Effects of Sucralfate and Its Components on Acid- and Pepsin-Induced Damage to Rat Gastric Epithelial Cells

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ABSTRACT—We have established models of cell damage induced by acid and pepsin using rat gastric epithelial cells (RGM1). In the present study, the effects of aluminum hydroxide [$Al(OH)_3$] and potassium sucrose octasulfate (KSOS), which are components of sucralfate, and sucralfate on cell damage and peptic activity of pepsin were examined. Pretreatment of cells with sucralfate (0.1-3 mg/ml) or $Al(OH)_3$ (0.1-1 mg/ml) for 2 hr prevented both acid- (pH 4.0) and pepsin- (pH 4.5) induced cell damage. However, KSOS (0.1-1 mg/ml) did not show any effects on two different types of cell damage. The peptic activity of pepsin at pH 4.5 was about 10% of that at pH 2.0. Sucralfate and KSOS slightly inhibited peptic activity at pH 4.5. $Al(OH)_3$ inhibited peptic activity by approximately 50%; however, no concentration-dependent pattern was observed. Pepstatin (0.003-0.1 mg/ml), a specific inhibitor of pepsin, inhibited the peptic activity in a concentration-dependent manner. Here, we confirmed that sucralfate and $Al(OH)_3$ have cytoprotective effects against acid- and pepsin-induced cell damage. The mechanism behind the cytoprotective effects of sucralfate seems to relate to adhesion of the cell surface and neutralization of hydrogen ion by aluminum that prevents the penetration of hydrogen ions into the cells.

Keywords: Sucralfate, Aluminum hydroxide, Potassium sucrose octasulfate, Rat gastric epithelial cell (RGM1), Cell damage

Sucralfate is known to inhibit cell damage caused by several necrotizing agents both in vivo (1-3) and in vitro (4, 5). Several mechanisms regarding the protective effects of sucralfate are proposed such as adhesion to gastric epithelial cell surface (5), production of prostaglandins (4), inhibition of peptic activity (6), stabilization of the growth factors in the stomach (7, 8), and neutralization of gastric acid (9). We previously have established new cell damage models caused by acid and pepsin (10), which can detect the effects of anti-ulcer drugs and growth factors (10, 11). In the present study, we examined the effects of aluminum hydroxide [$Al(OH)_3$] and potassium sucrose octasulfate (KSOS) (components of sucralfate) and sucralfate on acid- and pepsin-induced cell damage and on peptic activity to clarify the mechanisms of the protective effects of sucralfate.

MATERIALS AND METHODS

Cell culture

Rat gastric epithelial cells (RGM1) passaged in Ham's F12/Dulbecco's-MEM supplemented with 20% fetal bovine serum (FBS) were cultured at 1×10^4 cells/well in 96-well, flat-bottomed plates (Corning Costar, Corning, NY, USA). The cells were maintained at 37℃ under 5% CO_2 in air for 24 hr. Cells that reached confluency were used for the experiments.

Drug treatment

Sucralfate and $Al(OH)_3$ were suspended with Dulbecco's phosphate-buffered saline containing 0.9 mM $CaCl_2$ and 0.49 mM $MgCl_2$ [PBS(+)] supplemented with 1% fetal bovine serum, 10 mmol/l glucose (modified PBS). Potassium sucrose octasulfate was dissolved with modified PBS. The cells were incubated with these drugs at 37℃ for 2 hr.

Induction of cell damage

The test solution containing confluent cells was aspirated and the cells were washed with modified PBS three times. The cells were then placed in pH 4.0 PBS(+) or 0.75 mg/ml pepsin at pH 4.5 PBS(+). Thirty minutes

later, the cells were washed twice with modified PBS and were then subjected to the viability assay.

Estimation of cell viability

A colorimetric assay was performed with MTT [3-(4,5-dimethyl-2 thiazoyl)-2,5-diphenyl-2*H*-tetrazolium bromide] in 50 μl of culture medium. Briefly, 10 μl of the MTT solution (5 mg/ml in PBS) was added to the culture medium, and then the cells were incubated at 37℃ under 5% CO_2 in air for 4 hr. Subsequently, 150 μl of 0.04 mol/l HCl in isopropanol was added, and the mixture was held for 18 hr at room temperature. The color change was measured with a microplate reader (Molecular Devices, Menlo Park, CA, USA) at 595 and 650 nm (12).

Peptic activity

The peptic activity of the porcine pepsin was determined in several buffer pH values (phosphate-citrate-borate NaOH buffer), with bovine serum albumin (BSA) as a substrate, for 30 min. The digestive reaction of BSA was then stopped with trichloroacetic acid, followed by neutralization with a Na_2CO_3 solution. Tyrosine and tyrosine residues from BSA were detected by the addition of a Folin reagent (13). The effects of test drugs on the peptic activity were determined by incubation of 0.75 mg/ml of pepsin and 100 mg/ml of BSA in PBS(+) at pH 4.5 for 30 min.

Materials

Sucralfate and KSOS were gifts from Chugai Pharmaceuticals (Tokyo). $Al(OH)_3$ was purchased from Wako Pure Chemicals (Osaka). Pepstatin was a gift from Banyu Pharmaceuticals (Tokyo). MTT and porcine pepsin were purchased from Sigma (St. Louis, MO, USA). BSA fraction V was purchased from Nacalai Tesque (Kyoto).

Statistical analyses

The data are each the mean±S.E. of 4–6 samples. Statistical significance was evaluated by Dunnett's multiple comparison test or Student's *t*-test; a P value of <0.05 was regarded as significant.

RESULTS

Effects of sucralfate on acid- and pepsin-induced cell damage

Pretreatment of the cells with sucralfate for 2 hr did not change the cell viability compared to the control group (data not shown). However, sucralfate inhibited acid-induced cell damage in a concentration-dependent manner. At 0.3 and 1 mg/ml, the percentages of inhibition were 62.0% and 91.0%, respectively. At 3 mg/ml, complete inhibition was observed (Fig. 1A). Similar to the result obtained with acid-induced cell damage, sucralfate also inhibited pepsin- (pH 4.5) induced cell damage in a concentration-dependent manner. The efficacy on pepsin-induced cell damage was weaker than that on acid-induced cell damage to some extent. However, at concentrations $\geqq$0.3 mg/ml, the inhibition was significant as illustrated by the percentage inhibitions of 30.3%, 52.7% and 65.8% for concentrations of 0.3, 1 and 3 mg/ml, respectively (Fig. 1B).

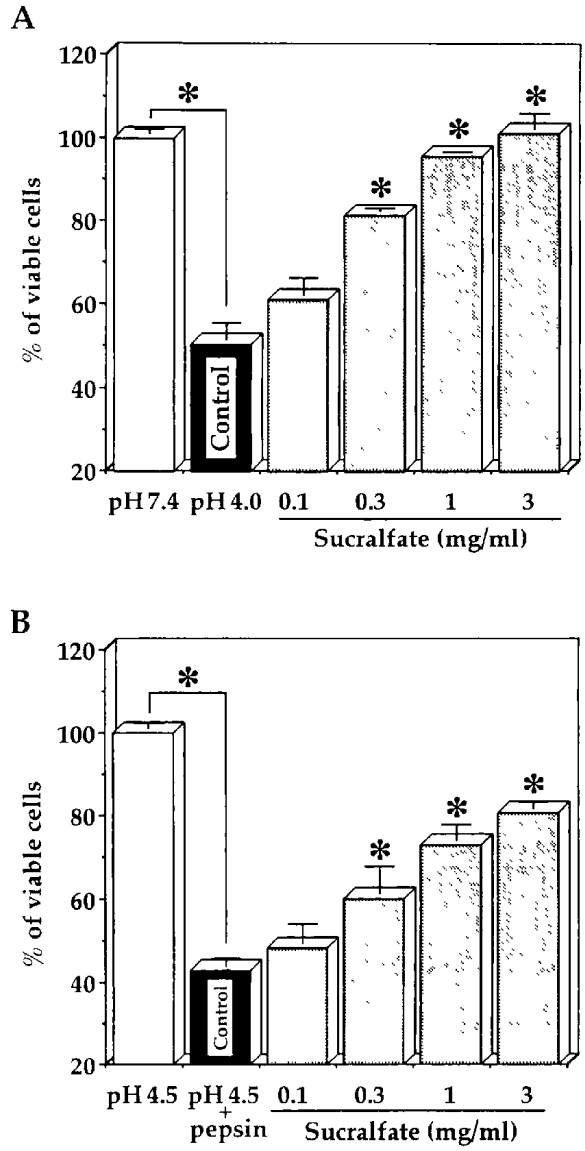

Fig. 1. Effects of sucralfate on acid- (A) or pepsin- (pH 4.5) (B) induced cell damage to RGM1 cells. Cell viability was determined by the MTT assay. Data are means±1 S.E. for 6 cultures. *Significantly different from the control at $P<0.05$.

Effects of $Al(OH)_3$ on acid- and pepsin-induced cell damage

Pretreatment of the cells with $Al(OH)_3$ for 2 hr did not change the cell viability compared with the control group (data not shown). Similar to the results with sucralfate, $Al(OH)_3$ inhibited acid- and pepsin- (pH 4.5) induced cell damage in a concentration-dependent manner. Percentage inhibitions at 0.3 and 1 mg/ml for acid-induced cell damage and pepsin-induced cell damage were 34.6% and 66.9% (Fig. 2A) and 32.6% and 56.9% (Fig. 2B), respectively.

Effect of KSOS on acid- and pepsin-induced cell damage

Pretreatment of the cells with KSOS for 2 hr did not change the cell viability compared to the control group (data not shown). In comparison with the results obtained from the two previously used drugs, KSOS did not inhibit the two different types of cell damage (Fig. 3: A and B).

pH dependency of peptic activity and the effects of test drugs on peptic activity

Peptic activity of pepsin at pH 4.5 was about 10% of the maximum activity at pH 2.0 (Fig. 4A). At pH 4.5,

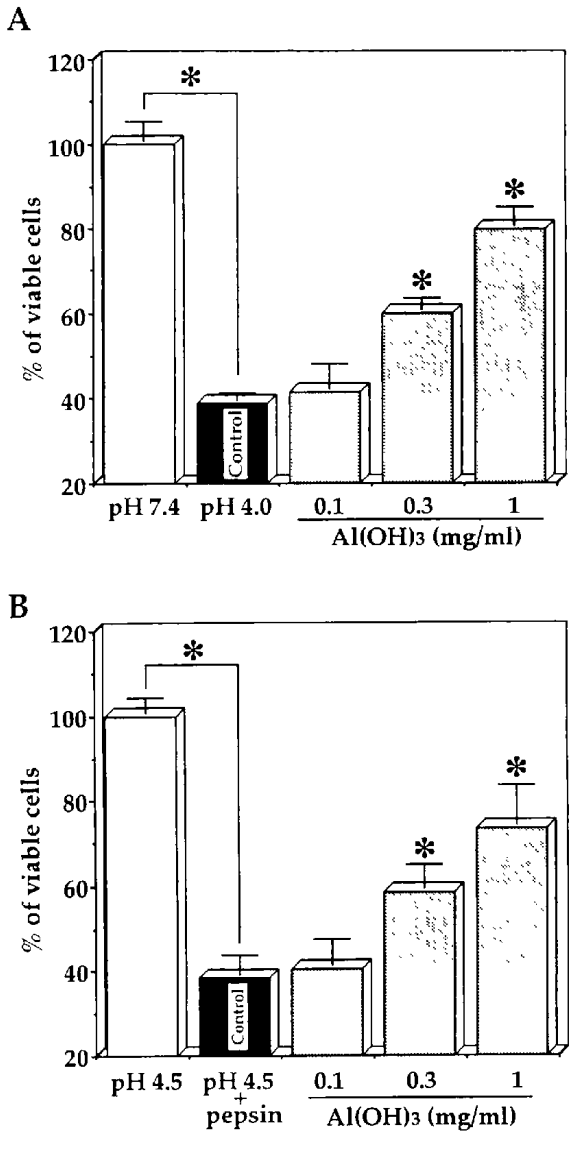

Fig. 2. Effects of $Al(OH)_3$ on acid- (A) or pepsin- (pH 4.5) (B) induced cell damage to RGM1 cells. Cell viability was determined by the MTT assay. Data are means ± 1 S.E. for 6 cultures. *Significantly different from the control at $P<0.05$.

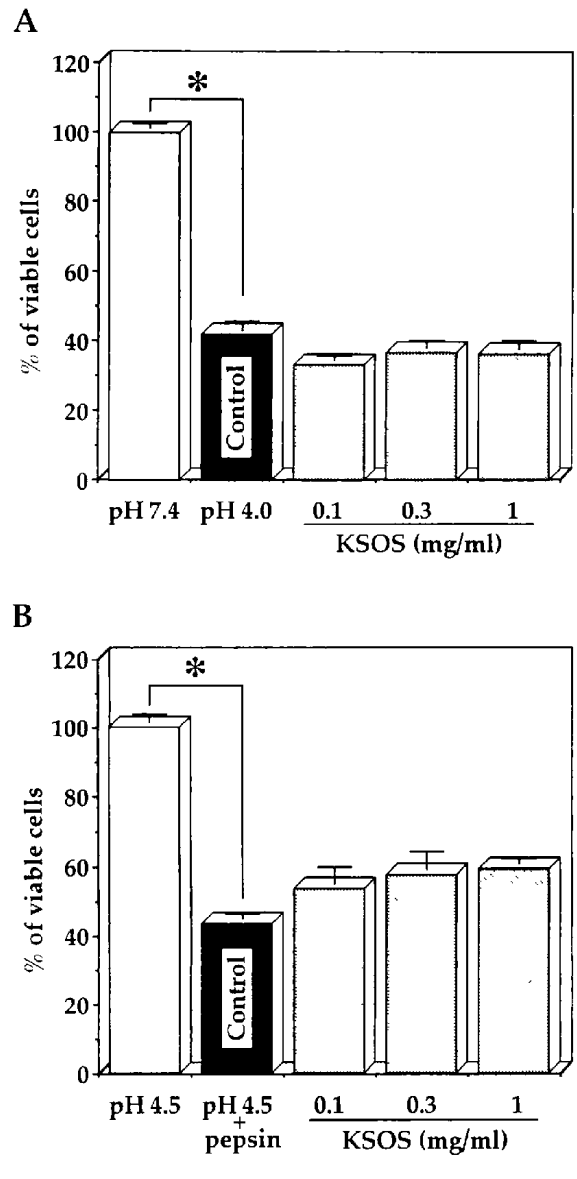

Fig. 3. Effects of potassium sucrose octasulfate (KSOS) on acid- (A) or pepsin- (pH 4.5) (B) induced cell damage to RGM1 cells. Cell viability was determined by the MTT assay. Data are means ± 1 S.E. for 6 cultures. *Significantly different from the control at $P<0.05$.

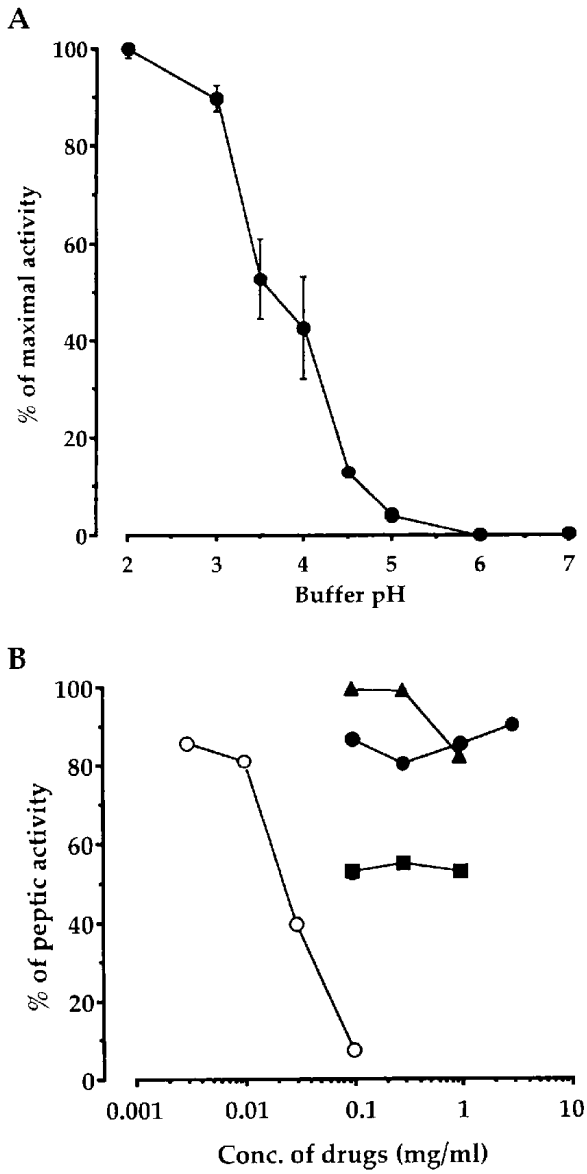

Fig. 4. Peptic activity of pepsin incubated at different pH for 30 min (A), and the effects of sucralfate (●), $Al(OH)_3$ (■), KSOS (▲) and pepstatin (○) on the activity at pH 4.5 (B). Data are means ± 1 S.E. for 4 samples.

pepstatin, a specific inhibitor of pepsin, inhibited peptic activity in a concentration-dependent manner. At 0.1 mg/ml, pepstatin almost completely inhibited the activity. Sucralfate and KSOS slightly inhibited peptic activity. $Al(OH)_3$ also inhibited the peptic activity (about 50%); however, no concentration dependency was observed (Fig. 4B).

DISCUSSION

In the present study, we confirmed that sucralfate and $Al(OH)_3$, but not KSOS, inhibit against both acid- and pepsin-induced cell damage.

Sucralfate inhibits several types of cell damage. It is likely that the mechanisms that underlie the protective effect of sucralfate is mediated by the production of prostaglandins (2, 3), stabilization of growth factors in the stomach (7, 8) and by the inhibition of peptic activity (6). Previously, we confirmed that the protective effect of sucralfate did not affect the pretreatment of cells with 10^{-5} mol/l indomethacin (10). It can be suggested that endogenous prostaglandins are not required for the effects of sucralfate.

Sucralfate is believed to stabilize growth factors in the stomach and accelerate ulcer healing (7). Furthermore, KSOS is a soluble derivative of sucralfate (7), and when growth factors are bound to it, the resulting complex enhances DNA synthesis of the cells (8). The RGM1 cells (14, 15) used in the present study can detect the action of growth factors and cytokines; actually, epidermal growth factors (EGF), basic fibroblast growth factor, transforming growth factor (TGF) alpha, hepatocyte growth factor and interleukin-1 enhance the DNA synthesis (ref. 14 and O. Furukawa et al., unpublished observations). Furthermore, EGF and TGF alpha inhibit acid- and pepsin-induced damage of RGM1 cells (11). However, KSOS did not show any effects against the two types of cell damage. It seems that sucralfate inhibits cell damage without mediating endogenous growth factors.

Sucralfate is known to inhibit the peptic activity of pepsin (6). In the present study, however, sucralfate does not inhibit the peptic activity. The mechanism of the inhibitory effect of sucralfate on peptic activity is thought to be a substrate inhibition (6). In general, the effects of drugs on the peptic activity of pepsin are measured at near pH 2.0, at which pepsin exhibits its maximum activity. In the present study, we used pH 4.5 PBS(+) when peptic activity was measured. The difference between our result and previous reports may be due to the different buffer pH that was used to measure peptic activity. On the other hand, $Al(OH)_3$ inhibits the peptic activity of pepsin by about 50% at 0.1 − 1 mg/ml; the reason for the lack of concentration-dependency is still not clear. However, it seems that the inhibition of peptic activity by aluminum is not related to the protective effect of sucralfate on pepsin-induced cell damage because of sucralfate itself scarcely inhibits the peptic activity.

The last possible mechanism of the protective effect of sucralfate is believed to be its adhesion to the cell surface, which directly inhibits contact with necrotizing agents to the cell surface. We confirmed that sucralfate remained on the cell surface even after washing three times.

Taking the results together, it seems that the mechanisms underlying the protective effect of sucralfate on two types of cell damage are: 1) its adherence to the cell sur-

face and 2) the neutralization of hydrogen ions by aluminum, which consequently prevents the penetration of hydrogen ions into the cells.

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