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Sucralfate effects on mucus synthesis and secretion by human gastric epithelium in vitro

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Abstract

Sucralfate has complex interactions with both the luminal and mucosal environments of the stomach. To study potential mechanisms of action, the effects of sucralfate on mucus synthesis and secretion were investigated by utilizing an in vitro system comprised of a human gastric carcinoma cell line, AGS. In this study, AGS cell synthesis and secretion of the major components of mucus, lipid and glycoproteins, were estimated using metabolic labeling methods. The results showed that sucralfate preferentially stimulated the synthesis and secretion of phosphatidylcholine and triglyceride, but did not alter the synthesis and secretion of glycoprotein. In addition, indomethacin blocked the sucralfate-induced lipid synthesis and secretion, possibly implicating prostaglandins as mediators of some of the drug's cytoprotective actions. In part, this work supports the proposed role of sucralfate's stimulatory effects on synthesis and secretion of lipids as contributing to enhancement of the mucosal barrier in ulcer treatment. Additionally, this study demonstrated that the AGS cell line is useful in vitro for studying the gastric epithelial cell mucus synthesis and secretion.

Keywords: Sucralfate; Gastric epithelium; AGS; Indomethacin; Mucus; Glycoprotein; Glycolipid

1. Introduction

The gel-like gastric mucus barrier is composed of several different biochemical components. Typically, mucus contains water, bicarbonate, proteins and various lipids (Miller, 1988; Marriott and Gregory, 1990). The polymeric form of the undegraded glycoprotein makes up a high viscous mucus gel which provides an unstirred layer retarding the diffusion of noxious agents to the mucosal cell surface. However, it seems unlikely to act as an absolute physical barrier to the diffusion of small ions, such as H^+ and HCO^{3-} (Gong et al., 1990). Phospholipids and other polar lipids may be stored as vesicles or micelles within the mucus gel layer, and deposited as an oriented monomolecular layer at the lumenal interface of

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the mucus gel to form a hydrophobic barrier. Phospholipid structures in the mucus gel may account for the hydrophobic nature of the gastric mucosal surface, and the lipids bound to gastric mucin may form a protective diffusion barrier to shield the gastric mucosal surface from excessive lumenal H^+ exposure and oxygen radical attack (Gong et al., 1990; Hiraishi et al., 1993). Serosiek et al. (1983) reported that removal of the bound lipid from mucin significantly increased the diffusion rate of H^+ through the gel layer in vitro. In addition, studies from Neutra et al. (1984) have indicated that, in the presence of peptic ulcers, gastric mucus has a greater acid glycoprotein content and is more easily degraded. Therefore, not only is mucus secretion is important, but the composition of mucus is also critical (Scheiman et al., 1992).

The protective effects of sucralfate on the gastroduodenal mucosa may include the stimulation of the production of mucus, bicarbonate, and the turnover of endogenous prostaglandins. The stimulatory effect of sucralfate on both the synthesis and secretion of mucus has been studied in vitro and in vivo (Shea-Donohue et al., 1986; Crampton et al., 1988; Slomiany et al., 1989; Shorrock and Rees, 1989; Scheiman et al., 1992). While sucralfate may increase mucus production to varying degrees, the effects of sucralfate on the composition of the mucus appear more relevant in explaining cytoprotective activities. Slomiany et al. (Slomiany et al., 1986; Slomiany et al., 1989) and Scheiman et al. (1992) have reported, for example, that upon sucralfate exposure, gastric mucus is enriched in phospholipid content, which in turn can promote the mucin gel hydrophobicity and retard H^+ diffusion to the epithelial cell surface.

The objective of this and a related studies (Zheng et al., 1994; Zheng et al., 1996) has been to further investigate the cytoprotective roles of sucralfate and related formulations using appropriate in vitro systems. A stable human adenocarcinoma cell line, AGS (Barranco et al., 1983), was identified and characterized as a mucus-secreting gastric epithelial cell system with which to examine proposed mechanisms of action for sucralfate. This homogenous cell line represents an appealing and convenient alternative to primary cultures by exhibiting rapid growth in culture and eliminating the need for donor animals. In this study, we specifically have focused on the role of sucralfate in regulating gastric epithelial cell mucus synthesis, secretion, and compositional changes in the AGS cell system.

2. Materials and methods

2.1. Materials

Ham's F-12 was purchased from JRH Biosciences (Lenexa, KS). Fetal bovine serum was obtained from Hyclone Laboratories, Inc. (Logan, UT). Silica-coated, thin-layer chromatography plates were purchased from Alltech Associates, Inc. (Deerfield, IL). [^{3}H]Glucosamine, [^{3}H]choline and [^{3}H]palmitic acid were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). The following reagents were purchased from Sigma Chemical Co. (St. Louis, MO): Dulbecco's Modified Eagle's medium, penicillin, streptomycin, *N*-[2-hydroxyethyl]-piperazine-*N*'-]2-ethanesulfonic acid, ethylenediaminetetraacetic acid, periodic acid-Schiff staining system, trichloracetic acid, trypsin, Alcian blue, $CaCl_2$, phosphotungstic acid, fatty acid, phosphotidylcholine, lysophosphotidylcholine, sphingomyelin, triglyceride, diglyceride, and monoglyceride. The following reagents were purchased from Fisher Scientific company (Fair Lawn, NJ): NaOH, ScintiVerse, Permount®, methanol, chloroform, acetic acid, hexane, ethyl ether, formaldehyde, microscope cover glasses (size, 18 × 18 mm, thickness No.2) and microscope slides (size 3" × 1" × 1.2 mm).

2.2. AGS cell culture

The human adenocarcinoma cell line (AGS; ATCC # CRL-1739) was obtained from American Type Culture Collection (ATCC) and used between passages 40 and 60. The cells were initially grown in 25-cm^2 tissue culture flasks with 1:1 (v:v) ratio of Ham's F12 and DMEM medium containing 100 μg/ml of penicillin, 100 μg/ml of

streptomycin, 15 mM of Hepes, 1.2 g/l of sodium bicarbonate and 5% of fetal bovine serum, and were maintained in an incubator at 37°C, 95% humidity and 5% CO_2. The culture medium was changed 2 days after seeding and every other day thereafter. Cells were harvested every 5 days with 0.25% trypsin plus 0.05% EDTA solution for subculture (Zheng et al., 1996). A phase-contrast microscope (Nikon TMS) was used to observe the cell's growth and morphology throughout the culture period.

2.3. Mucus staining

For Alcian blue staining, cells were seeded onto sterilized microscope cover glasses in a 100-mm Corning polystyrene tissue culture plate (5×10^5 cells/plate) and incubated for 2 days. When cells were near confluence, the cells were fixed with a formalin-ethanol solution (5 ml of 34% formaldehyde with 45 ml of 95% ethanol) for 1 min. The cells were immersed in 1% Alcian blue solution for 30 min (Ota et al., 1988) and washed three times by distilled water. The cover glasses were dried by air and mounted on a microscope slide with four small drops of Permount®.

For combined Alcian blue and PAS staining, cells were seeded onto sterilized microscope cover glasses in a 100-mm Corning polystyrene tissue culture plate (5×10^5 cells/plate) and incubated for 2 days. When cells were near confluence, the cells were fixed with formalin-ethanol solution (5 ml of 34% formaldehyde with 45 ml of 95% ethanol) for 1 min. The cells were first dyed by 1% Alcian blue solution. The procedure for Alcian blue staining was the same as the single Alcian blue staining procedure above. After cells were stained with Alcian blue and washed three times by distilled water, the cells were dyed by a periodic acid-Schiff staining system (Ota et al., 1988). The cover glasses were air dried and then mounted on a microscope slide with four small drops of Permount®.

2.4. Electron microscopy

AGS cells were cultured on a 60-mm Permanox® tissue culture plates (Nunc, Inc. Naperville, IL). After the cells formed a confluent monolayer, the cells were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate phosphate buffer (pH 7.3) for at least 4 h. After postfixing with 1% of OsO_4 for 1 h at 4°C, the cells were dehydrated and stained in 2% uranyl acetate and lead citrate for 10 min. Silver sections were observed with a JEOL 1200 EXII transmission electron microscope.

2.5. Glycoprotein synthesis and secretion

AGS cells were seeded into six-well Corning polystyrene plates at a density of 3×10^5 cells/well. After the cells formed a confluent monolayer (2–3 days at this seeding density), the cells were pulsed with [^{3}H]glucosamine (0.25 μCi/ml) in culture medium for a 24-h incubation. Fresh medium with or without test agents was added for an additional 4-h incubation at 37°C, the cells were washed three times with ice-cold PBS and scraped into a glass tube with a rubber policeman. A 2-ml aliquot of 10% trichloracetic acid:1% phosphotungstic acid solution was added to the cells and incubated at least 16 h at 4°C to precipitate proteins. The cell mixture was centrifuged at $1000 \times g$ for 10 min. The pellet was neutralized and dissolved in 0.3 N NaOH and then the solution was transferred to scintillation vials with 10 ml scintillation cocktail (Dial et al., 1991; Scheiman et al., 1992; Ota et al., 1994). The vials were counted in a liquid scintillation counter (Beckman Ls 7500, Beckman Inc., Arilngton Heights, IL).

Glycoprotein secretion was determined by the method of Scheiman et al. (1992). AGS cells were grown under the same conditions as for assaying glycoprotein synthesis above. After the cells were pulsed with [^{3}H]glucosamine (0.25 μCi/ml) in culture medium for 24 h, the medium was removed and the cells were washed quickly and thoroughly three times with PBS. The cells were then incubated with fresh culture medium with or without test agents added for an additional 4 h at 37°C. The medium in each well was then collected and centrifuged at $500 \times g$ for 5 min. The supernatants were counted in a liquid scintillation counter (Scheiman et al., 1992).

2.6. Lipid synthesis and secretion

Lipid synthesis and secretion were assayed as described by Dial et al. (1991). Briefly, AGS cells were seeded into six-well Corning polystyrene plates at a density of 3×10^5 cells/well with 2 ml culture medium. After the cells formed a confluent monolayer (2–3 days at this seeding density), the cells were pulsed with either [^{3}H]choline (0.125 μCi/ml) or [^{3}H]palmitic acid (0.125 μCi/ml) in culture medium for a 24-h incubation. Fresh medium with or without test agents was added for an additional 4-h incubation at 37°C. Following the incubation, the cells were washed three times with PBS and treated with a 0.25% trypsin/0.05% EDTA solution for 2–3 min at 37°C to detach the cells. The cell suspension was centrifuged at 1000 $\times$ g for 10 min. The pellet was treated with 1 part 100% methanol and 2 parts chloroform solution. Cell lipids were extracted according to the method of Folch et al. (1957). The phospholipid extract was then dissolved in either 500 or 100 μl of 100% methanol (1 part):chloroform (2 parts) solution, and then spotted (10 μl) onto silica-coated, thin-layer chromatography plates. The samples and standard solutions were run in a solvent system of chloroform:methanol:acetic acid:water (118:59:16:7). The neutral lipid extract was also dissolved in 100 μl of 100% methanol (1 part):chloroform (2 parts), and then spotted (10 μl) onto silica-coated, thin-layer chromatography plates, and the samples and standard lipids were run in a solvent system comprised of hexane:ethyl ether:acetic acid (40:10:1). After the plates were air dried, spots were visualized under iodine vapor. The scraped spots were transferred to scintillation vials with 10 ml scintillation cocktail. The vials were counted in a liquid scintillation counter (Dial et al., 1991).

For measurement of the phospholipid secretion (Dial et al., 1991), the AGS cells were grown under the same conditions as described above for assaying phospholipid synthesis. After the cells were pulsed with [^{3}H]choline (0.125 μCi/ml) or [^{3}H]palmitic acid (0.125 μCi/ml) in culture medium and incubated for 24 h at 37°C, the medium was removed and the cells were washed quickly and thoroughly three times with PBS. The cells were then incubated with fresh culture medium with or without test agents added for an additional 4 h at 37°C. The medium in each well was collected and centrifuged at 500 $\times$ g for 5 min. The supernatants were counted in a liquid scintillation counter (Dial et al., 1991; Scheiman et al., 1992).

2.7. Data analysis

Data were expressed as mean $\pm$ standard deviation for at least four replicates. The differences between treatment and control groups were expressed as their units or as a percentage of corresponding untreated control. A one-way ANOVA followed by either the Scheff's or the Dunnett's multiple comparison test was applied to analyze the significance of differences between treatment and control groups of raw data at the 0.05 level of significance.

3. Results

Confluent monolayers of AGS cells at light microscope level are shown in Fig. 1. The shape of cells was generally polygonal or cuboidal. Most cells exhibited intracelluar and intercelluar vesicu-

Fig. 1. Phase-contrast photomicrograph of AGS cells (magnification 100 $\times$).

Fig. 2. Electron micrograph of AGS cells (4000 ×). M, mucus vesicle; V, microvilli; J, intercellular junction.

lar inclusions. These vesicles usually accumulated at one end of the cytoplasmic matrix or gathered together to form 'lakes' between cells. The mucus vesicles remained essentially uncolored after Alcian blue staining alone, while some of the vesicles exhibited pink rings around the perimeter or circumference of the vesicles following additional staining with PAS (data not shown). The pink rimmed areas represented neutral glycoproteins while the bright white mucus drops represented acidic glycoproteins.

Fig. 2 shows a typical electron micrograph of AGS cells. The cells retained a gastric epithelial-like morphology, i.e., typically a teardrop appearance in cross-section, microvilli, and close apposition with adjoining cells and substantial interdigitation within the intercellular junctions. The cytosol of the cells contained frequent vesicles presumed to contain mucus.

The synthesis and secretion of glycoprotein reflected by incorporation of glucosamine in AGS cells was linear over a 24-h time period (data not shown). Results in Fig. 3 indicated that although exposure to low concentrations of sucralfate produced a small trend toward more glycoprotein synthesis and secretion by AGS cells, levels were not significant relevant to untreated control cells.

Both total phospholipid synthesis and secretion (data not shown) by AGS cells were nearly linear over a 24-h sampling period. The synthesis of sphingomyelin, phosphatidylcholine, and lysophosphatidylcholine, by AGS cells is illustrated in Fig. 4. The major part of [^{3}H]choline was incorporated into phosphatidylcholine (Fig. 4). The effect of sucralfate and sucralfate with indomethacin treatment on phosphatidylcholine

synthesis of AGS cells is shown in Fig. 5. AGS cells treated with low concentrations (< 1 μg/ml) sucralfate significantly enhanced synthesis of phosphatidylcholine by AGS cells. Phosphatidylcholine synthesis in the presence of both sucralfate and indomethacin was not significantly different from untreated controls ($p > 0.05$). The effect of sucralfate on the secretion of phospholipids was also stimulatory and altered by co-treatment with indomethacin as shown in Fig. 6. Phosphatidylcholine secretion by cells exposed to sucralfate and indomethacin was not significantly different from untreated controls ($p > 0.05$). Treatment of AGS cells with 100 μM indomethacin alone (data not shown) had no significant effect on either basal lipid synthesis and secretion or cell viability.

The incorporation of palmitic acid into the neutral lipids, phosphatidylcholine, triglycerides, diglycerides, monoglycerides and fatty acids, by AGS was nearly linear during a 4–24-h sampling period as illustrated in Fig. 7. The major part of

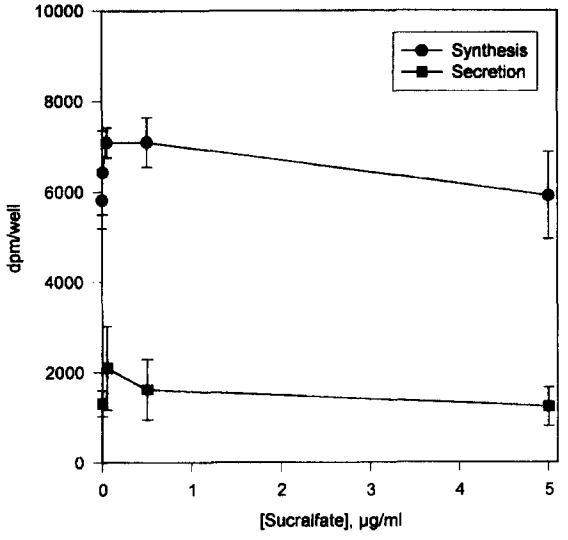

Fig. 3. The effect of sucralfate on the synthesis and secretion of glycoprotein. Two-day cultured AGS cells were pulsed with [^{3}H]glucosamine (0.25 μCi/ml/24 h) before adding test agents for a 4-h incubation. For secretion studies, after pulsing with [^{3}H]glucosamine (0.25 μCi/ml/24 h), AGS cells were washed and then incubated with test agents for 4-h before harvesting glycoprotein secreted into the extracellular medium. Values are means $\pm$ SD; $n = 6$.

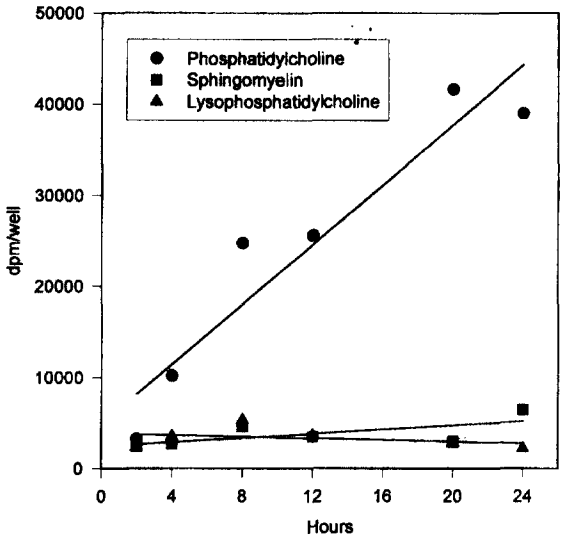

Fig. 4. The temporal synthesis of phospholipids by AGS cells. Two-day cultured cells were pulsed with [^{3}H]choline (0.125 μCi/ml/24 h), incubated with medium for indicated times, before harvesting and extracting lipids. Values are means $\pm$ SD, $n = 4$.

palmitic acid was incorporated into triglycerides (Fig. 7). Consistent with the effects of sucralfate on choline-labeled phospholipids, sucralfate significantly enhanced AGS triglyceride synthesis as shown in Fig. 8. In addition, the stimulatory effects of sucralfate on triglyceride synthesis could be reversed to untreated control levels by a co-incubation with indomethacin ($p > 0.05$). A similar pattern of sucralfate's stimulatory effects on triglyeride synthesis was observed on total neutral lipid secretion by the AGS cells as shown in Fig. 9.

4. Discussion

The AGS cells in culture have been described as mucus-secreting gastric epithelial-like cells at both light and electron microscope levels (Barranco et al., 1983). To qualitatively establish the mucus secretion properties of the AGS cells in our laboratory, we have utilized both PAS and Alcian blue stains in this study. The PAS reaction is used for the detection of glycogen and carbohydrate-containing molecules. Alcian blue is a cationic dye

that forms complexes with acidic glycoproteins and other negatively charged macromolecules (Slomiany et al., 1989). Supporting the observations of Barranco et al. (1983), we observed positive staining with both PAS and Alcian blue in the AGS cells. Additionally, these results were consistent with the observations with gastric mucus samples from the monkey (Shea-Donohue et al., 1986) and with primary cultures of rat gastric epithelium (Terano et al., 1982; Dial et al., 1991; Ota et al., 1994; Zheng et al., 1994).

Radiolabeled precursor markers, glucosamine, choline and palmitic acid were used to assay for cell-derived glycoproteins, phospholipids, and neutral lipids, respectively. For the AGS cells, as for observations with primary culture systems (Dial et al., 1991; Scheiman et al., 1992; Ota et al., 1994), glycoprotein and lipid production was found to be essentially linear over a 24-h period. The predominant lipid fraction labeled with choline in the AGS cells was phosphatidylcholine, the major lipid of the gastric mucosa in vivo (Wassef et al., 1979). After AGS cells were exposed to palmitic acid for 24 h, about 50% of labeled lipids appeared as triglycerides and 19% as

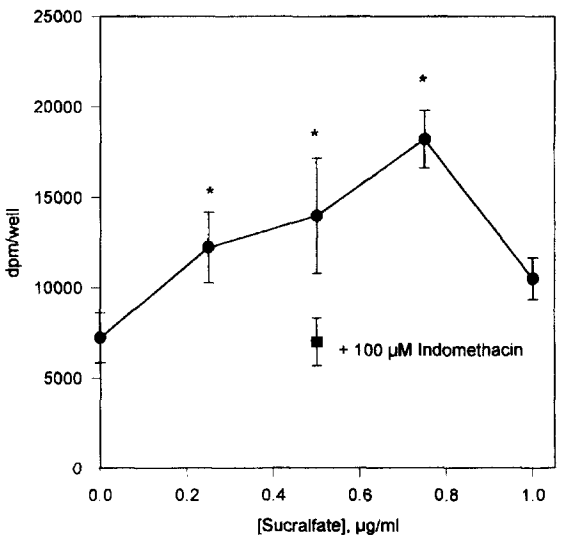

Fig. 5. The effect of sucralfate on phosphatidylcholine synthesis by AGS cells. Two-day cultured cells were pulsed with [^{3}H]choline (0.125 μCi/ml/24 h) and incubated with test agents for 4 h. Values are means $\pm$ SD, $n = 4$, $^*p < 0.05$ vs. untreated control.

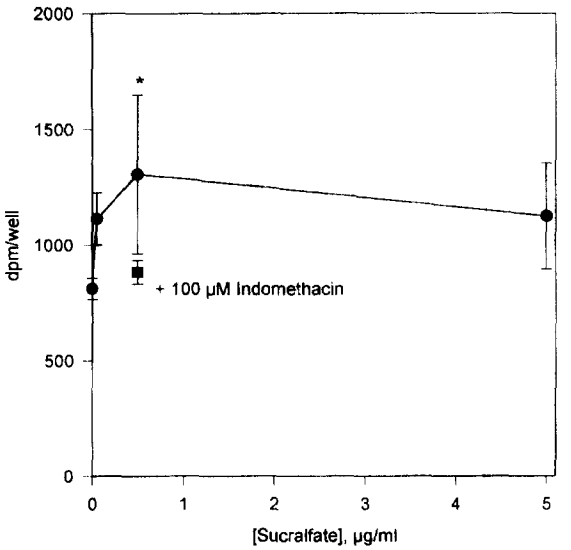

Fig. 6. The effect of sucralfate on phospholipid secretion by AGS cells. Two-day cultured cells were pulsed with [^{3}H]choline (0.125 μCi/ml/24 h). The cells were washed and incubated with test agents for 4 h before harvesting and extracting lipids. Values are means $\pm$ SD, $n = 4$. $^*p < 0.05$ vs. untreated control.

phosphatidylcholine. These results were also in good agreement with findings in primary cultures of gastric epithelial cells (Dial et al., 1991; Scheiman et al., 1991). Together, the results from the qualitative staining and the incorporation of radiolabeled markers confirmed that the AGS cell line was capable of synthesis and secretion of a mucus in vitro comprised of mucin and lipid fractions consistent with other gastric epithelial cell systems in vitro and in vivo.

The effects of sucralfate on gastric mucus production appears to affect not only the amount of mucus secretion, but also the composition or quality of mucus (Shea-Donohue et al., 1986; Slomiany et al., 1986; Tasman-Jones et al., 1989; Hollander and Tarnawski, 1990; Hills, 1991). Phospholipids and other polar lipids are important components in the mucosal defense barrier. These lipids appear to contribute to the hydrophobic nature of the gastric mucosal surface and aid in retarding the diffusion of H^+ to the cell surface (Serosiek et al., 1983; Slomiany et al., 1989; Scheiman et al., 1992). Slomiany et al. (1989) have investigated the chemical composition

of rat gastric mucus gel and found that with intragastric administration of sucralfate, the mucus gel exhibited a 14% lower protein content, a 62% higher content of carbohydrate, and an 18% higher neutral lipid content. Scheiman et al. (1992) also reported on the effects of sucralfate on components of mucosal barrier generated by cultured canine epithelial cells in vitro. They found that sucralfate (10^{-7} M or about 0.1 μg/ml) significantly increased phospholipid secretion (125% of control), but no effect on glycoprotein secretion or synthesis was noted. Although an apparent trend toward increases in glycoprotein synthesis by the AGS cells was observed in the presence of low concentrations of sucralfate, the effect (a maximal increase of 112% of control; Fig. 3) was not statistically significant. This observation was also consistent with minimal stimulation (108% of control) noted by Scheiman et al. (1992) for glycoprotein synthesis and secretion in the presence of 10^{-7} M sucralfate.

In studies with rats, researchers have noted that sucralfate produces gastric mucus changes in sites where obvious amounts of the drug have accumulated, as well as in adjacent tissue areas exposed

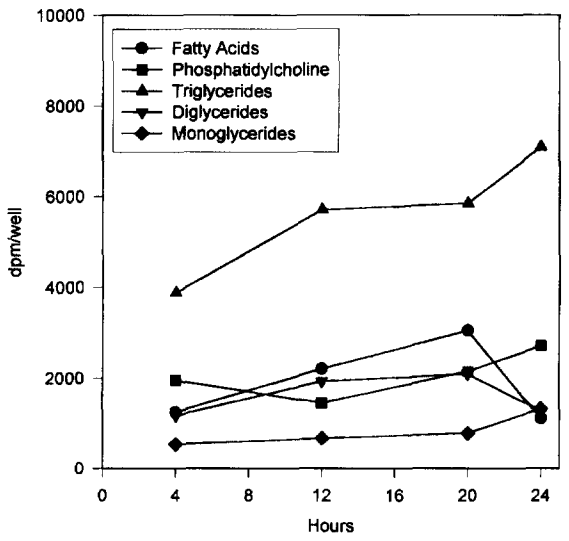

Fig. 7. The temporal synthesis of neutral lipids by AGS cells. Two-day cultured cells were pulsed with [^{3}H]palmitic acid (0.125 μCi/ml/24 h) and incubated with medium for indicated time before harvesting and extracting lipids. Values are means $\pm$ SD, $n = 4$.

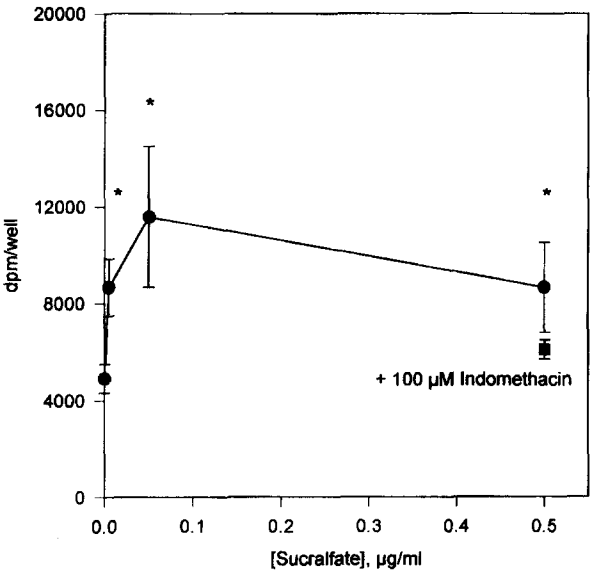

Fig. 8. The effect of sucralfate on triglyceride synthesis by AGS cells. Two-day cultured cells were pulsed with [^{3}H]palmitic acid (0.125 μCi/ml/24 h) and incubated with test agents for an additional 4 h before harvesting and extracting lipids. Values are means $\pm$ SD, $n = 4$, $^*p < 0.05$ vs. untreated control.

to much smaller amounts of the drug, suggesting that low concentrations of sucralfate have significant bioactivity (Morris et al., 1989). Lower concentrations of sucralfate have proven effective in modulating mucus production in cell systems in vitro (Slomiany et al., 1986; Slomiany et al., 1989; Scheiman et al., 1992). In our study, we found that low, μg/ml, concentrations of sucralfate significantly enhanced AGS cell synthesis of phosphotidylcholine (e.g., increases of 160–250% of untreated controls; Fig. 5) and triglycerides (e.g., increases of 170–230% of untreated controls; Fig. 8). The effects of sucralfate on lipid synthesis and secretion also did not occur in a typical dose-dependent fashion, i.e., sucralfate had greater effects at lower concentrations than that at higher concentrations. This may be due to the presence of excessive insoluble sucralfate at higher concentrations. At higher concentrations of sucralfate, the drug forms an insoluble layer over the cell monolayers that does not alter viability but may apparently reduce the responsiveness of the cells. Other researchers have noted a similar phenomenon in vitro (Scheiman et al., 1992).

The precise mechanisms mediating sucralfate regulation of the production of the mucus barrier are not clear. There are two major views in the literature. One view would suggest that sucralfate stimulates mucus production through a prostaglandin-independent 'irritant' response (Wallace and Williamson, 1989; Scheiman et al., 1992). The other view based on an in vivo study (Lichtenberger et al., 1983), would suggest that sucralfate enhances the lipid component of mucus production through a prostaglandin-dependent interaction. There seems to be little question that prostaglandins released on exposure to 'irritants' or physiological effectors regulate lipid production by gastric epithelial cells (Shorrock and Rees, 1989; Dial et al., 1991; Scheiman et al., 1991; Ota et al., 1994). We found that indomethacin can significantly block the stimulatory effects of sucralfate on lipid synthesis and secretion by AGS. As indomethacin is an inhibitor of prostaglandin biosynthesis and release, it is attractive to suppose that indomethacin blocked the effect of sucralfate on mucus production by decreasing prostaglandin availability. Although our findings in vitro supported prostaglandin-dependent interactions as mediating sucralfate effects on gastric epithelial cell production of the lipid components of mucus, we have not as yet directly verified that specifically prostaglandins were the mediators produced by AGS cells on exposure to sucralfate. Furthermore, Scheiman et al. (1992) have noted that indomethacin is capable of blocking sucralfate's stimulatory effects on lipid production but could not block sucrose octasulfate's stimulatory effect on lipid production. Therefore, it remains for future works to sort out an explanation for precisely how indomethacin and sucralfate interactions with the gastric epithelial cells might regulate lipid production.

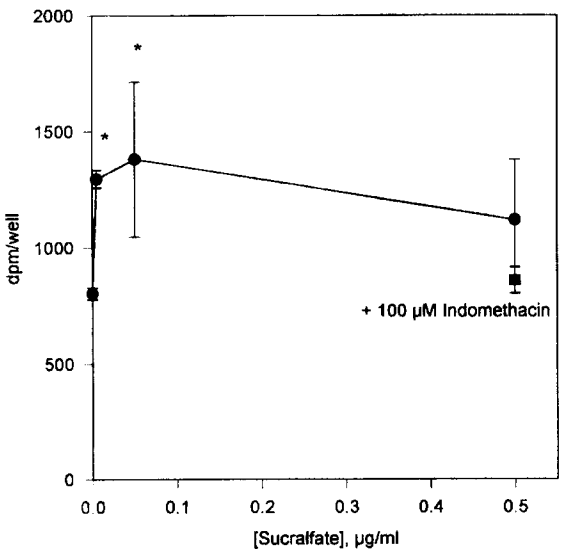

Fig. 9. The effects of sucralfate on neutral lipid secretion by AGS cells. Two-day cultured cells were pulsed with [^{3}H]palmitic acid (0.125 μCi/ml/24 h). The cells were washed and incubated with test agents for 4 h before collecting the extracellular medium and extracting lipids. Values are means $\pm$ SD, $n = 4$, $^*p < 0.05$ vs. untreated control.

In the literature, several lines of investigation focus on ulcer generation and wound repair. The AGS cell line has and could continue to be a convenient experimental model in those investigations concerned with mechanisms of action of anti-ulcer or cytoprotective agents. As an example, based on the evidence that infection with *Helicobacter pylori* has a major role in the pathogenesis of gastric diseases, Bull-Henry et al. (1991) have used the AGS cell line to demonstrate *H. pylori* invasion and alterations in gastric epithelium in vitro. Although not an antibiotic, sucralfate has shown activity in interfering with *H. pylori* infection and colonization by blocking the mucolytic action of the vector on the mucus barrier (Slomiany et al., 1994). Future studies could exploit the AGS system to establish precise mechanisms through which sucralfate and other agents might treat or prevent *H. pylori* infection of the gastric mucosa. As a second example, the repair mechanisms for ulcers are believed to involve secretion of a growth factor, basic fibroblast growth factor (bFGF), which stimulates angiogenesis and wound healing (Folkman et al., 1991). Coincidentally, sucralfate protects bFGF from the acidic lumenal contents of the stomach and promotes angiogenesis and ulcer repair in vivo (Folkman et al., 1991). Recent works from our laboratory have shown that the AGS cell line can also be used to follow the bioactivity of acid-treated growth factors, bFGF and epidermal growth factor, in the presence and absence of sucralfate and related formulations (Sheng et al.,

1996). These observations, along with findings described in this report and a related study (Zheng et al., 1996), indicate that the AGS cell culture system provides a useful alternative to primary cultures in the investigation and evaluation of certain cytoprotective drugs and related formulations or derivatives.

In summary, this study presents evidence generated with the AGS cell line that further supports a role for sucralfate in the regulation of the lipid composition of gastric mucus. In general, the results from this study also support the use of the AGS cell line as an in vitro system to investigate gastric epithelial cell functions and cell responses in the presence of irritants and certain cytoprotective agents.

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