Primary Culture of Rat Gastric Epithelial Cells as an *in Vitro* Model to Evaluate Antiulcer Agents

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Primary rat gastric cell cultures were investigated as an *in vitro* model for evaluating antiulcer agents. Following exposure to concentrations of up to 5 mg/mL of an antiulcer agent sucralfate, an aluminum hydroxide complex of sucrose octasulfate, cultured cells were treated with either pH 3.5 medium or 3.5 m*M* indomethacin. Cytoprotection was evaluated by colony forming efficiency, neutral red uptake, and 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) hydrolysis. By each measure, and depending on damaging agent, 2 and 5 mg/mL sucralfate provided partial (50% of untreated control) to near-complete (90% of untreated control) cytoprotection, respectively. Aluminum hydroxide also provided partial (55% of untreated control) to near-complete (more than 90% of untreated control) cytoprotection at 2 and 5 mg/mL, respectively, for the pH 3.5 medium-induced damage. Over a concentration range of 0.05 to 5 mg/mL, the potassium salt of sucrose octasulfate, KSOS, stimulated cell growth up to 40–60% over untreated controls but had little or no cytoprotective action in the presence of either 3.5 m*M* indomethacin or pH 3.5 medium. Overall results suggested that sucralfate may have at least two roles in influencing gastric epithelial cell function, cytoprotection and stimulation of cell growth *in vitro*. These observations serve as a basis for further study of *in vitro* models in evaluating the cytoprotective activity of antiulcer agents and their respective mechanisms of action.

KEY WORDS: gastric epithelium; cell culture; cytoprotection; sucralfate.

INTRODUCTION

Several primary gastric cell culture systems are currently under investigation as cell models for studying stomach physiology and for the evaluation of antiulcer or antiinflammatory agents. These models have been derived from rat gastric mucosa (1,2), rabbit parietal cells (3), canine gastric mucous cells (4), and human gastric mucosa (5). Cultured gastric epithelial cells offer a promising tool for evaluating the cytoprotective effect of antiulcer agents in the absence of gastric and systemic factors and, thus, may provide a suitable model for studying the mechanisms by which these drugs influence gastrointestinal epithelial cell functions.

Sucralfate, an aluminum hydroxide complex of sucrose octasulfate, has been shown in clinical studies to be effective in the treatment of duodenal and gastric ulcers (6,7) and in the prevention of acute gastric mucosal lesions induced by experimental injury (5,8–11). The proposed mechanisms include direct binding to mucosa (12,13), inactivation of pepsin, binding of bile salts (14,15), and decreased penetration of luminal irritants into the mucosa (16). The diverse chemical properties of sucralfate, however, have resulted in multiple effects and the precise mechanisms of action of the drug remain unclear.

In our study, primary cultured rat gastric mucosal cells were investigated as an *in vitro* model to measure the cytoprotective activities of antiulcer agents including sucralfate. The purposes of this study were (i) to evaluate the effect of sucralfate, potassium sucrose octasulfate (KSOS), and aluminum hydroxide on the damage brought about by either acid or indomethacin treatment *in vitro*, (ii) to compare the cytoprotective effect of different formulations of sucralfate, including Carafate (i.e., the commercial formulation of sucralfate) and acid-solubilized sucralfate, and (iii) to establish the cultured rat gastric cell system as a suitable *in vitro* model for preliminary evaluation of antiulcer agents.

MATERIALS AND METHODS

Chemicals

Sucralfate (aluminum sucrose octasulfate), Carafate (commercial formulation of sucralfate), KSOS (potassium sucrose octasulfate heptahydrate), aluminum hydroxide [$Al(OH)_3$], and 5-aminosalicylic acid were obtained from Marion Merrell Dow Laboratories (Kansas City, MO). 3-(4,5-Dimethyl-2-thiazoyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) was purchased from Eastman Kodak (Rochester, NY). Neutral red, HCl, isopropanol, acetic acid, and ethanol were obtained from Fisher (St. Louis, MO). All other chemicals and biochemicals were supplied by Sigma (St. Louis, MO).

Cell Culture

Gastric mucosal cells from 1- to 2-week-old Sprague–Dawley rats were isolated as described by Terano *et al.* (17). In brief, the gastric mucosal surface was washed thoroughly with sterile cotton and Hank's balanced salt solution (HBSS) and then rinsed with HBSS before being minced into approx. 1-mm^3 pieces. The minced tissues were incubated in HBSS containing 0.1% collagenase and 0.05% hyaluronidase at 37°C in a shaking water bath for 60 min, then pipetted several times and filtered through a sterile nylon mesh (Nytex, Tetko). The filtrate was washed twice with HBSS by centrifugation (200*g* for 5 min) and resuspended in Coon's modified Ham's F-12 culture medium containing 100 μg/mL penicillin, 100 μg/mL streptomycin, 50 μg/mL gentamycin, 15 m*M* HEPES, 2 μg/mL fibronectin, and 10% fetal bovine serum. Cells were seeded at a density of $1.5–2 \times 10^5$ or $1–2 \times 10^4$ cells/cm^2 directly onto either 96-well plates or 6-well plates, respectively, and maintained in a Steri-Cult incubator at 37°C in a humidified atmosphere with 5% CO_2. The medium was changed daily. The confluent monolayers were formed after 4–5 days in 96-well plates.

Phase-Contrast Microscopy

Collagenase-dissociated gastric mucosal cells were ob-

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served under the phase-contrast microscope to confirm general epithelial cell morphology as described by Terano *et al.* (17). Histochemical identification of the gastric cells was performed with the periodic acid–Schiff (PAS) reaction which produces granules in fundic mucus-producing cells (Sigma Kit No. 395-B, Sigma Chemical Co.).

Protocols for Drug Preparation and Use in Growth and Cytoprotective Assays

To measure the effects of individual drugs on the viability of gastric mucosal cells, cells were incubated with 0.02–5 mg/mL drugs in culture medium for either 2 or 48 hr for the MTT assay or up to 8 days for the colony-forming efficiency assay as described below.

To measure the cytotoxicity of indomethacin and acidified medium to gastric mucosal cells, cells were incubated in either serum-free medium containing 0.5–10 m*M* indomethacin for 1 hr or in pH 3.5 medium for 10–30 min. Indomethacin was prepared as a 50 m*M* solution in $NaCO_3$ (0.2 *M*), then diluted in serum-free culture medium to the desired concentrations; the pH was adjusted to 7.4 with 1 *N* HCl before experiment.

To study the effects of different sucralfate formulations (Carafate, sucralfate suspension and acid-solubilized sucralfate), aluminum hydroxide [$Al(OH)_3$], and KSOS on indomethacin-induced damage, cells were incubated in culture medium containing these drugs for 1 hr. The drug suspensions were then aspirated away, followed by another hour of treatment with 3.5 m*M* indomethacin. Alternatively, cells were treated with drugs and 3.5 m*M* indomethacin concurrently for 1 hr. Acid-solubilized sucralfate was prepared immediately prior to each experiment by dissolving the drug in 1 *N* HCl first, then diluting it in culture medium before adjusting it back to pH 7.4. This treatment was made to simulate the conditions the drug encounters in the stomach.

The effects of sucralfate, $Al(OH)_3$, KSOS, and 5-aminosalicylic acid on acid-induced damage were investigated by incubating the cells with the agents for 2 hr and then exposing them to pH 3.5 medium for 10 to 30 min.

MTT Colorimetric Assay

Cells in 96-well plates were treated as above with the different drugs and then incubated in 100 μL of culture medium containing 10 μL of an MTT stock solution (5 mg/mL in PBS) for 4 hr at 37°C according to the method of Mosmann (18). Following the incubation, 100 μL acid-isopropanol (0.04 *N* HCl in isopropanol) was added to wells and incubated overnight at room temperature. The color changes were recorded at 540 nm on a microplate reader (Cambridge Series 700, Cambridge Technology, Inc., Watertown, MA). To exclude the disturbance of precipitates in some samples, samples were centrifuged and only the supernatants were read. For each experiment, a standard curve was generated by measuring the relationship of absorbance to a series of viable cell numbers.

Neutral Red Uptake Assay

Neutral red was prepared as a 1% stock solution in distilled water and diluted to 0.035% in HBSS immediately before each experiment. The cells were treated with the drugs and then stained with 0.1 mL of 0.035% neutral red for 30 min (19). The stain was discarded and the cells were washed twice in HBSS before the addition of 200 μL/well of acidified alcohol solution (50%, v/v, ethanol/water containing 0.5%, v/v, acetic acid). After a 2-hr incubation at room temperature, the color changes were measured on a microplate reader at 540 nm as described for the MTT assay.

Colony-Forming Efficiency Assay

The cells which were seeded into six-well plates were incubated in culture medium containing 0.02 to 5 mg/mL of individual drugs for 8 days. Cells were then fixed in 10% formalin and stained with 1% aqueous crystal violet as described by Sundqvist *et al.* (20). Colonies formed on each well were counted (crystal violet stains cell nuclei) and compared with those formed on drug-free wells.

Statistical Analysis

Data are expressed as mean ± standard error. A one-way analysis of variance (ANOVA) followed by Scheffe's post hoc test (ABSTAT, Anderson Bell software) was used to test the significance between control and drug-treated samples. Differences were considered significant at $P < 0.05$.

RESULTS

Phase-Contrast Microscopy

Collagenase-dissociated gastric mucosal cells were slow-growing and morphologically epithelial-like (Fig. 1). Histochemical identification of the gastric cells as fundic mucus-producing (versus parietal acid-producing cells) was confirmed by periodic acid-Schiff (PAS) reaction.

Effects of Sucralfate, Indomethacin, and Acid on Cell Viability

Exposure to either sucralfate suspension or Carafate at

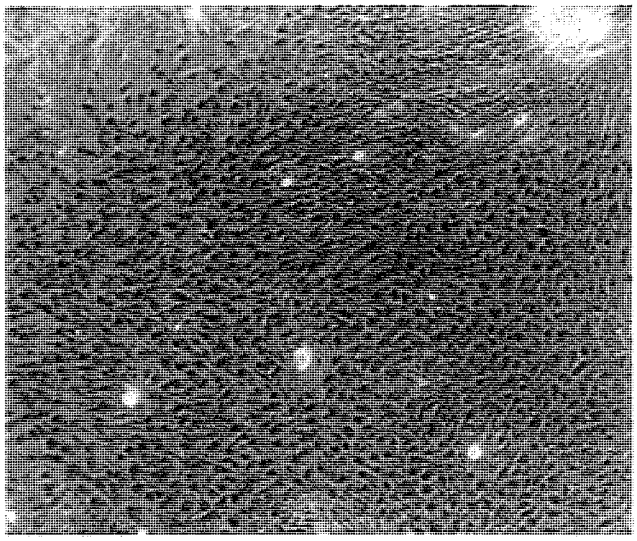

Fig. 1. Phase-contrast photomicrograph of a 4-day-old primary culture of rat gastric cells. ×100; reduced 17% for reproduction.

Table I. Effect of Sucralfate and Carafate on the Viability of Cultured Gastric Mucosal Cells[a]

Drug	Concentration (mg/mL)	% living cells
Sucralfate	0.5	103 ± 2.6
	2.0	95 ± 4.2
	5.0	92 ± 1.8
Carafate	0.2	98 ± 2.2
	0.5	97 ± 2.3
	2.0	102 ± 2.6
	5.0	99 ± 1.4

[a] Cells were incubated in medium containing drugs for 2 hr, and then measured by MTT assay. Values are means ± SE for six cultures.

0.02–5 mg/mL did not cause a significant loss of cell viability (Table I). Other agents used in this study also did not alter cell viability (data not shown). In contrast, indomethacin treatment resulted in a dose-dependent cytotoxicity at concentrations from 0.5 to 10 m*M* (Fig. 2a). The concentration producing about a 50% decrease in viability was estimated as 3–3.5 m*M*. Acidifying the medium (pH 3.5) reduced cell viability in a time-dependent manner (<10% at 30 min; Fig. 2b).

Effect of Selected Agents on Cell Growth

Cells were exposed to KSOS, sucralfate suspension, or Carafate for 48 hr. Only KSOS, the potassium salt of sucrose octasulfate that is soluble at neutral pH, was demonstrated to be effective in stimulating the growth of rat gastric cells in tissue culture as determined by MTT and neutral red assays (Fig. 3). When cells were incubated in 0.2 mL medium containing 0.2 mg/mL KSOS for 48 hr, the cell numbers were increased 40% (about 1.2×10^5 cells/well) as measured by MTT assay, which was significantly higher ($P < 0.05$) than for drug-free controls (about 8.5×10^4 cells/well). Similar results were observed in the neutral red assay, with a maximal increase in cell numbers of 33% at 0.5 mg/mL KSOS. A measure of the colony-forming efficiency of rat gastric cells was qualitatively consistent with results of the MTT and neutral red assays. Compared with untreated controls in Fig. 4, KSOS stimulated colony formation by 150% at 0.02 mg/mL ($P < 0.05$) and 175% at 0.05 mg/mL ($P < 0.01$) after 8 days in culture.

To exclude the possibility of growth stimulation induced by potassium and sucrose, the primary components of KSOS, potassium chloride and sucrose, were checked for stimulatory effects on cell growth. No significant ($P > 0.05$) growth stimulation was observed in any of the three assays over concentration ranges of either 0.01–1 mg/mL for potassium chloride or 0.016–1.6 mg/mL for sucrose (data not shown).

Protective Effects of Sucralfate and Other Agents on Indomethacin-Induced Damage

Pretreatment with any of the sucralfate formulations reduced indomethacin-induced cell damage. As shown in Fig. 5, when cells were pretreated with 5 mg/mL of the drugs prior to exposure to 3.5 m*M* indomethacin, Carafate, and sucralfate suspensions provided for survival of cells of up to 82% ($P < 0.001$) and 78% ($P < 0.001$) of the control cell number, respectively. Acid-solubilized sucralfate provided significant protection beginning at lower concentrations ($P < 0.05$ at 0.5 mg/mL; $P < 0.001$ at 2 mg/mL). However, acid-solubilized sucralfate at 5 mg/mL apparently was not cytoprotective. There was the possibility that the higher ion concentrations needed to adjust the pH of a 5 mg/mL sucralfate

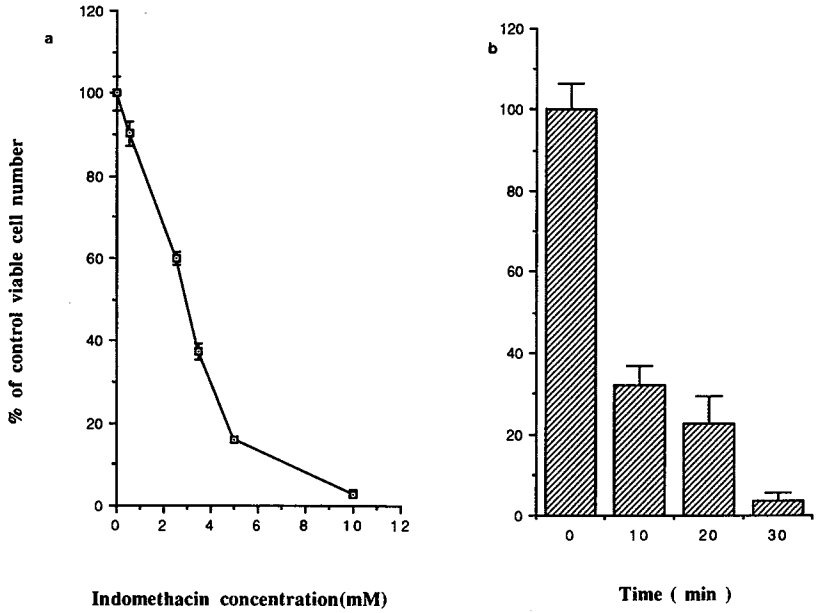

Fig. 2. (a) Dose dependence of indomethacin-induced cell damage. Cultured gastric cells were exposed to indomethacin solutions for 1 hr before performing MTT assay. Values are means ± SE for six cultures. (b) Time dependence of acid medium-induced damage. Cultured gastric cells were incubated in pH 3.5 medium for 10, 20, and 30 min before performing MTT assay. Values are means ± SE for four cultures.

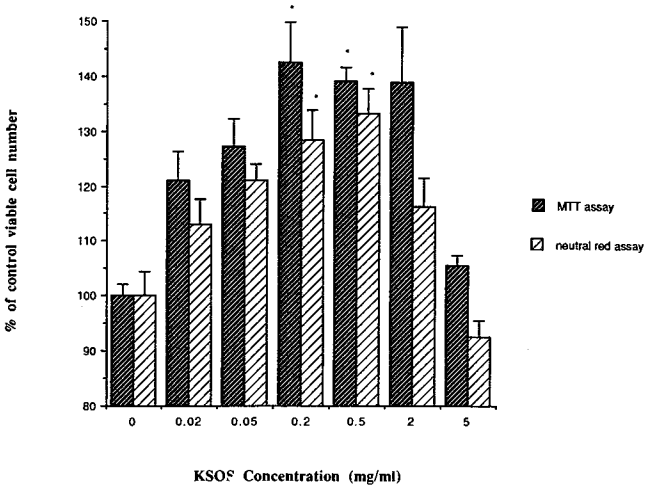

Fig. 3. The stimulatory effect of potassium sucrose octasulfate (KSOS) on the growth of gastric mucosal cells. Cell growth was measured by MTT and neutral red assays after 48 hr in culture in KSOS solutions. Values are means ± SE for six cultures. (*) Significant differences compared with control (no exposure to KSOS) values: $P < 0.05$.

suspension may have resulted in an osmotic shock for the cells, thus dropping cell viability to essentially zero.

Treatment of cultured cells with drugs and 3.5 m*M* indomethacin concurrently protected the cells against the insult more effectively (Fig. 6). At 5 mg/mL, Carafate and acid-solubilized sucralfate virtually completely blocked indomethacin-induced cytotoxicity ($P < 0.001$). A dose-dependent increase in the survival rate of cells brought about by KSOS was also typical, with an 80% survival rate at a 5 mg/mL concentration. In these experiments, aluminum hydroxide was not capable of providing a protective effect.

Effects of Sucralfate and Other Agents on Acid-Induced Damage

As illustrated in Fig. 7, sucralfate suspension reduced the loss of cell viability produced by pH 3.5 medium in a dose-dependent manner. In the drug-free wells, less than 10% of the cells survived, while more than 60% of cells survived a 30-min exposure to acidified medium following treatment with 5 mg/mL sucralfate. In these studies, controls were not exposed to either low-pH conditions or drugs. The number of surviving cells was approximately 58% of controls at 2 mg/mL and 38% of controls at 0.5 mg/mL, respectively. The protective effect was greater, 80%, when the exposure time to the acidified medium was decreased to 10 min.

Among three other drugs tested against pH 3.5 medium challenge, only aluminum hydroxide significantly blocked the acid-induced cell damage (Fig. 8), increasing the surviving cells to about 80% of control at 30 min. The protective effect of Carafate paralleled that of sucralfate as described earlier. KSOS and 5-aminosalicylic acid failed to prevent acid-induced damage in these experiments over a concentration range of 0.05 to 5 mg/mL.

DISCUSSION

Terano *et al.* previously characterized primary cultures of fetal rat fundic mucosal epithelial cells as a valuable model to examine cellular functions of gastric mucosa. Collagenase-dissociated gastric mucosal cells are morphologically epithelial-like (>90%), do not exhibit acid-secreting properties of parietal cells, and do not require pentagastrin for growth (17). This study examines the use of the primary cultures as a model to evaluate the formulations and mechanisms of the cytoprotective action of selected antiulcer agents. Results of the present study demonstrated that sucralfate can protect cultured gastric mucosal cells against loss of cell viability produced by either acidified medium or indomethacin. Indomethacin-induced damage, but not acidified medium-induced damage, was also partially prevented by KSOS, the potassium salt of sucralfate. Thus, there may be more than one mechanism responsible for the cytoprotective effects of sucralfate.

Nagshima (21) indicated that sucralfate possesses an acid-buffering capacity and reacts slowly with acid due to its basicity. Aluminum, a basic metal with strong positive charge, could bring basic character to these drugs. Orlando *et al.* also reported that sucralfate could protect against acid injury in the esophagus by pH buffering through its aluminum hydroxide content (22). In our study, aluminum hydroxide, as well as sucralfate, exerted a dose-dependent protective effect against acid damage, supporting these earlier observations.

Acid plays another important role in the special effects of sucralfate in the gastrointestinal tract (12,21,23). On encountering gastric acid in stomach, sucralfate becomes a highly condensed, viscous substance which adheres tightly to the ulcerated mucosa and acts as an effective barrier to the penetration of acid, pepsin, and bile salts. Konturek *et al.* (23) reported that sucralfate acidified to pH 2.0 showed sig-

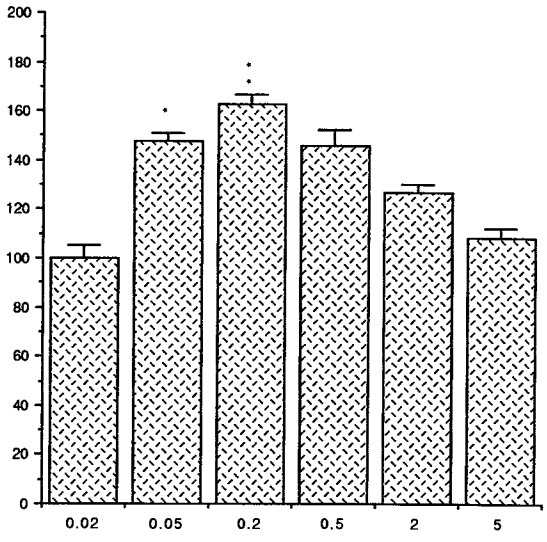

Fig. 4. The stimulatory effect of potassium sucrose octasulfate (KSOS) on the colony-forming efficiency of gastric mucosal cells. Cells were incubated in medium containing KSOS, and colonies were counted after 8 days in culture. Values are means ± SE for four cultures. Significant differences compared with the control (no exposure to KSOS) values: (*) $P < 0.05$; (**) $P < 0.01$.

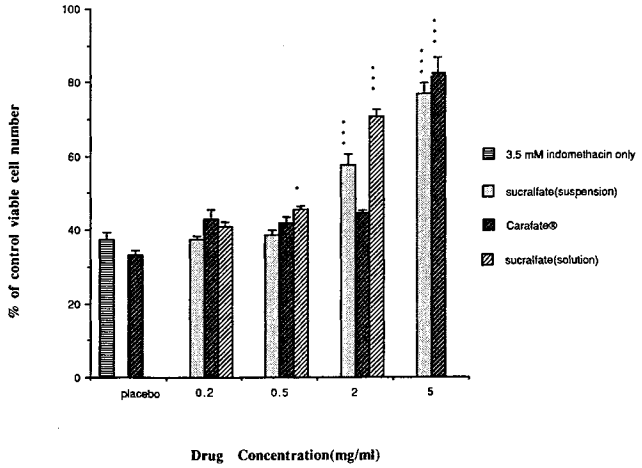

Fig. 5. Cytoprotective effect of Carafate, sucralfate suspension, and acid-solubilized sucralfate against indomethacin-induced damage. Cells were treated with drugs for 1 hr before being exposed to 3.5 m*M* indomethacin for 30 min and then performing an MTT assay. Values are means ± SE for six cultures. The placebo contains supplemental reagents in Carafate but not the active ingredient, sucralfate. Significant differences compared with indomethacin treatment in the absence of drugs: (*) $P < 0.05$; (**) $P < 0.001$.

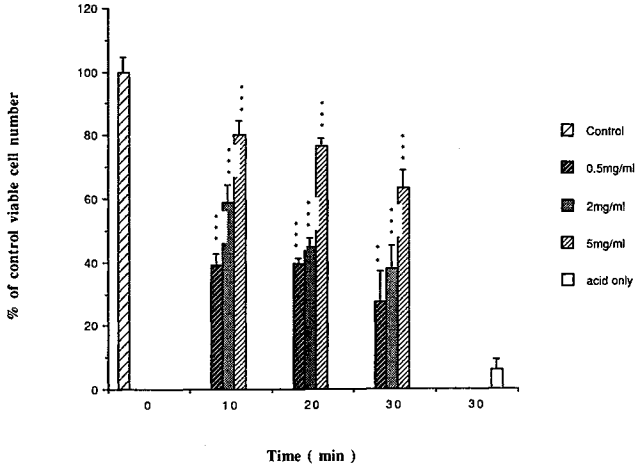

Fig. 7. Protective effect of sucralfate on gastric mucosal cells against acidified medium over various times. The cells were incubated with sucralfate suspensions for 2 hr and then treated with pH 3.5 medium for 10, 20, and 30 min before performing the MTT assay. Values are means ± SE for six cultures. "Control" means no exposure to acidified medium or drugs. Significant differences compared with acid medium treatment in the absence of drugs: (**) $P < 0.01$; (**) $P < 0.001$.

nificant protective activity against ethanol, acidified aspirin, taurochlate, or stress *in vivo*. Compared to sucralfate suspension, the sucralfate solution (5 mg/mL) was significantly ($P < 0.05$) more protective against the cytotoxic effect of indomethacin in this study (Fig. 6). The number of surviving cells under the protection of acid-solubilized sucralfate was almost doubled compared to the sucralfate suspension-treated cells. This result seems to conform with the importance of intragastric pH in sucralfate-induced gastroprotection. The consistency of our *in vitro* cell model with the animal model also provides evidence that cultured gastric cells may serve as a suitable model for studying these antiulcer drugs.

Indomethacin is an inhibitor of the generation of prostaglandins, which are considered important in enhancing mucus and bicarbonate secretion. Romano *et al.* reported that 10^{-6} to 10^{-4} *M* indomethacin inhibited the secretion of prostaglandin E_2 significantly in a human gastric epithelial cell line (24). Sucralfate effectively prevented the indomethacin-

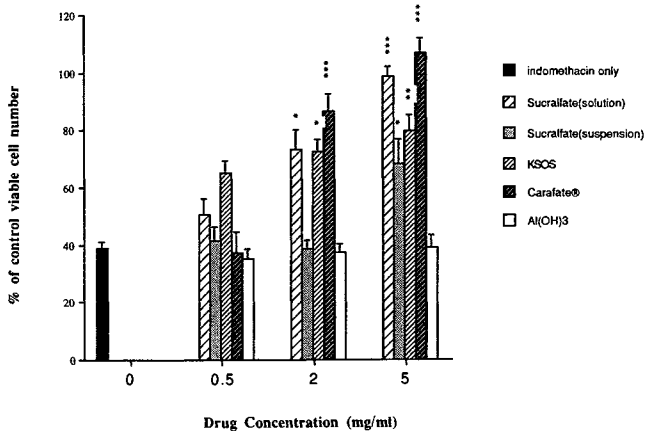

Fig. 6. Effect of Carafate, sucralfate suspension, acid-solubilized sucralfate, potassium sucrose octasulfate (KSOS), and aluminum hydroxide on indomethacin-induced damage when gastric mucosal cells were incubated with these drugs and 3.5 m*M* indomethacin concurrently for 1 hr before performing the MTT assay. Values are means ± SE for six cultures. Significant differences compared with indomethacin treatment in the absence of drugs. (*) $P < 0.05$; (**) $P < 0.01$; (**) $P < 0.001$.

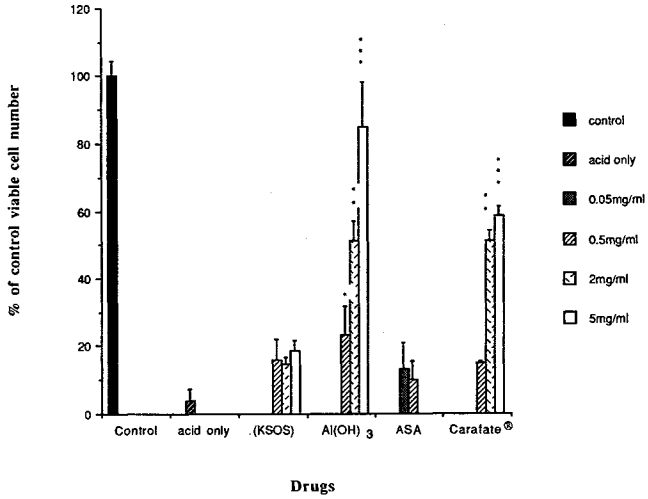

Fig. 8. Effect of Carafate, potassium sucrose octasulfate (KSOS), aluminum hydroxide, and 5-aminosalicylic acid on gastric mucosal cells against the loss of cell viability caused by acidified medium. The cells were incubated with drugs for 2 hr and then with pH 3.5 medium for 30 min before performing the MTT assay. Values are means ± SE for six cultures. Significant differences compared with acid medium treatment in the absence of drugs: (*) $P < 0.05$; (**) $P < 0.01$; (**) $P < 0.001$.

induced injury dose dependently in this study, regardless of whether it was applied to the cells concurrently with or prior to indomethacin treatment. The 3.5 m*M* concentration of indomethacin used in our study would be more than sufficient to suppress any prostaglandin secretion. Thus, it is less likely that the cytoprotective role of sucralfate could be mediated through regulation of prostaglandin effects. This observation supports the observations of Romano *et al.* (1) and Konturek *et al.* (23).

KSOS has not been used as an antiulcer drug and its protective effect was apparently lower than that of sucralfate against an indomethacin insult. Effect of an acid insult on the cells was not prevented by KSOS. However, KSOS did stimulate gastric cell growth and proliferation as demonstrated by all of the three assays in our study. Neither potassium chloride nor sucrose, primary components of KSOS, exhibited similar properties (data not shown). This important character of KSOS suggests the possibility that it may promote cell growth and recovery of epithelia, a potentially important action for sucralfate on the healing rate of ulcer wound *in vivo*. Sucralfate has been shown to elevate the levels of an epithelial cell mitogen, basic fibroblast growth factor (bFGF), in the ulcer bed without affecting gastric acid secretion (25). Sucralfate and KSOS, the salts of sucrose octasulfate, have dramatically different solubility characteristics. Perhaps sucralfate, unlike KSOS, was not able to promote the cell growth due to its poor solubility in culture medium. On the other hand, the insolubility of sucralfate may play an important role in coating ulcer bed and binding bFGF, thus protecting the wound and mitogen from the acidic environment (25).

In conclusion, sucralfate provided significant protection for cultured rat gastric cells against acid and indomethacin-induced damage in a concentration-dependent manner. Carafate and acid-solubilized sucralfate were apparently more effective than a simple sucralfate suspension in culture medium. Aluminum hydroxide and KSOS were cytoprotective but only to acid and indomethacin-induced damage, respectively. In addition, KSOS stimulated gastric epithelial cell growth and proliferation. The present study suggests that primary rat gastric epithelial cells can serve as a suitable *in vitro* model for preliminary evaluation of the cytoprotective actions of antiulcer agents.

ACKNOWLEDGMENT

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REFERENCES

1. M. Romano, M. Razandi, and K. J. Ivey. Effect of sucralfate and its components on taurocholate-induced damage to rat gastric mucosal cells in tissue culture. *Digest. Dis. Sci.* 35:467–476 (1990).
2. S. Ota, M. Razandi, S. Sekhon, A. Terana, H. Hiraishi, and K. J. Ivey. Cytoprotective effect of acetaminophen against taurocholate-induced damage to rat gastric monolayer cultures. *Digest. Dis. Sci.* 33:938–944 (1988).
3. R. A. Levine, J. Nandi, and R. L. King. Nonsalicylate nonsteroidal antiinflammatory drugs augment prestimulated acid secretion in rabbit parietal cells. *Gastroenterology* 101:756–765 (1991).
4. J. M. Scheiman, E. R. Kraus, L. A. Bonnville, P. A. Weinhold, and C. R. Boland. Synthesis and prostaglandin E_2-induced secretion of surfactant phospholipid by isolated gastric mucous cells. *Gastroenterology* 100:1232–1240 (1991).
5. M. M. Cohen, R. Bowdler, P. Gervais, G. P. Morris, and H. Wang. Sucralfate of human gastric mucosa against acute ethanol injury. *Gastroenterology* 96:292–298 (1989).
6. F. Martin, A. Farley, M. Gagnon, and D. Bensemana. Comparison of the healing capacities of sucralfate and cimetidine in the short-term treatment of duodenal ulcer: A double-blind randomized trial. *Gastroenterology* 82:401–405 (1982).
7. S. K. Lam, W. Y. Lau, and C. L. Lai. Efficacy of sucralfate in corpus, prepyloric and duodenal ulcer-associated gastric ulcers. *Am. J. Med.* 79 (Suppl. 2C):24–31 (1985).
8. S. Okabe, K. Takeuchi, H. Kunimi, M. Kanno, and M. Kawashima. Effects of an antiulcer drug, sucralfate (a basic aluminum salt of sulfated disaccharide), on experimental gastric lesions and gastric secretion in rats. *Digest. Dis. Sci.* 28:1034–1042 (1983).
9. A. Tarnawaki, D. Hollander, H. Gergely, and J. Stachura. Comparison of antacid sucralfate, cimetidine and ranitidine in protection of gastric mucosa against ethanol injury. *Am. J. Med.* 79:19–23 (1985).
10. D. Hollander, A. Tarnawski, W. Krause, and H. Gergely. Protective effect of sucralfate against alcohol-induced gastric mucosa injury in the rat. Macroscopic, histologic, ultrastructural and functional time sequence analysis. *Gastroenterology* 88:366–374 (1985).
11. L. D. Bighley and D. H. Giesing. Mechanism of action studies of sucralfate. In W. F. Caspary (ed.), *Duodenal Ulcer, Gastric Ulcer: Sucralfate. A New Therapeutic Concept,* Urban & Schwarzenberg, Baltimore, 1981, pp. 3–12.
12. R. Nagashima. Mechanism of action of sucralfate. *J. Clin. Gastroenterol.* 3 (Suppl. 2):117–127 (1981).
13. D. H. Giesing, L. D. Bighley, and R. L. Fles. Effect of food and antacid on binding of sucralfate to normal and ulcerated gastric and duodenal mucosa in rats. *J. Clin. Gastroenterol.* 3 (Suppl. 2):111–116 (1981).
14. I. M. Samloff, and C. O'Dell. Inhibition of peptic activity by sucralfate. *Am. J. Med.* 79 (Suppl. 2C):15–18 (1985).
15. D. Y. Granam, J. W. Sackman, D. H. Giesing, and D. J. Runser. In vitro absorption of bile salts and aspirin to sucralfate. *Digest. Dis. Sci.* 29:402–406 (1984).
16. B. L. Slomiany, W. Laszewicz, and A. Slomiany. Effect of sucralfate on the viscosity of gastric mucus and the permeability to hydrogen ion. *Digestion* 33:146–151 (1986).
17. A. Terano, K. J. Ivey, J. Stachura, S. Sekhon, H. Hosojima, W. N. Mckenzie, Jr., W. J. Krause, and J. H. Wyche. Cell culture of rat gastric fundic mucosa. *Gastroenterology* 83:1280–1291 (1982).
18. T. Mosmann. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immunol. Methods* 65:55–63 (1983).
19. C. R. Parish and A. Müllbacher. Automated colorimetric assay for T cell cytotoxicity. *J. Immunol. Methods* 58:225–237 (1983).
20. K. Sundqvist, Y. Liu, J. Nair, H. Bartsch, K. Arvidson, and C. Grafstrom. Cytotoxic and genotoxic effects of areca nut-related compounds in cultured human buccal epithelial cells. *Cancer Res.* 49:5294–5298 (1989).
21. R. Nagshima. Development and characteristics of sucralfate. *J. Clin. Gastroenterol.* 3 (Suppl. 2):103–110 (1981).
22. R. C. Orlando, N. A. Turjman, N. A. Tobey, V. J. Schreiner, and D. W. Powell. Mucosal protection by sucralfate and its components in acid-exposed rabbit esophagus. *Gastroenterology* 93(2):352–361 (1987).
23. S. J. Konturek, T. Brzozowski, D. Drozdowicz, J. Garlicki, J. Majka, and J. Pytko-Polonczyk. Role of acid milieu in the gastroprotective and ulcer healing activity of sucralfate. 6th International Sucralfate Symposium, Gold Coast, Queensland, Australia, Aug. 19–21 (1990).
24. M. Romano, M. Razandi, S. Sekhon, W. J. Krause, and K. J. Ivey. Human cell line for study of damage to gastric epithelial cells in vitro. *J. Lab. Clin. Med.* 111:430–440 (1988).
25. J. Folkman, S. Szabo, M. Stouroff, P. McNeil, W. Li, and Y. Shing. Duodenal ulcer. *Ann. Surg.* 214:414–427 (1991).