Effects of Sucralfate on Gastric Irritant-Induced Necrosis and Apoptosis in Cultured Guinea Pig Gastric Mucosal Cells

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We previously reported that several gastric irritants, including ethanol, hydrogen peroxide, and hydrochloric acid, induced both necrosis and apoptosis in cultured gastric mucosal cells in vitro.1—4) We recently reproduced such apoptosis and necrosis in vitro using primary cultures of guinea pig gastric mucosal cells. Several gastric irritants (nonsteroidal antiinflammatory drugs [NSAIDs], ethanol, hydrogen peroxide, and hydrochloric acid) have been shown to induce apoptosis or necrosis when gastric mucosal cells are treated with low concentrations of these irritants for a long period or with high concentrations for a short period, respectively.5—7) We have also found that these gastric irritants induce apoptosis through a common pathway in which mitochondrial dysfunction and caspase-8 activation play important roles.7)

Since primary cultures of guinea pig gastric mucosal cells are thought to mimic gastric mucosal cells closely in vivo,8,9) we consider that this in vitro system of gastric irritant induced apoptosis and necrosis is useful for evaluation of the action of gastroprotective drugs. Most gastroprotective drugs are inducers or derivatives of prostanoids (PGs), which are known to protect the gastric mucosa via various mechanisms.10—13) Using primary cultures of guinea pig gastric mucosal cells, we recently reported that PGs inhibited the induction of apoptosis, but not of necrosis, produced by various gastric irritants.14) Furthermore, geranylgeranylacetone, another type of gastroprotective drug and an inducer of heat shock proteins (HSPs), was shown to inhibit both the apoptosis and necrosis induced by various gastric irritants.7,9,13—17)

Sucralfate, an aluminum hydroxide complex of sucrose octasulfate, is another type of gastroprotective drug. In addition to its ability to induce PG synthesis,18,19) this drug was shown to adhere to gastric mucosa directly and form a physical barrier against gastric irritants,20,21) which distinguish it from the actions of other gastroprotective drugs. In addition to various mechanisms for its cytoprotective effects, such as detoxification and stimulation of mucus secretion and synthesis,22—24) sucralfate was shown to protect gastric mucosal cells from gastric irritants (NSAIDs and taurocholate) directly in vitro,25,26) suggesting that this drug can inhibit the processes of necrosis and apoptosis. In this study, we found that sucralfate inhibits gastric irritant-induced necrosis in vitro. These results suggest that one of the mechanisms of the cytoprotective effects of sucralfate is its inhibitory effect on gastric irritant-induced necrosis.

**Key words** necrosis; apoptosis; gastric mucosal cell; gastric irritants; sucralfate

**MATERIALS AND METHODS**

**Chemicals and Media** RPMI 1640 was obtained from Nissui Pharmaceutical Co. (Tokyo, Japan). Fetal calf serum (FCS) and trypsin solution were purchased from Gibco (Grand Island, NY, U.S.A.). Pronase E and type I collagenase were purchased from Kaken Pharmaceutical Co. (Kyoto, Japan) and Nitta Gelatin Co. (Osaka, Japan), respectively. 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was from Sigma Co. (Tokyo, Japan). The sucralfate used was a kind gift from Chugai Pharmaceutical Co. (Tokyo, Japan).

The experiments and procedures described here were approved by the Animal Care Committee of Okayama University.

**Preparation and Culture of Gastric Mucosal Cells** Male guinea pigs (4 weeks of age) were purchased from Shimizu Co. (Kyoto, Japan). Gastric mucosal cells were isolated from guinea pig fundic glands as described previously.8) Isolated gastric mucosal cells (2×10⁵ cells/well) were cultured for 48 h in RPMI 1640 containing 0.3% FCS, penicillin 100 U/ml, and streptomycin 100 µg/ml in type-I collagen-coated plastic culture plates (Iwaki, Tokyo, Japan) under 5% CO₂/95% air and at 37 °C. After removing nonadherent cells by washing with RPMI 1640, cells that were attached to plates at about 50% confluence were used. Guinea pig gastric mucosal cell preparations cultured under these conditions have been previously characterized, with the majority (about 90%) of cells being identified as pit cells.8)

**Treatment of Cells with Gastric Irritants and Sucralfate** Cells were exposed to gastric irritants (ethanol, hydrogen peroxide, or indomethacin) in the presence or absence of

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sucralfate by replacing the entire bathing medium with fresh medium containing one of the irritants.

**Cell Viability Assay** Cell viability was examined using the MTT assay. After exposure to one of the gastric irritants, cells were incubated for 2 h with MTT solution at a final concentration of 1 mg/ml in phosphate-buffered saline (PBS). Isopropanol and hydrochloric acid were added at final concentrations of 50% and 20 mM, respectively. The optical density at 570 nm was determined by spectrophotometric analysis using a reference wavelength of 630 nm.27)

**Statistical Analysis** All values are expressed as the mean±S.E.M. A Student’s t-test for paired results was used for the evaluation of differences between groups. Differences were considered to be significant at values of p<0.05.

**RESULTS AND DISCUSSION**

**Effect of Sucralfate on Ethanol-Induced Necrosis in Cultured Gastric Mucosal Cells** We previously reported that treatment of guinea pig gastric mucosal cells in primary culture with 7—8% ethanol for 1 h caused necrosis. This conclusion was based on results showing that cell death occurred without apoptotic DNA fragmentation or chromatin condensation.6) We thus examined the effect of sucralfate on such ethanol-induced necrosis in the present study. Guinea pig gastric mucosal cells in primary culture were preincubated for 2 h with various concentrations of sucralfate and further incubated for 1 h with 7.5% ethanol. We confirmed that this treatment with ethanol in the absence of sucralfate did not induce apoptotic DNA fragmentation or chromatin condensation, but there was an obvious decrease in cell viability (data not shown), indicating that this ethanol treatment caused necrosis. As shown in Fig. 1, sucralfate (5 mg/ml) clearly inhibited the cell death induced by exposure of cells to 7.5% ethanol. Lower concentrations of sucralfate did not prevent the decrease in cell viability seen in the presence of 7.5% ethanol. The concentration of sucralfate required for a cytoprotective effect against ethanol (see Fig. 1) is similar to that previously reported for the cytoprotective effect of sucralfate against indomethacin and taurocholate.25,26) The results presented in Fig. 1 thus show that sucralfate is able to suppress ethanol induced necrotic cell death.

**Requirement of Simultaneous Treatment of Cells with Sucralfate for Its Inhibitory Effect on Ethanol-Induced Necrosis** Gastric mucosal cells were first incubated with sucralfate (preincubation step) and then incubated with ethanol in the presence of the same concentration of sucralfate (incubation step) as that used for the experiments described in Fig. 1. We subsequently examined whether both preincubation and incubation with sucralfate were required for the inhibitory effect of sucralfate on ethanol-induced necrosis. When sucralfate was omitted from the incubation step but included in the preincubation step, ethanol-induced necrosis was not inhibited (Fig. 2, second column from the right). On the other hand, when sucralfate was omitted from the preincubation step but included in the incubation step, ethanol-induced necrosis was inhibited (Fig. 2, right-hand column) to the same extent as when sucralfate was included in both steps (Fig. 2, center column). It would therefore appear that sucralfate must be present simultaneously with the irritant to inhibit its necrosis-inducing effects, but the preincubation of cells with sucralfate is not required for its cytoprotective effect against gastric irritants. This property is different from other types of gastroprotective drugs (HSP-inducers and PG-related drugs)6,14—17) and may prove useful in the clinical setting. In subsequent experiments, we added sucralfate only in the incubation step when examining the mechanisms of its cytoprotective effects.

**Effect of Sucralfate on Hydrogen Peroxide or Indomethacin Induced Necrosis in Cultured Gastric Mucosal Cells** Because the gastric mucosa in vivo is exposed to numerous gastric irritants other than ethanol, we have also shown that irritants such as hydrogen peroxide and indomethacin are also capable of inducing necrosis in cultured gastric mucosal cells.5,6) We therefore examined here the effect of sucralfate on necrosis induced by these two irritants. As shown in Fig. 3, cell death induced by treatment for 1 h with hydrogen peroxide (0.8 mM) or indomethacin (2.5 mM) could be suppressed in the presence of sucralfate. We confirmed that these treatments caused necrosis (in the absence of sucralfate) by showing the lack of apoptotic DNA fragmentation and chromatin condensation (data not shown),

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![Fig. 1. Effect of Sucralfate on Ethanol-Induced Necrotic Cell Death](image)

Cultured gastric mucosal cells were preincubated for 2 h with the indicated concentrations of sucralfate. Cells were further incubated for 1 h with 7.5% ethanol (EtOH) in the presence of the same concentration of sucralfate as that used in the preincubation step. Cell viability was determined using the MTT assay. Values are expressed as mean±S.D., n=3. **p<0.001.

![Fig. 2. Requirement of the Presence of Sucralfate in the Incubation Step Only for Highlighting Its Inhibitory Effect on Ethanol-Induced Necrotic Cell Death](image)

Cultured gastric mucosal cells were preincubated in the presence or absence of sucralfate 5 mg/ml for 2 h (preincubation step). Cells were further incubated for 1 h with or without 7.5% ethanol (EtOH) in the presence or absence of sucralfate 5 mg/ml (incubation step). Cell viability was determined by the MTT assay. Values are expressed as mean±S.D., n=3. ***p<0.001.

![Fig. 3. Effect of Sucralfate on Hydrogen Peroxide or Indomethacin Induced Necrosis in Cultured Gastric Mucosal Cells](image)

Because the gastric mucosa in vivo is exposed to numerous gastric irritants other than ethanol, we have also shown that irritants such as hydrogen peroxide and indomethacin are also capable of inducing necrosis in cultured gastric mucosal cells.5,6) We therefore examined here the effect of sucralfate on necrosis induced by these two irritants. As shown in Fig. 3, cell death induced by treatment for 1 h with hydrogen peroxide (0.8 mM) or indomethacin (2.5 mM) could be suppressed in the presence of sucralfate. We confirmed that these treatments caused necrosis (in the absence of sucralfate) by showing the lack of apoptotic DNA fragmentation and chromatin condensation (data not shown),
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ethanol for 4 h caused a decrease in cell viability (Fig. 4). We
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Concentrations of irritants but for longer periods compared
previously reported that various gastric irritants induced
as described above, we
Cultured Gastric Mucosal Cells

which is consistent with previous results. The concentra-
tion of sucralfate required for the cytoprotective effect
against hydrogen peroxide or indomethacin was the same as
that against ethanol (Fig. 1). Furthermore, the results in Fig.
3B are similar to those reported in a previous paper, in
which it appeared that necrosis occured, based on our inter-
pretation of the experimental conditions used by those
authors. These results suggest that the inhibitory effect of
sucralfate on necrosis is not specific for ethanol but is a com-
mon mechanism associated with necrosis induced by gastric
irritants in general.

Effect of Sucralfate on Ethanol-Induced Apoptosis in
Cultured Gastric Mucosal Cells As described above, we
previously reported that various gastric irritants induced
apoptosis when gastric mucosal cells were treated with lower
concentrations of irritants but for longer periods compared
with the conditions used for necrosis induction. Treatment
of guinea pig gastric mucosal cells in primary culture with
4% ethanol for 4 h was shown to induce apoptosis. We
subsequently examined the effect of sucralfate on ethanol-
duced apoptotic cell death. Treatment of cells with 4% etha-
hol for 4 h caused a decrease in cell viability (Fig. 4). We
confirmed that both apoptotic DNA fragmentation and chro-
matin condensation occurred under the experimental condi-
tions employed (data not shown), showing that cell death was
induced by apoptosis. Sucralfate showed a weak protective
effect against ethanol induced apoptotic cell death (Fig. 4),
and the concentration required for the cytoprotective effect
against ethanol induced apoptotic cell death was lower than
that for protection against necrotic cell death (Fig. 1). Since
sucralfate of doses of more than 0.5 mg/ml showed little tox-
icity in 4 h incubation (data not shown), we used sucralfate
doses of less than 0.5 mg/ml to obtain the results shown in
Fig. 4.

We next attempted to examine the effect of sucralfate on
ethanol-induced apoptotic DNA fragmentation, chromatin
condensation, and caspase activation. Sucralfate prevented
the recovery of DNA and caspases from cells, as well as the
staining of cells with Hoechst 33342, thus rendering it im-
possible to reveal whether ethanol-induced apoptotic DNA
fragmentation, chromatin condensation, and caspase activa-
tion were suppressed by sucralfate.

To the best of our knowledge, this paper reports the first
attempt to examine the effect of sucralfate on gastric irritant-
induced cell death by distinguishing between apoptosis and
necrosis. We found that sucralfate exhibited a strong cytopro-
tective effect against gastric irritant-induced necrosis of cul-
tured gastric mucosal cells. These results suggest that the
cytoprotective effects of sucralfate on gastric mucosa in vivo
can be partly explained by its inhibitory effect on gastric irri-
tant-induced necrosis. The results in Fig. 4 suggest that su-
cralfate can also inhibit gastric irritant-induced apoptosis;
however, since we could not examine the effect of this drug
on ethanol-induced apoptotic DNA fragmentation, chromatin
condensation, and caspase activation we cannot conclude this
point at present.

As for the mechanism of the suppression of cell death by
sucralfate, we believe that adherence of sucralfate to the cell
membrane and formation of a physical barrier rather than
stimulation of PG synthesis by this drug is responsible for its
cytoprotective action. This is because the mechanism of ac-
tion of the cytoprotective effect of sucralfate on gastric mu-
cosal cells in vitro is different from that seen with PGS. For
example, the cytoprotective effect of PGS but not of su-
cralfate required that cells be preincubated with PGS prior to
treatment with gastric irritants. Furthermore, sucralfate,
but not PGS, shows a cytoprotective effect against gastric irri-
tant-induced necrosis.

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