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## EFFECTS OF SUCRALFATE AND ITS COMPONENTS ON INDOMETHACIN-INDUCED DAMAGE TO CULTURED RABBIT GASTRIC MUCOSAL CELLS

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We examined the effects of sucralfate and its components on indomethacin-induced damage to cultured rabbit gastric mucosal cells. Cell viability was assessed by colorimetric and dye exclusion methods. Exposure of mucosal cells to indomethacin at 50 and 500  $\mu\text{M}$  for 4 hr significantly reduced their viability, the viability loss being about 40% and about 90% respectively. Pretreatment of the cells with sucralfate for 30 min prevented the reduction in viability caused by 50  $\mu\text{M}$  indomethacin in a dose-related manner. Furthermore, sucralfate also significantly protected mucosal cells against severe damage caused by 500  $\mu\text{M}$  indomethacin. However, neither sucrose octasulfate nor  $\text{Al}(\text{OH})_3$  (the components of sucralfate) significantly prevented the reduction in viability caused by 50 and 500  $\mu\text{M}$  indomethacin, although these components tended to inhibit the viability loss. When mucosal cells were incubated with sucralfate or its components for 4 hr, the drugs did not affect the cell viability. These results indicate that sucralfate directly protects gastric mucosal cells against cell damage caused by indomethacin, and that the protective effect of sucralfate might be expressed as a synergistic action of its components.

**Key words:** *gastric mucosal cells, sucralfate, KSOS,  $\text{Al}(\text{OH})_3$ , cytoprotection.*

### INTRODUCTION

Sucralfate, an aluminium hydroxide complex of sucrose octasulfate, has ulcer-healing effect on chronic ulcers (1—3) and protective effect against various acute mucosal injury (4—7) in animal experiments. The mechanism underlying the protective effect to sucralfate still remains unclear. Some investigators reported that the drug protects rat gastric mucosal cells against drug-induced damage (8, 9). Zheng et al. (9) reported the protective effect of sucralfate on gastric cell damage caused by indomethacin, but their damage was induced rapidly (within 1 hr) and severely at millimolar concentrations of indomethacin. Recently, we established a new cell damage model using rabbit gastric mucosal cells by indomethacin at micromolar concentrations (10). In the present study, we determined whether or not sucralfate and its components exert cytoprotection on indomethacin-induced damage to rabbit gastric mucosal cells.

## MATERIALS AND METHODS

*Preparation of Gastric Mucosal Cells*

Gastric mucosal cells were prepared from rabbit stomachs according to the method of Watanabe et al. (11). Briefly, male Japanese white rabbits (Nihon S.L.C., Shizuoka, Japan), weighing 2.0 to 3.0 kg, were anesthetized with pentobarbital (50 mg/kg, i.v.; Abbott, North Chicago, IL). After their stomachs had been excised, the surface of the oxyntic mucosa was removed with a razor blade and minced immediately. The minced tissue was incubated in Hank's balanced salt solution containing 0.05% collagenase (Wako Chemicals, Osaka, Japan) for 15 min at 37°C, and then washed with Ca<sup>2+</sup>, Mg<sup>2+</sup> — free Hank's solution containing 1 mg/ml bovine serum albumin and 1 mM EDTA. These procedures were repeated twice. The mucosal cells were obtained by filtration through a metal mesh (diameter, 300 µm). The viability of the isolated cells was more than 85%, as determined by the dye exclusion test (12).

*Cell Culture*

Coon's modified Ham's F12 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 units/ml streptomycin and 0.25 µg/ml amphotericin B, and culture plate coated with rat collagen type I (Sumitomo Bakelite, Tokyo, Japan) were used. Gastric mucosal cells were inoculated onto a 24-well plate (2 × 10<sup>5</sup> cells/0.5 ml). The cultures were maintained at 37°C under 5% CO<sub>2</sub> in air, the medium being changed every day. The cells reached confluence 2—3 days later. Most of the cultured cells were morphologically epithelial-like, and 80—90% of the cells were confirmed to be mucus-producing ones by periodic acid-Schiff staining (13—15).

*Effects of Sucralfate and its Components on Indomethacin-induced Mucosal Cell Damage*

Indomethacin-induced damage to gastric mucosal cells was induced according to the method described previously (10). After mucosal cells grown to confluence were washed twice with Ca<sup>2+</sup>, Mg<sup>2+</sup> — free phosphate-buffered saline (PBS), the cells were treated with the indicated concentrations of various drugs or the vehicle for 30 min. After wash with PBS, the cells were further incubated in 0.5 ml of medium containing 50 or 500 µM indomethacin for 4 hr at 37°C. The medium alone without cells was used as a blank. Thereafter, cell viability was determined as described below.

Cell viability was assessed by the colorimetric method involving 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT; Sigma Chemicals, St. Louis, MO) (16). After mucosal cells had been washed with PBS, 0.1 ml of an MTT solution (5 mg/ml in distilled water) was added. Two hours later, the MTT was extracted with 1.5 ml of isopropanol containing 0.04 N HCl, and the color change of the extract was measured at 595 nm.

Alternatively, cell viability was also assessed by the dye exclusion method (17). After the medium had been gently removed, 0.1 ml of a trypan blue solution (0.1% in PBS) was added. Three minutes later, the numbers of stained and non-stained cells were counted in four randomly chosen fields in each well under a microscope (Olympus CK2, Tokyo, Japan; × 100). Cell viability was calculated as follows:

$$\text{Viability (\%)} = [\text{non-stained cells}/(\text{stained cells} + \text{non-stained cells})] \times 100$$

Cell viability was expressed as a percentage of the corresponding control value.

## Drugs

Sucralfate, potassium sucrose octasulfate (KSOS), and  $\text{Al}(\text{OH})_3$  were provided by Chugai Pharmaceutical Co. (Tokyo, Japan). Sucralfate and  $\text{Al}(\text{OH})_3$  were suspended in distilled water. KSOS was dissolved in distilled water. Ten microliter of the suspension or solution was added to wells to the desired concentrations. Indomethacin (Sigma Chemicals) was dissolved in 0.2 M  $\text{Na}_2\text{CO}_3$ . All other chemicals used were of reagent grade.

## Statistical Analysis

The data are presented as means  $\pm$  S.E. ( $n = 8$ ). Statistical difference was evaluated using the Student's t-test or Dunnett's multiple comparison test, a P value of  $< 0.05$  being regarded as significant.

## RESULTS

### Effect of Sucralfate on Indomethacin-induced Damage to Gastric Mucosal Cells

Exposure of rabbit gastric mucosal cells to indomethacin at  $50 \mu\text{M}$  for 4 hr caused a significant loss of viability, as determined by both the MTT and dye exclusion methods (Fig. 1). The viability loss was 44.4% by the MTT method

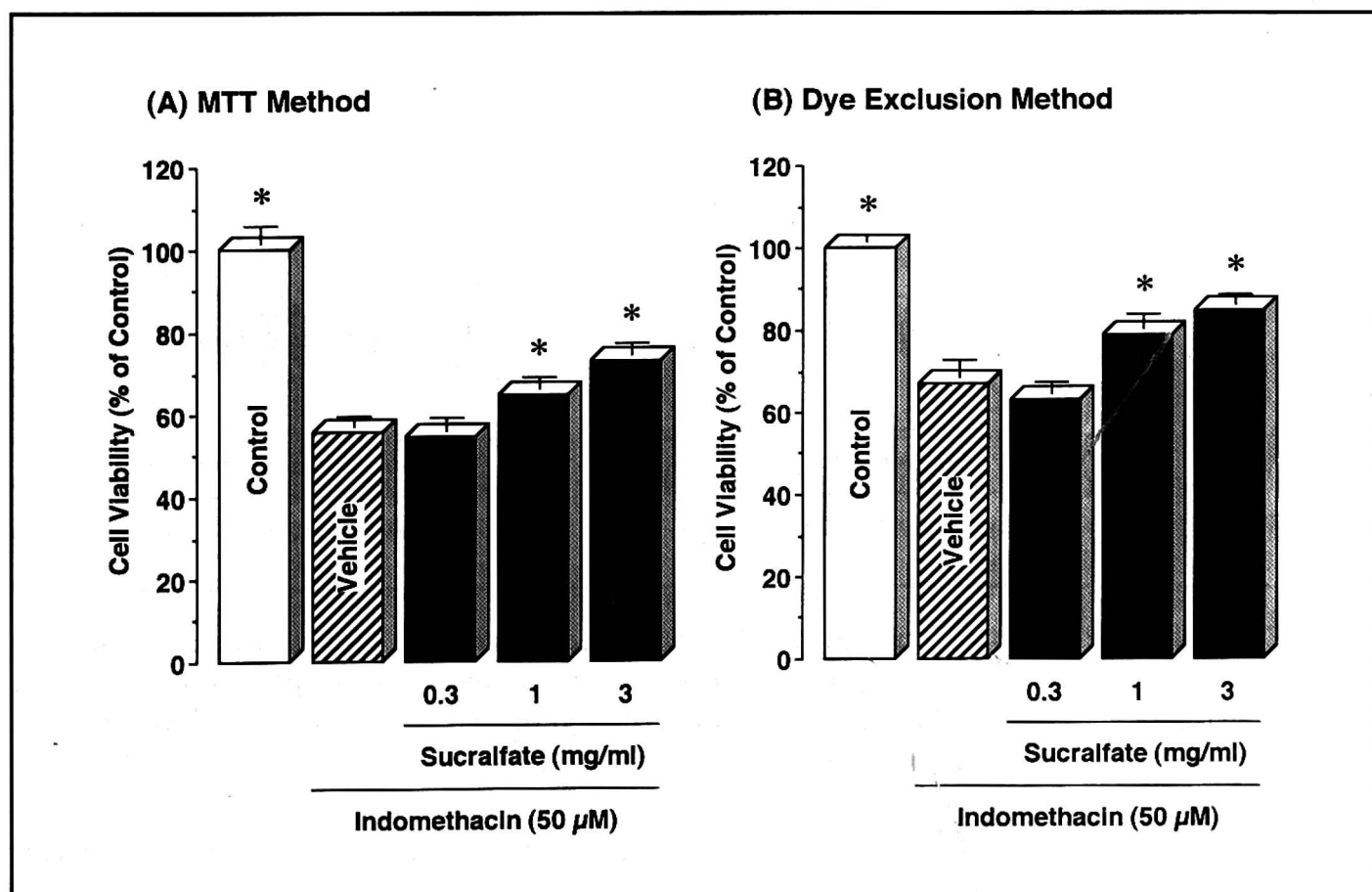


Fig. 1. Effect of sucralfate on indomethacin ( $50 \mu\text{M}$ )-induced gastric cell damage. Mucosal cells were pretreated with sucralfate at 0.3-3 mg/ml for 30 min, and then exposed to  $50 \mu\text{M}$  indomethacin for 4 hr. Cell viability was assessed by the MTT method (A) and the dye exclusion method (B). Data are presented as means  $\pm$  S.E. ( $n = 8$ ). \* Significantly different from the vehicle,  $P < 0.05$ .

and 32.9% by the dye exclusion method. Pretreatment of the cells with sucralfate for 30 min prevented the viability loss caused by 50  $\mu$ M indomethacin in a dose-related manner. Sucralfate at 1 and 3 mg/ml significantly inhibited the reduction in cell viability, the inhibition being 21.4% at 1 mg/ml and 39.3% at 3 mg/ml, as determined by the MTT method. The protective effect of sucralfate was observed with the dye exclusion method, the inhibition by sucralfate at 1 mg/ml and 3 mg/ml being 35.9% and 53.6%, respectively.

In addition, sucralfate dose-relatedly prevented the severe damage to gastric mucosal cells caused by 500  $\mu$ M indomethacin (Fig. 2). The viability loss caused by 500  $\mu$ M indomethacin was 88.1% by the MTT method and 88.7% by the dye exclusion method. Sucralfate at 1 mg/ml significantly inhibited the viability loss by 11.0%, as determined by the dye exclusion method, while the drug at 3 mg/ml further attenuated the cell damage, the inhibition being 32.4% by the MTT method and 23.3% by the dye exclusion method.

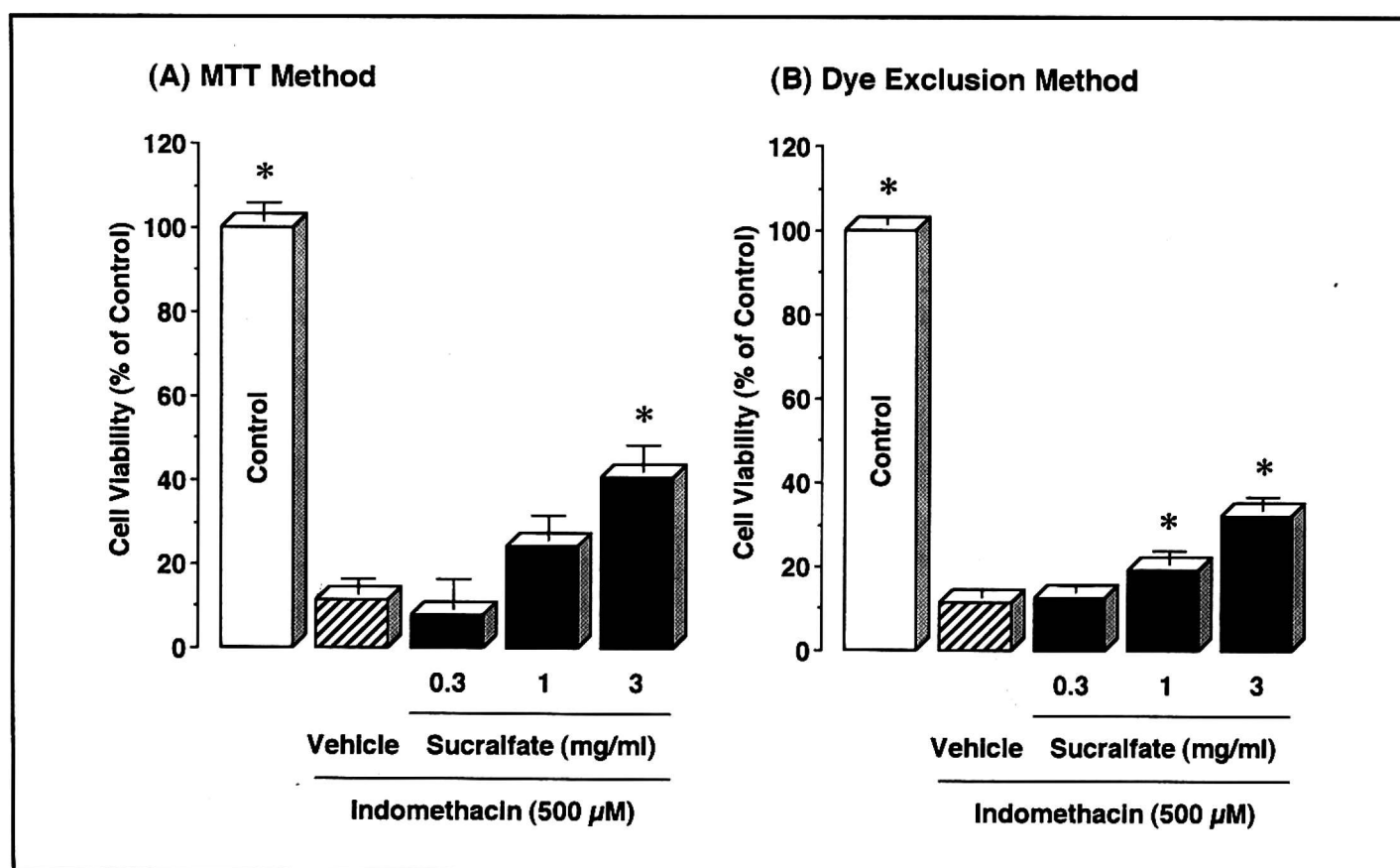
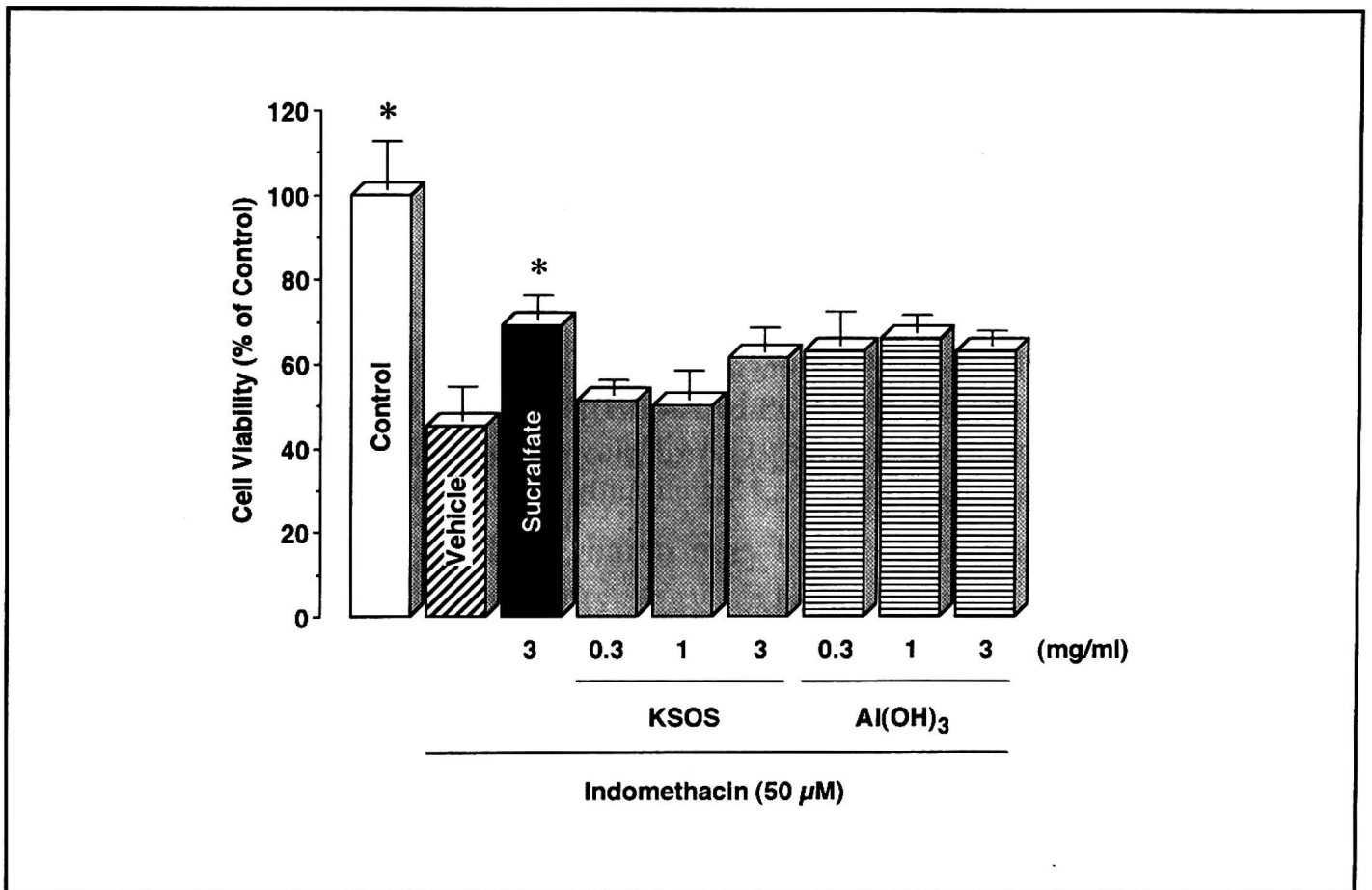


Fig. 2. Effect of sucralfate on indomethacin (500  $\mu$ M)-induced gastric cell damage. Mucosal cells were pretreated with sucralfate at 0.3–3 mg/ml for 30 min, and then exposed to 500  $\mu$ M indomethacin for 4 hr. Cell viability was assessed by the MTT method (A) and the dye exclusion method (B). Data are presented as means  $\pm$  S.E. (n = 8). \* Significantly different from the vehicle, P < 0.05.

### Effects of KSOS and Al(OH)<sub>3</sub> on Indomethacin-induced Damage to Gastric Mucosal Cells

Since sucralfate was found to prevent indomethacin-induced damage to rabbit gastric mucosal cells, the effects of its components, KSOS and Al(OH)<sub>3</sub>, were examined. Pretreatment of mucosal cells with KSOS at 0.3 and 1 mg/ml

for 30 min did not affect the reduction in viability caused by 50  $\mu\text{M}$  indomethacin, while KSOS at 3 mg/ml tended to prevent cell damage, as determined by the MTT method (*Fig. 3*).  $\text{Al}(\text{OH})_3$  at 0.3—3 mg/ml also tended to inhibit the viability loss caused by 50  $\mu\text{M}$  indomethacin. However, the inhibition by KSOS and  $\text{Al}(\text{OH})_3$  was lower than that by sucralfate (3 mg/ml sucralfate, 43.4%; 3 mg/ml KSOS, 30.3%; 1 mg/ml  $\text{Al}(\text{OH})_3$ , 38.2%). Similarly, pretreatment with KSOS at 3 mg/ml and  $\text{Al}(\text{OH})_3$  at either 0.3 or 1 mg/ml slightly prevented the marked loss of viability caused by 500  $\mu\text{M}$  indomethacin, but their inhibition was much weaker compared to that by sucralfate (*Fig. 4*).



*Fig. 3.* Effects of KSOS and  $\text{Al}(\text{OH})_3$  on indomethacin (50  $\mu\text{M}$ )-induced gastric cell damage. Mucosal cells were pretreated with KSOS or  $\text{Al}(\text{OH})_3$ , at 0.3—3 mg/ml, for 30 min, and then exposed to 50  $\mu\text{M}$  indomethacin for 4 hr. Cell viability was assessed by the MTT method. Data are presented as means  $\pm$  S.E. ( $n = 8$ ). \* Significantly different from the vehicle,  $P < 0.05$ .

#### *Effect of Sucralfate and its Components on Viability of Gastric Mucosal cells*

It was examined whether or not sucralfate and its components alone, without indomethacin, affect viability of gastric mucosal cells. After the cells were incubated with each drug for 4 hr, cell viability was determined by the MTT method (*Table 1*). Sucralfate and its components at 1 and 3 mg/ml did not significantly affect viability of the cells, although  $\text{Al}(\text{OH})_3$  at 3 mg/ml slightly reduced the viability.

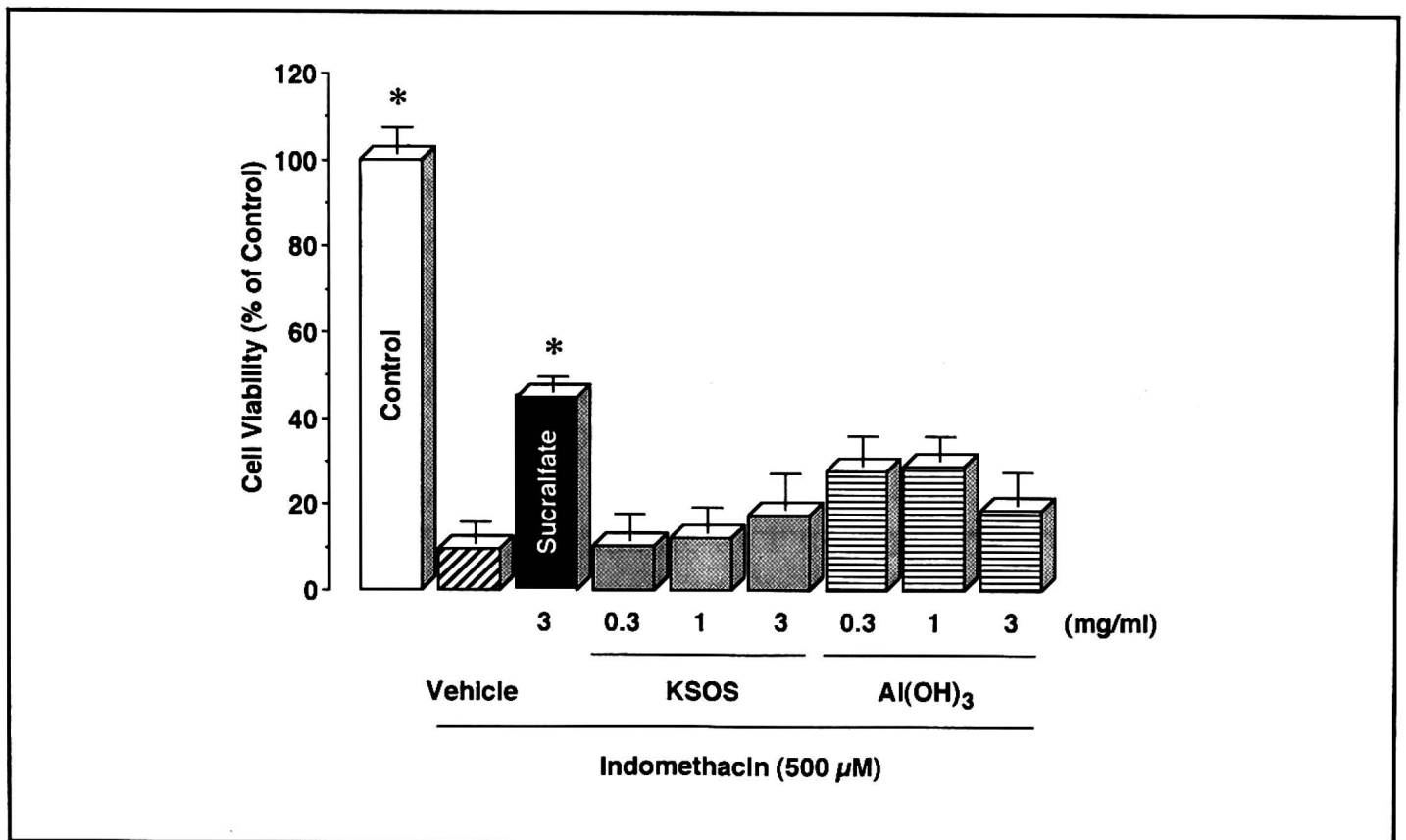


Fig. 4. Effects of KSOS and Al(OH)<sub>3</sub> on indomethacin (500 µM)-induced gastric cell damage. Mucosal cells were pretreated with KSOS or Al(OH)<sub>3</sub>, at 0.3–3 mg/ml, for 30 min, and then exposed to 500 µM indomethacin for 4 hr. Cell viability was assessed by the MTT method. Data are presented as means ± S.E. (n = 8). \* Significantly different from the vehicle, P < 0.05.

Table 1. Effects of sucralfate, KSOS and Al(OH)<sub>3</sub> on viability of gastric mucosal cells. Mucosal cells were incubated with sucralfate, KSOS, Al(OH)<sub>3</sub> at 1 and 3 mg/ml or vehicle for 4 hr, and then cell viability was assessed by the MTT method. Data are presented as means ± S.E. (n = 8).

Treatment	Cell Viability (% of Control)
Control	100.0 ± 7.4
Sucralfate 1 mg/ml	94.9 ± 5.7
3 mg/ml	96.1 ± 5.6
KSOS 1 mg/ml	92.6 ± 7.9
3 mg/ml	105.9 ± 7.3
Al(OH) <sub>3</sub> 1 mg/ml	101.1 ± 8.3
3 mg/ml	80.2 ± 8.8

## DISCUSSION

We confirmed that sucralfate directly acts on rabbit gastric mucosal cells, leading to the protection of cells against indomethacin-induced damage. The cytoprotection by sucralfate is quite effective, since the drug has the ability to protect mucosal cells against severe damage caused by 500  $\mu\text{M}$  indomethacin. Zheng et al. (9) reported that sucralfate prevented indomethacin-induced damage to rat gastric epithelial cells. Concerning sucralfate cytoprotection, their results and our results are consistent. However, the effects of the components of sucralfate were different. In this study, both  $\text{Al}(\text{OH})_3$  and KSOS exerted only a slight cytoprotective effect, suggesting that the cytoprotection by sucralfate might be expressed as a synergistic effect of its components. In contrast, Zheng et al. showed that KSOS, not  $\text{Al}(\text{OH})_3$ , provided nearly complete protection in rat gastric mucosal cells, suggesting that KSOS mainly contributes to the cytoprotection by sucralfate. The difference is thought to be due to the experimental conditions. In our model, mucosal cells prepared from rabbit stomachs were exposed to indomethacin at micromolar concentration for 4 hr. On the other hand, Zheng et al. treated rat gastric cells with indomethacin at millimolar concentrations for 1 hr. Of note was that cell damage by indomethacin appeared slowly in our model and rapidly in their model. Accordingly, it can be considered that the mechanisms of cell damage caused by indomethacin may be quite different between the two models, so that the components may exhibit different protective effects. Alternatively, it is possible that species difference may be related to the different results.

The mechanism underlying the cytoprotection by sucralfate remains unknown. We ruled out that prostaglandins are involved in the cytoprotective effect of sucralfate, because sucralfate did not affect prostaglandin production by the cells (15). Similar results were reported by Romano et al. (8). They reported that sucralfate but not its components, protects rat gastric mucosal cells against taurocholate-induced damage, independent of endogenous prostaglandin synthesis. We recently confirmed that treatment with sucralfate for 4 and 8 hr significantly stimulates mucus secretion and synthesis by gastric mucosal cells (15). However, the effect of sucralfate was not observed when the cells were exposed to the drug for 2 hr. The cytoprotection by sucralfate was expressed after only a 30-min pretreatment, indicating that secreted mucus does not contribute to the cytoprotective effect of sucralfate. In the present study,  $\text{Al}(\text{OH})_3$  rather than KSOS was more effective. Microscopically, sucralfate and  $\text{Al}(\text{OH})_3$  were found to adhere tightly to the cell surface despite frequent washings. It is suggested that  $\text{Al}(\text{OH})_3$  covering over the cells may be important to inhibit an interaction between the cells and necrotizing agents, and

that KSOS, in concert with  $\text{Al}(\text{OH})_3$ , may exert a significant protective effect. In fact, sucralfate was reported to act by adhering to ulcerated tissue and forming a physical barrier to reduce injury by acid, pepsin and bile salts (7, 18, 19).

In conclusion, sucralfate, but not its components (KSOS and  $\text{Al}(\text{OH})_3$ ), directly protects rabbit gastric mucosal cells against indomethacin-induced damage. The results suggest that the cytoprotection by sucralfate might be expressed as a synergistic effect of its components.

*Acknowledgements:* We wish to thank N.J. Halewood for critical reading of the manuscript and K. Ichimaru and K. Hirose for their technical assistance.

#### REFERENCE

- Hollander D. Efficacy of sucralfate for duodenal ulcers.: A multicenter, double blind trial. *J Clin Gastroenterol* 1981; 3 (Suppl 2): 153—157.
- Lam SK, Law WY, Lai CL. Efficacy of sucralfate in corpus, prepyloric and duodenal ulcer-associated gastric ulcers. *Am J Med* 1985; 79 (suppl 2C): 24—31.
- McCarthy DM. Sucralfate. *N Engl J Med* 1991; 325: 1017—1025.
- Okabe S, Takeuchi K, Kunimi M, Kanno M, Kawashima M. Effects of an antiulcer drug, sucralfate (a basic aluminum salt of sulfated disaccharide), on experimental gastric lesions and gastric secretion in rats. *Dig Dis Sci* 1983; 28: 1034—1042.
- Tarnawski A, Hollander D, Gergely H, Stachura J. Comparison of antacid sucralfate, cimetidine and ranitidine in protection of gastric mucosa against ethanol injury. *Am J Med* 1985; 79: 19—23.
- Hollander D, Tarnawski A, Krause W, Gergely H. Protective effect of sucralfate against alcohol-induced gastric mucosa injury in the rat.: Macroscopic, histologic, ultrastructural and functional time sequence analysis. *Gastroenterology* 1985; 88: 366—374.
- Szabo S, Hollander D. Pathways of gastrointestinal protection and repair: Mechanism of action of sucralfate. *Am J Med* 1989; 86 (Suppl 6A): 23—31.
- Romano M, Razandi M, Ivey KJ. Effect of sucralfate and its components on taurocholate-induced damage to rat gastric mucosal cells in tissue culture. *Dig Dis Sci* 1990; 35: 467—476.
- Zheng H, Shah PK, Audus KL. Primary culture of rat gastric epithelial cells as an in vitro model to evaluate antiulcer agents. *Pharm Res* 1994; 11: 77—82.
- Takahashi S, Okabe S. The cytoprotective effect of leminoprazole on indomethacin-induced damage to rabbit gastric mucosal cells. *J Pharmacol Exp Ther* (in press).
- Watanabe S, Hirose M, Yasuda T, Miyazaki A, Sato N. Role of actin and calmodulin in migration and proliferation of rabbit gastric mucosal cells in culture. *J Gastroent Hepatol* 1994; 9: 325—333.
- Phillips HJ. Dye exclusion tests for cell viability. In Krause PF and Patterson MK, eds. *Tissue culture methods and applications*. New York: Academic Press, 1973; pp. 406—408.
- Takahashi S, Nakamura E, Okabe S. Stimulatory effect of leminoprazole on secretion and synthesis of mucus by rabbit gastric mucosal cells. *J Pharmacol Exp Ther* 1995; 275: 1396—1401.
- Takahashi S, Okabe S. A histamine  $\text{H}_2$  receptor antagonist, roxatidine, simulates mucus secretion and synthesis by cultured rabbit gastric mucosal cells. *J Physiol Pharmacol* 1995; 46: 503—511.



15. Takahashi S, Okabe S. Stimulatory effects of sucralfate on secretion and synthesis of mucus by rabbit gastric mucosal cells.: Involvement of phospholipase C. *Dig Dis Sci* 1996; 41: 498—504.
16. Mosmann T. Rapid colorimetric assay for cellular growth and survival.: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; 65: 55—63.
17. Terano A, Ivey KJ, Stachura J, Sekhon S, Hosojima H, McKenzie Jr WN, Krause WJ, Wyche JH. Cell culture of rat gastric fundic mucosa. *Gastroenterology* 1982; 83: 1280—1291.
18. Nagashima R. Mechanism of action of sucralfate. *J Clin Gastroenterol* 1981; 3 (Suppl 2): 117—127.
19. McGraw BF, Caldwell EG. Sucralfate. *Drug Intell Clin Pharm* 1981; 15: 578—580.

Received: August 3, 1996

Accepted: October 9, 1996

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