Utilization of a Human Intestinal Epithelial Cell Culture System (Caco-2) for Evaluating Cytoprotective Agents

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Human intestinal epithelial cells (Caco-2) were cultured as confluent monolayers on polycarbonate membranes in Transwells for investigating their applicability in evaluating the cytoprotective activity of sucralfate. The control experiments established a reproducible chemical method (using 0.5 m*M* indomethacin in Hanks' balanced salt solution) for inducing damage to the Caco-2 cell monolayers. Damage was determined by measuring changes in transepithelial electrical resistance (TEER). Twenty-day-old Caco-2 cell monolayers were significantly and reproducibly damaged (compared to buffer alone) ($P < 0.001$) by application of 0.5 m*M* indomethacin to the apical side for 1 hr. While sucralfate, at a 0.5, 2, or 5 mg/mL concentration in the buffer, was shown not to reverse (treat) the damage caused by indomethacin in this cellular model, it was able to protect (prevent) the cells from indomethacin-induced damage ($P < 0.001$). We observed that indomethacin-induced damage to the Caco-2 cell monolayers greatly affected the paracellular pathway since the percentage transport of [^{3}H]methoxyinulin was significantly elevated. In contrast, protection of the Caco-2 cells with 5 mg/mL sucralfate in the presence of the damaging agent resulted in transport of the paracellular marker similar to that in the control (HBSS-treated) cell monolayers. This direct cytoprotective effect was thus independent of vascular factors at neutral pH and was observed to be dose dependent (0.5 to 5 mg/mL) when sucralfate was applied to the cells in the presence of the damaging agent. These findings, which are consistent with those observed for sucralfate *in vivo* (Okabe *et al., Digest. Dis. Sci.* 28:1034–1042, 1983), demonstrate the feasibility of using Caco-2 cell monolayers as an *in vitro* cell culture system which may serve to identify and rapidly screen the cytoprotective activity of potential drugs and their pharmaceutical formulations.

KEY WORDS: Caco-2; cytoprotection; sucralfate; indomethacin; transepithelial electrical resistance.

INTRODUCTION

A human intestinal epithelial cell culture system (Caco-2) has been employed previously to study intestinal drug transport (1). This cell culture system has been determined to exhibit morphological and biochemical characteristics similar to those of human intestinal mucosa such as the expression of cell polarity and the presence of tight junctions and marker enzymes (1). Hence, cultured monolayers of Caco-2 cells have been extensively used to study the barrier properties of human intestinal epithelium to drug transport (2). This cell culture model has been used to study the carrier-mediated transport of various solutes including bile acids (3), amino acid-like drugs (4), and vitamins (5). Furthermore, the transcellular passive diffusion of lipophilic solutes such as steroids or peptides (2,6) and the paracellular flux of hydrophilic solutes such as inulin or mannitol (2) utilizing these cultured human intestinal cells have also been reported. Recently, the transport and metabolism of fatty acids (7), the transport of iron (8), and the glucuronide conjugation of *p*-nitrophenol (9) by Caco-2 cells were demonstrated.

In this study we have investigated the possible applicability of cultured Caco-2 cell monolayers grown onto polycarbonate membranes in Transwells as an *in vitro* model system for evaluating cytoprotective agents (e.g., sucralfate, an aluminum hydroxide complex of sucrose octasulfate). Such cellular model systems may serve to identify and screen rapidly the cytoprotective activity of potential antiulcer drugs and to evaluate the efficacy of their pharmaceutical formulations *in vitro*.

MATERIALS AND METHODS

Materials

The Caco-2 cell line originating from a human colorectal carcinoma was obtained from American Type Culture Collection (Rockville, MD). Sucralfate was a gift from Marion Merrell Dow, Inc. (Kansas City, MO). Dulbecco's modified Eagle medium (DMEM), nonessential amino acids (NEAA), L-glutamine (200 mM), penicillin G (10,000 U/mL)–streptomycin sulfate (10,000 μg/mL) solution, and 0.25% trypsin (1:250)–0.02% EDTA disodium solution were obtained from JRH Biosciences (Lenexa, KS). Fetal bovine serum (FBS) was purchased from Intergen (Cambridge, MA), and rattail collagen (Type I) from Collaborative Research (Lexington, MA). Transwell clusters, PVP free, 24.5 mm in diameter (4.71-cm^2 surface area), and 3.0 μm in pore size were purchased from Costar Corporation (Bedford, MA). Hanks' balanced salt solution, powder form (HBSS), *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), D-(+)-glucose, indomethacin, and sodium bicarbonate were purchased from Sigma Chemical Co. (St. Louis, MO). The Evom Epithelial Voltohmmeter and STX-2 electrode were bought from World Precision Instruments Inc. (New Haven, CT). The Olympus inverted microscope (model CK 2) was purchased from Olympus Optical Co. (Tokyo). Preparation of HBSS solutions and cell culture medium has been reported previously (1). Indomethacin (0.5 m*M*) solution and sucralfate (0.5, 2, and 5 mg/mL) suspensions in either the cell culture medium or HBSS were freshly prepared before use.

Caco-2 Cell Culture

Caco-2 cells were plated and grown according to previously published procedures (1). Briefly, the cells were grown in 165-cm^2 culture flasks in culture medium consisting of DMEM with 100 U/mL penicillin and 100 μg/mL streptomy-

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cin, 1% NEAA, and 10% FBS. Before reaching confluency, the Caco-2 cells were trypsinized with 0.25% trypsin and 0.02% EDTA. The cells were then plated at a density of 63,000/cm^2 in the culture medium on Transwell polycarbonate membranes (3.0-μm pore size) which were previously coated with rattail collagen. The Caco-2 cells were fed with the culture medium every other day for 7 days and then daily for 20 or 30 days by replacing 1.5 mL of the medium in the apical (AP) chamber and 2.5 mL of the medium in the basolateral (BL) chamber. The cells were maintained at 37°C in an atmosphere of 5% CO_2 and 90% relative humidity in the incubator. The cell monolayers used in this study were between passage 69 and passage 89 and 20 or 30 days postseeding. They were checked for confluency by observation under the microscope and by measurement of their transepithelial electrical resistance (TEER) values in HBSS. Typical TEER values for 20- or 30-day-old confluent Caco-2 cell monolayers were found to be 440 ± 6 and 600 ± 4 Ω·cm^2, respectively.

Cytotoxicity and Cytoprotective Studies

Prior to the experiments, the cell culture medium was removed from the AP and BL sides of the cell monolayers. The cell monolayers were washed twice with HBSS (at 37°C) and their TEER values were measured in HBSS at $t = 0$. During the experiment, the cell monolayers were incubated under 5% CO_2 and 90% humidity at 37°C in the incubator (Steri-Cult, Forma Scientific, OH). The AP and BL solutions were removed prior to TEER measurements and the TEER values (in fresh HBSS) were obtained from the same position over the cell monolayer in the Transwell. They were corrected for the blank (polycarbonate membrane without the cell monolayer).

Various control experiments were first carried out in order to establish a reproducible chemical method using 0.5 m*M* indomethacin in HBSS on the AP side to induce damage to 20- or 30-day-old Caco-2 cell monolayers. In these experiments, 0.5 m*M* indomethacin in HBSS was applied for 1 or 6 hr to the AP surface of the Caco-2 cell monolayers (phase I), followed by the application of medium for up to 12 hr (phase II). The BL chamber contained HBSS for 1 or 6 hr (phase I), followed by the cell culture medium for up to 12 hr (phase II). In separate studies, HBSS or 5 mg/mL sucralfate in HBSS was applied to the cell monolayers to confirm that cell damage was not caused by HBSS or 5 mg/mL sucralfate (the highest concentration of the cytoprotective agent used in this study).

The sucralfate study was divided into treatment and prevention protocols. These studies were conducted similar to those reported by Romano *et al.* (10).

Treatment Protocol. In the treatment studies, damage was first induced by applying 0.5 m*M* indomethacin in HBSS for 1 hr to the AP side of the cell monolayers (phase I), followed by the application of medium in either the absence (control) or the presence of 0.5, 2 or 5 mg/mL sucralfate for up to 12 hr (phase II). The BL chamber contained HBSS for 1 hr (phase I), followed by medium for up to 12 hr (phase II). These experiments were designed to investigate dose dependency of sucralfate in treating the damage caused by application of indomethacin to the AP surface of the Caco-2 cells.

Prevention Protocol. The prevention studies were designed to determine the protective effect of 0.5, 2, or 5 mg/mL sucralfate on the cell monolayers in the presence of the damaging agent. In these experiments, 0.5 m*M* indomethacin in either the absence (control) or the presence of 0.5, 2, or 5 mg/mL sucralfate in HBSS was applied to the AP surface of the Caco-2 cells for 1 hr (phase I), followed by the application of 0.5 m*M* indomethacin in either the absence (control) or the presence of 0.5, 2, or 5 mg/mL sucralfate in medium for up to 12 hr (phase II) to the Caco-2 cell monolayers. Similar to the treatment studies, in these experiments the BL side contained HBSS for 1 hr (phase I) followed by medium for up to 12 hr (phase II).

Electron Microscopy (EM)

Transmission (TEM) and scanning (SEM) electron microscopy studies were conducted to visualize the cytoprotective effect of sucralfate against the cellular damage induced by indomethacin in the Caco-2 cells. For TEM, the Caco-2 cell monolayers were fixed with 2.5% glutaraldehyde in HBSS, pH 7.4, for 24 hr at 4°C and rinsed three times with HBSS (4°C) followed by postfixation for 1 hr at 25°C in HBSS, pH 7.4, containing 1% OsO_4. Silver cross sections through the Caco-2 cell monolayers were examined with a JEOL 1200 EX II transmission electron microscope operated at 80 kV (1).

For SEM, the Caco-2 cell monolayers were similarly fixed and postfixed as described above. After dehydration and drying they were mounted on an aluminum stud and coated with a 300-Å Au-Pd sputter coat in a Hummer II. The monolayers were examined in a Hitachi 570 LaB_6 scanning electron microscope.

Transport Studies

Fluxes of the radiolabeled hydrophilic (methoxyinulin) and lipophilic (testosterone) solutes across the Caco-2 cell monolayers were monitored for 2 hr after subjecting them to various prevention protocols. These studies were conducted in order to determine the effect of cytoprotection afforded by sucralfate against indomethacin induced cellular damage as reflected by the flux of hydrophilic and lipophilic markers.

In these experiments, fluxes of [^{3}H]methoxyinulin (0.5 μCi/mL) and [^{14}C]testosterone (0.2 μCi/mL) across nontreated and treated Caco-2 cell monolayers were monitored by sampling the BL radioactivity at 30, 60, 90, and 120 min. The percentage radioactivity on the BL side at 2 hr was taken as a measure of the Caco-2 cell monolayer integrity.

Statistical Analysis

The percentage TEER values were expressed as mean ± SE. Significance was evaluated by analysis of variance (ANOVA). When the F value was significant, Duncan's multiple-range test was applied (11). Differences were considered significant when $P < 0.001$.

RESULTS AND DISCUSSION

The choice of 0.5 m*M* indomethacin as an ulcerogenic agent in our *in vitro* study utilizing human intestinal (Caco-2) cells was based on earlier observations that it provided a

reproducible chemical method for inducing gastric damage *in vitro* and *in vivo* (10,12). Further, the direct cellular damage due to indomethacin or other necrotizing agents and the development of gastric lesions have been correlated to the decrease in transmucosal electrical potential difference (PD) of the membrane barrier accompanied by net fluxes of Na^+, Cl^-, and H^+ ions across the gastrointestinal mucosa (13). PD as a measure of the integrity of the membrane barrier has also been reported in evaluating the damage caused by agents such as glycocholate, ethanol, or acid and the protective effect of sucralfate on the gastric mucosal barrier *in vitro* and *in vivo* (14). In addition, TEER measurements have been reported to indicate the integrity of a cell monolayer and its barrier properties (1,15). Therefore, we used TEER values to monitor cell damage produced by indomethacin and recovery induced by application of sucralfate (cytoprotective) to the Caco-2 cells.

We used 20- or 30-day-old Caco-2 cell monolayers in our control experiments first to identify the basic protocol that would demonstrate cell damage (as given by TEER values) upon indomethacin application. Confluent Caco-2 cell monolayers cultured for 30 days were subjected to 0.5 m*M* indomethacin in HBSS for either 1 or 6 hr. The resulting changes in TEER values were monitored. The 30-day-old Caco-2 cells were not damaged after 1 hr of indomethacin treatment but showed significant damage only after a 6-hr exposure to indomethacin (data not shown). In contrast to 30-day-old Caco-2 cell monolayers, significant cell damage could be induced by treatment for 1 hr with 0.5 m*M* indomethacin in 20-day-old Caco-2 cell monolayers (Fig. 1). For example, the TEER values at 150 min (after initiating the experiment) were approximately 65 and 85% $\Omega \cdot cm^2$ compared to the control (100%) value for the 20- and 30-day-old cells, respectively, when the cell monolayers were damaged with indomethacin for 1 hr. Furthermore, the damage done to 20-day-old Caco-2 cell monolayers by indomethacin was greater after 12 hr (data not shown) than after a 1-hr exposure (Fig. 1) of the intestinal cells to the damaging agent. Thus, these experiments demonstrated the direct cytotoxic potential of indomethacin against Caco-2 cells *in vitro*.

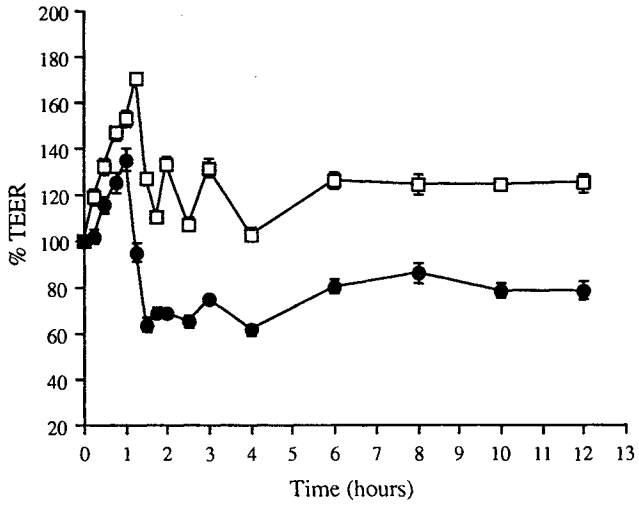

Fig. 1. Indomethacin-induced damage to 20-day-old Caco-2 cell monolayers. Changes in percentage TEER values as a function of time after application of indomethacin to the AP side of 20-day-old Caco-2 cell monolayers for 1 hr. The AP side of the Caco-2 cell monolayers was treated with HBSS in the absence (□) or presence (●) of 0.5 m*M* indomethacin for 1 hr (Phase I) and then incubated in medium for up to 12 hr (Phase II). Data are averages of nine determinations. Average TEER values at t = 1 min were considered as 100% (SEM) and were 392 (±30) $\Omega \cdot cm^2$.

Since the electrode required about 1 min to stabilize, we have reported the TEER values at 1 min as 100%. The maximum drop in TEER occurred at 150 min after application of indomethacin to the Caco-2 cells. Therefore, the TEER values at 150 min were evaluated for the extent of damage to the Caco-2 cells due to indomethacin and the protection afforded to the cells by the application of medium (control) or various concentrations (0.5 to 5 mg/mL) of sucralfate. On the other hand, the TEER values at 720 min served as an indication of the recovery of indomethacin-induced damage to the Caco-2 cells due to the application of medium (control) or various concentrations (0.5 to 5 mg/mL) of sucralfate.

Sucralfate was chosen as a model cytoprotective drug in this study due to its proven efficacy in the treatment of acute peptic ulcer disease (16,17). It has been shown to reduce the recurrence of gastric ulcers in a clinical study in man (18) and to protect the gastric mucosa against several types of acute experimentally induced injury (12). In addition, sucralfate has shown cytoprotective effectiveness against not only gastric but also duodenal ulcers (16). It is believed to exert its cytoprotective action by various mechanisms including (a) creating a physical protective barrier due to selective binding to the ulcer site (19), (b) inhibiting the action of pepsin and bile and blocking the "back-diffusion" of gastric acid and pepsin across the gastric membrane barrier *in vivo* (19), and (c) affecting the quantity and nature of mucus production by the gastric epithelium (20). Furthermore, sucralfate has been reported to preserve the integrity of the microvasculature and promote angiogenesis in its mechanism of cytoprotective action (21).

We observed that there was no damage to the Caco-2 cell monolayers due to either HBSS (Fig. 1) or 5 mg/mL sucralfate (the highest concentration of the drug used in this study; data not shown) in HBSS as determined by the TEER values of the cell monolayers. There was no significant decrease in TEER values with HBSS application to the AP surface of the Caco-2 cell monolayers after either 1 or 12 hr (data not shown) compared to control values at 1 min. As in our studies, sucralfate at 5 mg/mL was not observed to cause damage to cultured gastric mucosal cells *in vitro* (10). Additionally, *in vitro* and *in vivo* studies in rats have shown that sucralfate preserves the gastric membrane barrier integrity and does not significantly damage the luminal gastrointestinal epithelium (14,22).

Our results from the various control experiments demonstrated that 0.5 m*M* indomethacin was responsible for significant and reproducible damage to the Caco-2 cells, while neither HBSS nor sucralfate at the highest concentration (5 mg/mL) used in this study caused any damage to the cells according to the TEER values. Hence, we investigated the cytoprotective activity of sucralfate in the treatment and prevention protocols as described under Materials and Methods. The treatment studies were designed to investigate dose dependency of sucralfate in treating the damage caused by AP application of indomethacin to the Caco-2 cell monolay-

ers for 1 hr prior to the treatment with various concentrations (0.5, 2, or 5 mg/mL) of sucralfate. These experiments indicated the potential of sucralfate to enhance repair of the damage induced by indomethacin to the Caco-2 cells. In contrast, the prevention studies were designed to determine the protective effect due to the application of 0.5, 2, or 5 mg/mL sucralfate on the AP surface of the Caco-2 cell monolayers in the presence of the damaging agent. These prevention studies were designed to demonstrate the potential of sucralfate in preventing indomethacin-induced damage to the Caco-2 cells.

In the treatment studies, the Caco-2 cells were first damaged by indomethacin for 1 hr, followed by the application of 0.5, 2, or 5 mg/mL sucralfate in medium to determine if this cytoprotective agent induced recovery. The results from these treatment (and the control) experiments are presented in Fig. 2. When indomethacin was applied to the AP side of the Caco-2 cell monolayers for 1 hr, TEER values dropped significantly, to approximately 65%, at 150 min (phase I). When sucralfate at various doses (0.5 to 5 mg/mL) was included in phase II (1–12 hr) of the treatment, no significant enhancement of recovery was noted as measured by TEER values. These findings are similar to other *in vitro* and *in vivo* observations that sucralfate was unable to treat gastric ulcers induced by indomethacin but, rather, needs to be administered prior to the necrotizing agent in order to be cytoprotective (14,23).

The results obtained when sucralfate was used in the prevention studies are shown in Fig. 3. In contrast to the

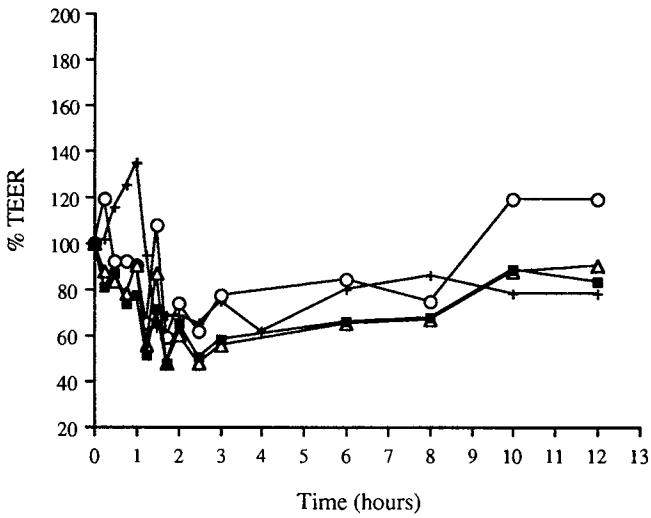

Fig. 2. Inability of sucralfate in treating (reversing) indomethacin-induced damage to 20-day-old Caco-2 cell monolayers. Changes in percentage TEER values as a function of time after application of indomethacin to the AP side of 20-day-old Caco-2 cell monolayers for 1 hr followed by treatment with various concentrations of sucralfate for up to 12 hr. The AP side of the Caco-2 cell monolayers was treated with 0.5 m*M* indomethacin in HBSS for 1 hr (Phase I) and then treated in the absence (+) (control) or presence of 0.5 (○), 2 (△), or 5 (■) mg/mL sucralfate in medium for up to 12 hr (Phase II). Data are averages of nine determinations. Average TEER values at t = 1 min were considered as 100% (SEM) and were 480 (±30) Ω·cm^2. The TEER values are not significantly different ($P > 0.005$) at 150 min and ($P > 0.03$) at 720 min compared to the control at 150 and 720 min, respectively.

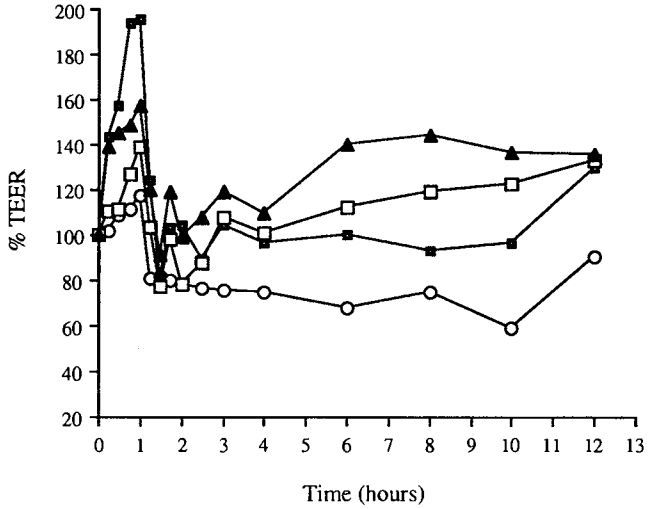

Fig. 3. Dose-dependent effectiveness of sucralfate in protecting (preventing) 20-day-old Caco-2 cell monolayers against indomethacin-induced damage. Changes in percent TEER values as a function of time after application of indomethacin to the AP side of 20-day-old Caco-2 cell monolayers for 12 hr in the presence of various concentrations of sucralfate. The AP side of the Caco-2 cell monolayers was treated with 0.5 m*M* indomethacin in the absence (○) (control) or presence of 0.5 (■), 2 (□), or 5 (▲) mg/mL sucralfate in HBSS for 1 hr (Phase I). During Phase II (1–12 hr) of the experiment, the cell monolayers were treated with the same concentration of indomethacin (control) or indomethacin in the presence of sucralfate as indicated above but HBSS was replaced by medium. Data are averages of nine determinations. Average TEER values at t = 1 min were considered as 100% (SEM) and were 405 (±24) Ω·cm^2. The TEER values are significantly greater ($P < 0.001$) compared to the control at 150 and 720 min, respectively.

treatment protocol, sucralfate was able to protect the Caco-2 cell monolayers significantly from indomethacin-induced damage (compared to the control) when applied to the cells in the presence of the damaging agent, as measured by changes in TEER values. This cytoprotective effect of sucralfate was dose dependent, with maximal protective effect achieved with 5 mg/mL of the drug (Fig. 3). Our observations that sucralfate is able to prevent damage to the human intestinal epithelial cells *in vitro* in a dose-dependent manner are consistent with a number of reported observations (12,24) which have demonstrated that sucralfate is an effective cytoprotective drug when administered orally before inducing gastric damage with indomethacin or other necrotizing agents. For instance, Okabe *et al.* (12) observed a dose-dependent cytoprotective effect of sucralfate in inhibiting indomethacin-induced gastric erosions when sucralfate was administered orally before subcutaneous injection of indomethacin to rats. Significant gastric protection by sucralfate against aspirin-induced gastric ulceration upon subcutaneous injection of aspirin and simultaneous administration of sucralfate was also reported in rhesus monkeys (24). Thus, it appears that based on the results obtained from this study in the Caco-2 model system and other literature reports (14,23), sucralfate is more effective in protecting against gastroduodenal damage than in treating the damage caused by prior administration of the damaging agent.

In an attempt to visualize the damage produced by in-

domethacin and the protective effect of sucralfate, TEM and SEM studies were conducted. The TEM and SEM studies were carried out after the prevention experiments in which 5 mg/mL sucralfate had shown significant protection against indomethacin-induced cellular damage as determined by the TEER values. Although SEM did not show conclusively the protection by sucralfate, TEM indicated that sucralfate was able to prevent gross intracellular damage (characterized by granulation), damage and (or) reduction in the number of microvilli induced by indomethacin (data not shown).

To determine if indomethacin induced changes in solute flux across Caco-2 cell monolayers, the transport of a hydrophilic ([^{3}H]methoxyinulin) and a lipophilic ([^{14}C]testosterone) solute was determined using the prevention protocols. The results shown in Fig. 4 indicate that damage due to the application of indomethacin alone to the Caco-2 cell monolayers for 12 hr resulted in a significant increase in the transport of the hydrophilic [^{3}H]methoxyinulin but no change in the flux of the lipophilic [^{14}C]testosterone compared to the control (Fig. 4). Compared to indomethacin-induced damage to Caco-2 cell monolayers, the presence of sucralfate in the indomethacin-treated cells caused a significant reduction in the flux of [^{3}H]methoxyinulin (Fig. 4). Therefore, in this study we observed that the transport of [^{3}H]methoxyinulin across indomethacin-damaged Caco-2 cell monolayers was greatly affected due to the influence of indomethacin on the paracellular pathway. In contrast, indomethacin-induced damage to the cells did not change the transcellular transport of the lipophilic [^{14}C]testosterone compared to the control (Fig. 4). Finally, our radiotracer ([^{3}H]methoxyinulin) transport data (Fig. 4) are consistent with the protection afforded by sucralfate against indomethacin-induced damage to Caco-2 cell monolayers as determined by the TEER values (Fig. 3) and the TEM results (data not shown).

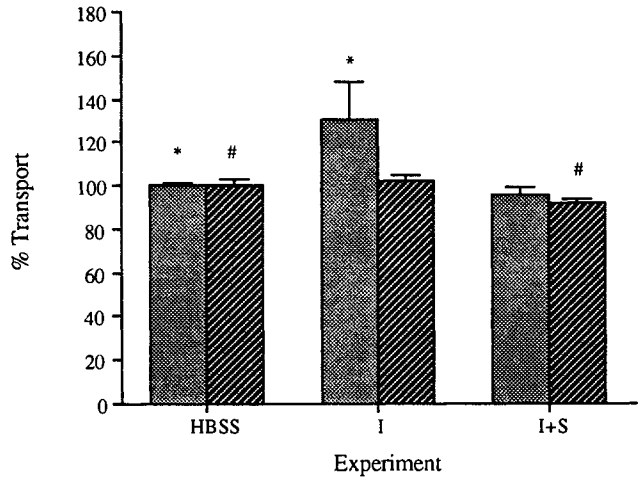

Fig. 4. Influence of indomethacin in the absence or presence of sucralfate on the transport of a hydrophilic and a lipophilic solute across 20-day-old Caco-2 cell monolayers. Percentage transport of [^{3}H]methoxyinulin (0.5 μCi/mL) (■) and [^{14}C]-testosterone (0.2 μCi/mL) (▨) relative to the control (HBSS) at 2 hr across 20-day-old Caco-2 cell monolayers after their exposure to 0.5 m*M* indomethacin (I) or 0.5 m*M* indomethacin in presence of 5 mg/mL sucralfate (I + S) for 720 min according to the protocols described under Materials and Methods. Percentage transport values of [^{3}H]methoxyinulin and [^{14}C]testosterone for the control (HBSS-treated) cell monolayers at 2 hr were 0.23 (0.003) and 43.78 (1.19), respectively. Data are averages of three determinations (±SEM). * and #, are significantly different according to unpaired *t* test ($P < 0.5$).

Keenan and Morris (14) observed that the cytoprotective effect of sucralfate against ethanol and HCl treatment in rats *in vivo* was evident only after formation of a continuous adherent coat of the drug over gastric epithelium. During our studies, we observed that the Caco-2 cell monolayers were coated by a white layer of sucralfate which was resistant to washing with HBSS. There have been other reports that sucralfate adheres to ulcerated tissue sites to form a physical protective barrier against injury induced by acid, pepsin, and bile salts (19). Similarly, the selective binding of sucralfate to gastric ulcer lesions in man was demonstrated by Nakazawa *et al.* (25). Gastric damage due to indomethacin has been proposed to occur as a result of a dual insult: by indomethacin-mediated direct acidic damage to the gastric cell membranes, thus increasing their permeability, followed by the deleterious effect of prostaglandin inhibition (26). It is likely that in this Caco-2 cell monolayer study, sucralfate significantly reduced or counteracted the direct indomethacin-induced damage to the human intestinal epithelial cells due to the presence of a sucralfate layer in the prevention studies. Hence, we observed significant direct cytoprotective activity of sucralfate in the prevention rather than the treatment experiments.

A characteristic feature that was noted during these experiments was the significant rise in the TEER values during the first hour after application of sucralfate to the Caco-2 cells in the prevention protocol (Fig. 3). Tarnawski *et al.* (27) had reported that oral administration of sucralfate to rats *in vivo* causes functional and morphological changes in the normal gastric mucosa such as the apical release of mucus and increased mitotic activity, including the adherence of sucralfate to the gastric mucosal surface. Furthermore, in animal and human studies, long-term treatment with antacids was shown to increase the mucosal layer thickness and to exert a trophic effect on the gastric mucosa (28). In this study, whether the rapid increase and high TEER values of the Caco-2 cell monolayers observed at 1 hr after application of sucralfate (Fig. 3) could be due to the direct effect of sucralfate on the morphology of the Caco-2 cells is not clear.

Our results with sucralfate indicate that it can produce cytoprotective effects at neutral pH. Such direct protective action of sucralfate on gastric mucosal cells *in vitro* has been previously reported (10). Danesh *et al.* (29) also reported significant cytoprotective ability of sucralfate under both acidic and neutral pH conditions *in vivo* in rats. Other investigators (30) have reported that sucralfate provided gastric mucosal cytoprotection at lower intragastric pH (1 or 2) but not at neutral pH.

It should be noted that although we observed the direct cytoprotective effect of sucralfate on the Caco-2 cells, this *in vitro* cellular model does not include the normal vascular factors and proliferative effect of lower layers in the epithelial tissue that contribute to the healing effect *in vivo* (21). It has been suggested that this endogenous contribution toward

the total cytoprotection can be highly significant once sucralfate forms the protective physical barrier (adherent coat) over the ulcerous sites *in vivo* (21). Thus, sucralfate has been reported to promote the growth of blood vessels (angiogenesis) at the ulcer site, to enhance the mucosal blood flow, and to preserve the integrity of the microvasculature (21), all of which contribute significantly toward the total cytoprotection afforded by sucralfate against gastric mucosal ulcers *in vivo*.

The effect of passage numbers of the Caco-2 cells on the cytotoxic or cytoprotective response to various agents is not known. Furthermore, this Caco-2 cell line, which is a human colorectal carcinoma cell line, differs from primary cell cultures (such as the gastric cells) (10) or *in vivo* cytoprotective models (12) reported in the literature for assessing cytoprotective activity. These differences may or may not allow direct comparison of cytotoxic or cytoprotective responses observed in this cell culture model with various *in vitro* (10,14) and *in vivo* (12) models.

In conclusion, our findings suggest that human intestinal epithelial (Caco-2) cell monolayers may be a useful *in vitro* cell culture model for rapid screening and evaluating potential cytoprotective drugs including their pharmaceutical preparations. This cell culture model could also be used effectively for studying the mechanism of induced damage and cytoprotection due to various necrotizing and cytoprotective agents, respectively. Further, this study illustrates the utilization of this experimental system for monitoring cellular damage, recovery, and cytoprotective activity of agents by measuring transepithelial electrical resistance of confluent monolayers of cultured human intestinal epithelial (Caco-2) cells.

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