by indomethacin. Sucralfate had little or no effect on the PGE_2 level in normal gastric mucosa and did not restore the reduced PGE_2 level around ulcers in response to indomethacin. Therefore, it is most unlikely that the enhancement

of ulcer healing by this drug is due to the restoration of the PGE_2 level in the gastric mucosa.

Nagashima and Yoshida (1) first reported the buffering capacity of sucralfate against HCl in vitro experiment. In addition, Delchier (18) showed that the increase in the intragastric pH induced by this drug may contribute to the mucosal protective action. We also confirmed in the present study a significant and persistent increase in the gastric pH after sucralfate treatment (> 300 mg/kg). The increase in pH might be due to acid-neutralizing activity and a mucus/alkaline secretory response of this drug (1, 19). In addition, we found that this drug produced a two-fold increase in the volume of the gastric contents. Wallace et al. (7) reported that sucralfate breaks down the mucosal barrier of the stomach. Therefore, it is possible that water might passively leak through the mucosa after treatment with sucralfate. Since the increase in the volume and pH caused by sucralfate was not affected by pretreatment with indomethacin, the response appears to be independent of endogenous PGs.

The increased pH caused by sucralfate lasted for up to 8 h with over 300 mg/kg. As previously reported, the healing of acetic acid-induced gastric ulcers was significantly accelerated by proton pump inhibitors such as omeprazole, lansoprazole and NC-1300 (20—22), suggesting that the potential and persistent inhibition of gastric acid might be important in accelerating ulcer healing. Therefore, it is likely that the effect of sucralfate in ulcer healing might be partly due to the persistent reduction of gastric acidity caused by this drug. Certainly, the binding activity of sucralfate to the ulcer base and antipeptic activity (1, 2) seem to also be involved in the underlying mechanism.

We conclude that the mechanism by which sucralfate enhances the healing of gastric ulcers, particularly the delayed healing in response to indomethacin, appears not to be related to endogenous PGs but to the antacid property.

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