Effects of phenylbutazone, indomethacin, prostaglandin E_2, butyrate, and glutamine on restitution of oxidant-injured right dorsal colon of horses in vitro

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Objective—To study the effects of phenylbutazone, indomethacin, prostaglandin E_2 (PGE_2), glutamine, and butyrate on restitution of oxidant-injured right dorsal colon of horses in vitro.

Sample Population—Right dorsal colon from 9 adult horses euthanatized for reasons other than gastrointestinal tract disease.

Procedures—Mucosal segments from the right dorsal colon were injured via exposure to HOCl and incubated in Ussing chambers in solutions containing phenylbutazone, indomethacin, indomethacin and PGE_2, glutamine, and butyrate. Transepithelial resistance and mucosal permeability to mannitol were measured, and all mucosal segments were examined histologically.

Results—The HOCl-injured mucosa had lower resistance and higher permeability to mannitol, compared with control tissue. Histologic changes were also evident. Resistance of HOCl-injured mucosa recovered partially during the incubation period, and glutamine improved recovery. Phenylbutazone and indomethacin increased resistance, but these increases were not significant. Butyrate and PGE_2 had no effects, compared with nontreated HOCl-injured tissues. Mucosal permeability to mannitol was lower in glutamine-treated tissue, compared with nontreated tissue. Histologic changes reflected the resistance and permeability changes.

Conclusions and Clinical Relevance—According to our findings, phenylbutazone and indomethacin do not seem to interfere with restitution of oxidant-injured mucosa of equine colon in vitro, and glutamine could facilitate mucosal restitution. *(Am J Vet Res* 2004;65:1589–1595)

In horses, 2 life-threatening and common diseases of the colon, strangulating obstruction and colitis, are characterized by diffuse loss of surface epithelium and variable damage to crypt cells. Repair of epithelium is crucial for recovery and involves 2 rapid processes that occur within hours: epithelial restitution and tightening of intercellular junctions (paracellular pathways) in remaining epithelial cells.[1,2] During epithelial restitution, cells at the edge of the area denuded of epithelial cells migrate over it until the defect is covered with flattened epithelial cells.[1,2] A slower process of repair that occurs within days of injury involves proliferation of surviving crypt cells.[1] Processes leading to restitution of epithelium and decreased tight junction permeability (increased paracellular resistance) are of particular interest because they begin immediately after an injury occurs and can be influenced by pharmacologic agents.[3]

The role of prostaglandins in epithelial restitution is not clear. Prostaglandins can protect mucosal epithelium against injury in rat small intestine and porcine and human colon.[4,5,a] The nonspecific **cyclooxygenase (COX)** inhibitor, indomethacin, increased the severity of mucosal epithelial damage induced in porcine colon by bile salts, but the protective effects of endogenous prostaglandins in this tissue were not mediated through an effect on restitution.[5] Prostaglandins do appear to be involved in intestinal epithelial restitution stimulated by growth factors.[6] The effects of prostaglandins and COX inhibitors on repair of equine intestinal mucosal epithelium are important because most horses with gastrointestinal tract disease are routinely treated with **nonsteroidal anti-inflammatory drugs (NSAIDs)** which are predominantly nonspecific COX inhibitors. These drugs can be toxic to equine gastrointestinal mucosal epithelium and may have a role in the pathogenesis of gastric and colonic ulceration, right dorsal colitis, large colon impaction, and cecal impaction and rupture.[7,8]

Nonsteroidal anti-inflammatory drugs can interfere with mucosal epithelial repair in equine and porcine jejunum in in vivo and in vitro models of epithelial injury[9-11,b]; however, these effects may not be applicable to the colon.[12,13] In healthy segments of equine large colon, indomethacin, phenylbutazone, and flunixin-meglumine treatment resulted in epithelial cell necrosis and apoptosis, decreases in transepithelial resistance, and increases in epithelial permeability to mannitol.[12,13,c] In bile-injured mucosa from the pelvic flexure of horses, flunixin meglumine and etodolac had no effect on recovery of transepithelial electrical resistance.[13] The differences in response between injured colon and jejunum in vitro could relate to differences in water and ion transport in these tissues.[14]

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Glutamine is the most abundant amino acid in mammalian plasma and is found in high concentrations in cells.[15] It serves as a nutrient for enterocytes[16] and induces proliferation of enterocytes via stimulation of DNA synthesis.[17,18] Glutamine is required for glutathione synthesis, and IV infusion of glutamine partially maintained gastrointestinal glutathione concentrations and resulted in decreased mucosal injury that resulted from ischemia-reperfusion injury in rats, compared with controls.[19] In an epithelial cell line exposed to heat and an oxidant, glutamine protected cells in part via induction of heat shock protein, HSP70.[20] Glutamine also increased transepithelial resistance and decreased mannitol permeability of acid-injured rat colonic mucosa in vitro[21] and has been proposed as a potential treatment to promote intestinal repair in animals with enteritis.[22]

Butyrate is a short-chain fatty acid that serves as a nutrient for epithelial cells. Butyrate may have an important role in support of epithelial repair mechanisms[23] and stimulates cell proliferation in colonic crypts.[24-26] In cell culture, butyrate reduces paracellular permeability[d] and accelerates wound healing.[e] Infusion of short-chain fatty acids into the proximal colon accelerated the rate of healing of distal colonic anastomoses in rats.[27] However, in 2 in vitro models of HCl- or deoxycholate-induced injury of rat colonic mucosa, butyrate failed to influence epithelial restitution.[21,f]

The purpose of the study reported here was to evaluate the effects of phenylbutazone, indomethacin, **prostaglandin E_2 (PGE_2)**, glutamine, and butyrate on restitution of oxidant-injured mucosal epithelium of equine right dorsal colon in vitro. We hypothesized that phenylbutazone and indomethacin would impair restitution and that glutamine and butyrate would increase the rate of epithelial restitution. We chose phenylbutazone because it is widely used in horses for the treatment of a variety of diseases and has been associated with NSAID-related toxicoses. Indomethacin was used because it has been the standard NSAID in previous in vitro studies[28,g] of equine colon. The right dorsal colon was examined because it is a target organ for harmful effects of NSAIDs in horses[29-31] and the site of severe mucosal changes in horses with colonic ischemia.[7,32]

Materials and Methods

Tissue preparation—The study was approved by the Institutional Animal Care and Use Committee of the University of Illinois. Nine adult horses were euthanatized for reasons other than gastrointestinal tract disease, via administration of pentobarbital sodium (88 mg/kg, IV). Immediately after euthanasia, a full-thickness segment of colon was removed from the lateral aspect of the right dorsal colon, close to its attachment to the base of the cecum; intestinal contents were removed via rinsing with isotonic saline (0.9% NaCl) solution. The tissue was divided into 2 full-thickness segments, and each segment was transported to the laboratory in cold (4°C) **Krebs-Ringer-bicarbonate (KRB)** solution (112mM NaCl, 25mM $NaHCO_3$, 10mM glucose, 5mM KCl, 3mM Na acetate, 2.5mM $CaCl_2$, 1.2mM $MgSO_4$, 1.2mM KH_2PO_4, and 0.01mM mannitol).

Preincubation conditions—To induce mucosal epithelial injury, full-thickness segments of colon were submerged in 3mM HOCl for 10 minutes. Flasks containing tissues and HOCl were placed in a water bath at 37°C, and contents were perfused with 95% O_2 and 5% CO_2. When 10 minutes had elapsed, each tissue segment was rinsed with KRB solution and pinned onto a rubber surface, mucosal surface facing up, and submerged in fresh KRB solution at 20° to 22°C. While the solution was being constantly perfused with 95% O_2 and 5% CO_2, mucosal sheets were removed via sharp dissection and mounted in Ussing chambers. These chambers had an aperture of 1.13 cm^2, and a solution volume of 7.0 mL bathed each tissue surface. In the chambers, the bathing solution was maintained at pH 7.4 via constant perfusion with 95% O_2 and 5% CO_2 and at 37°C by circulating the solution by use of a gas lift through water-jacketed reservoirs.

Incubation conditions and measurements—Tissue segments were assigned to 7 treatment groups, and each horse provided tissues for an entire set of experiments. One tissue that served as a control was not exposed to HOCl; mucosal sheets were dissected from control tissue and incubated in KRB solution only, in Ussing chambers. A mucosal sheet from one of the tissues exposed to HOCl was incubated in KRB solution only; this nontreated HOCl-injured tissue was used to compare effects of treatments on restitution of HOCl-injured mucosal epithelium. Mucosal sheets from the remaining tissues exposed to HOCl were each incubated in KRB solution that contained one of the following (final concentrations): 1 mg of phenylbutazone/mL, 10^{-6}M indomethacin; 10^{-6}M indomethacin and 10^{-6}M PGE_2, 20mM butyrate; and 2mM glutamine. All tissues were incubated for 4 hours.

During the incubation period, the **short circuit current (Isc)** was recorded on voltage clamps through Ag-$AgCl_2$ electrodes connected to 4% agar bridges of bathing solution. The ends of the bridges were placed within 1 to 2 mm of the tissue surface. The **potential difference (PD)** between the calomel electrodes, when the tissue was not in place, was nullified by an offsetting voltage, and the transepithelial PD, with the tissue mounted, was recorded by use of a potentiometer.[h] The transepithelial PD was automatically and continuously nullified by the passage of an external current (ie, Isc) across the tissue, by use of an automatic voltage-current clamp amplifier,[i] except for 10-second periods at 15-minute intervals, when spontaneous tissue PD was measured. The transepithelial electrical resistance was calculated according to Ohm's law (resistance = PD/Isc) by use of spontaneous PD and Isc. The effect of fluid resistance on the Isc was automatically corrected.[h] Resistance in Ohms • cm^2 was used as a measure of integrity of the colonic mucosa and permeability of the paracellular pathway.

Mannitol permeability—Radiolabeled (^3H) mannitol[j] was added to the bathing solution on the epithelial side of each tissue segment at the beginning of the experiment. For scintillation counting, solution samples (250 mL each) were collected from both sides of the tissue after a 60-minute equilibration period and again 2 hours later.[33] Scintillation counting was performed by use of a commercial scintillation counter.[k] The transmucosal flux of mannitol was expressed as percentage of scintillation counts detected on the epithelial side of the tissue that moved to the serosal side of the tissue per hour.[33] Mannitol permeability (determined by the transmucosal flux of mannitol) was determined as an additional measure of integrity of the colonic mucosa and permeability of the paracellular pathway.[33]

Histologic examination—Fresh tissues, tissues exposed to 3mM HOCl for 10 minutes, and all tissues collected after the 4-hour incubation period were fixed in 4% formalin, embedded in paraffin, and cut into 5-µm thick sections. Slides were stained with H&E. An investigator (AKR) unaware of the identity of treatment groups performed all histologic evalua-

tions. This investigator was trained and supervised during the study by a veterinary histologist (JACE) and a board-certified veterinary pathologist (MW).

Histomorphometric measurements—A computer-based software program[l] was used for histomorphometric analysis of the images obtained via light microscopy. For all measurements, 3 microscopic fields (at various magnifications) of each tissue were examined. The magnifications were the result of combining the microscopic magnification and the enlargement provided by the software program. Mucosal height (measured at a magnification of 260×) was expressed as the mean vertical distance between tracings of the muscularis mucosae and the epithelial surface.[13] Epithelial cell height was expressed as the mean vertical perpendicular (to the basement membrane) distance between tracings of the basement membrane and the cell apex (1,040×).[13] Mean cell width was calculated by use of the width of 5 clearly identifiable epithelial cells in 3 sets in each field (1,040×). The length of mucosal surface denuded of epithelium was measured (650×) and expressed as a percentage of the total surface length of the mucosa in the section. Lifted epithelium was defined as a group of at least 5 epithelial cells that were separated from the mucosal surface at the level of the basement membrane but were still attached to adjacent epithelial cells that held them in place. The length of lifted epithelium was expressed as a percentage of the total surface length of the mucosa in the section (650×). Detached cells were defined as cells that were morphologically similar to healthy cells but were separated from the basement membrane in groups of at least 5 cells and were completely detached from adjacent epithelium. The length of detached cells was measured (650×) and expressed as a percentage of the total surface length of the mucosa in the section. Sloughed cells were defined as cells undergoing necrosis and sloughing individually from the epithelial surface. The number of sloughed cells was counted in each field (1,040×), and the mean number per 0.1-mm mucosal surface length was calculated.

Statistical analyses—Data were expressed as least square means ± SEM. A value of $P < 0.05$ was considered significant. A statistical software program[m] was used for analysis. Data that were not normally distributed were log transformed or ranked before repeated measures ANOVA was performed. Whenever a significant *F* test for treatment, time, or interaction was found, appropriate Bonferroni adjusted *P* values were used for each family of comparisons. For histomorphometric measurements, the following comparisons were made: fresh tissue compared with control tissue after 4 hours of incubation, fresh tissue compared with tissue immediately after HOCl exposure, tissue immediately after HOCl exposure with nontreated HOCl-injured tissue after 4 hours of incubation, and all treated HOCl-injured tissues after 4 hours of incubation with nontreated HOCl-injured tissue after 4 hours of incubation.

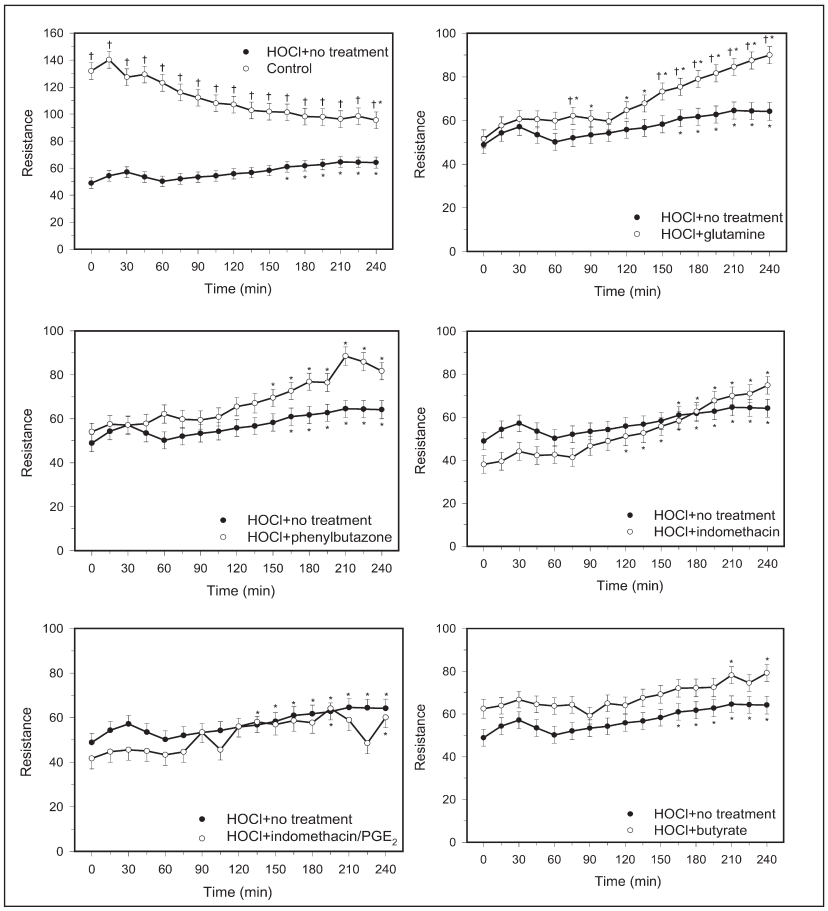

Figure 1—Resistance (Ohms • cm^2) of mucosa from right dorsal colon of 9 horses, in control (not exposed to HOCl) and treatment groups (exposed to HOCl and treated with glutamine, phenylbutazone, indomethacin, indomethacin and prostaglandin E_2 [PGE_2], or butyrate) compared with nontreated HOCl-injured mucosa over a 4-hour incubation period. Data are expressed as least squares mean ± SEM. *Significant ($P < 0.05$) difference in resistance between that time point and the time point at beginning of the incubation period (0 minutes) for each group. †Significant ($P < 0.05$) difference between groups at each time point.

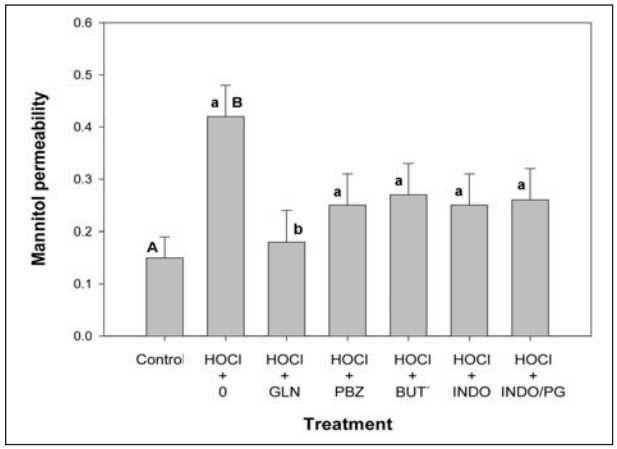

Figure 2—Permeability to mannitol (% per hour) in control mucosa (not exposed to HOCl); nontreated HOCl-injured mucosa (HOCl + 0 [no treatment]); and mucosa exposed to HOCl and treated with glutamine (HOCl + GLN), phenylbutazone (HOCl + PBZ), butyrate (HOCl + BUT), indomethacin (HOCl + INDO), and indomethacin and prostaglandin E_2 (HOCl + INDO/PG) from the right dorsal colon of 9 horses. Data are expressed as least squares mean ± SEM. Values for groups labeled with a or b are significantly ($P < 0.05$) different than values for groups labeled with corresponding A or B.

Results

Transepithelial electrical resistance—Exposure to HOCl resulted in significantly lower transepithelial

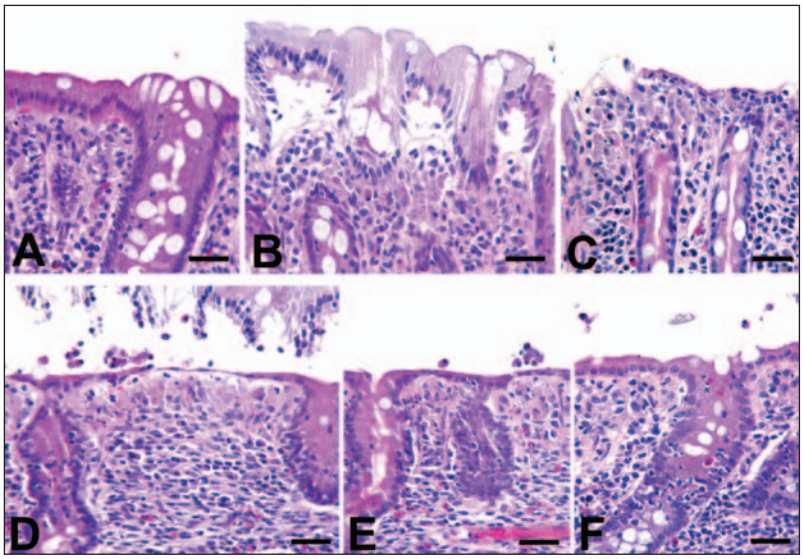

Figure 3—Photomicrographs of histologic sections of mucosa of fresh right dorsal colon (A) and the same tissue exposed to HOCl immediately before (B, C) and 3 (D) and 4 hours (E, F) after incubation in Krebs-Ringer-bicarbonate solution. Notice epithelial cells in fresh tissue are tall and narrow and firmly attached to the basement membrane (A). In tissue exposed to HOCl, epithelial cells are lifted from the basement membrane (B) and eventually slough, leaving a denuded mucosal surface (C). Remaining epithelial cells from crypt regions migrate across the denuded mucosal surface, covering it with short, wide cells (D). As the number of epithelial cells on the mucosal surface increases, the individual cells become taller and acquire a cuboidal shape (E, F). H&E stain; Bar = 50 mm.

electrical resistance, compared with control tissues (**Figure 1**; $P < 0.001$). During the 4-hour incubation period, resistance in HOCl-injured tissues was able to partially recover; however, values never reached those for control tissues. Incubation with glutamine resulted in a faster recovery and higher resistance values ($P = 0.042$) than incubation in KRB after HOCl-induced injury, and incubation with phenylbutazone had a similar effect, although the increase in resistance was not significantly ($P = 0.073$) different than nontreated HOCl-injured tissue. Treatment with indomethacin resulted in a similar nonsignificant ($P = 0.28$) effect, and the addition of PGE_2 had no effect on the response to indomethacin ($P = 0.28$). Butyrate did not change resistance measurements ($P = 0.17$).

Mannitol permeability—Tissue permeability to mannitol was significantly ($P < 0.001$) greater after exposure to HOCl, compared with the control group. Only tissue treated with glutamine had significantly ($P < 0.001$) lower mannitol permeability, compared with

Table 1—Histomorphometric measurements of fresh mucosa; control mucosa (not exposed to HOCl); nontreated HOCl-injured mucosa (HOCl + nontreated); and mucosa exposed to HOCl and treated with glutamine (HOCl + GLN), phenylbutazone (HOCl + PBZ), butyrate (HOCl + BUT), indomethacin (HOCl + INDO), and indomethacin and prostaglandin E_2 (HOCl + INDO/PG) from the right dorsal colon of 9 horses, before (0 minutes) and after incubation (240 minutes) in Krebs-Ringer-bicarbonate solution. Data are expressed as least squares mean (SEM).

Group/ time (min) (mm)	Mucosal height (mm)	Epithelial cell height (μm)	Epithelial cell width (mm)	Denuded mucosa (%)	Lifted epithelium (%)	Detached epithelium (%)	Sloughed epithelial cells/0.1 mm
Fresh/0	380.9 (19)	31.0 (1.6)	5.5 (0.3)	0 (1.5)	0 (2.8)	0 (5.9)	0.1 (1.0)
Control/240	306.6[a] (21.2)	17.8[a] (1.8)	7.2[a] (0.4)	4.9 (1.7)	0 (3.2)	0.7[c] (6.6)	3.4[a] (1.1)
HOCl + nontreated/0	426.6 (21)	31.9 (1.6)	6.1 (0.3)	0.8 (1.7)	14.8[a] (3.1)	0.5 (6.5)	0 (1)
HOCl + nontreated/240	348.7[b] (19.7)	15.3[b] (1.6)	8.2[b] (0.3)	1.8 (1.6)	0[b] (2.9)	14.3[b] (6.1)	6.1[b] (1)
HOCl + GLN/240	247.7[c] (16)	13.5 (1.4)	8.2 (0.3)	1.8 (1.3)	0 (2.4)	23.5 (5.0)	3.5 (0.8)
HOCl + PBZ/240	273.5[c] (16)	17.4 (1.4)	6.8[c] (0.3)	2.2 (1.3)	0 (2.4)	23.1 (5.2)	4.9 (0.8)
HOCl + BUT/240	306.3 (21.2)	16.3 (1.8)	7.7 (0.4)	2.6 (1.7)	7.9 (3.2)	20.9 (6.6)	2.9 (1.1)
HOCl + INDO/240	235.5[c] (16)	16.1 (1.4)	7.3 (0.3)	1.0 (1.3)	0 (2.4)	15.4 (5.0)	4.6 (0.8)
HOCl + INDO/PG/240	226.8[c] (22.3)	16.6 (1.9)	7.5 (0.4)	2.9 (1.8)	4.7 (3.3)	9.0 (6.9)	5.5 (1.1)

[a]Significant ($P < 0.05$) difference, compared with fresh tissue. [b]Significant ($P < 0.05$) difference, compared with tissue immediatly after HOCl exposure. [c]Significant ($P < 0.05$) difference, compared with nontreated HOCl-damaged tissue after 240 min of incubation.
Bonferroni adjusted P values were used for each family of comparisons.

HOCl-injured nontreated tissue. All other treated tissues had lower mannitol permeability; however, the differences were not significant (**Figure 2**).

Histomorphometric measurements—Compared with fresh tissue (**Figure 3**), control tissue after 4 hours of incubation had lower mucosal height ($P = 0.010$; **Table 1**), greater epithelial cell width ($P = 0.002$), lower epithelial cell height ($P < 0.001$), and a greater number of sloughed epithelial cells ($P < 0.001$). Exposure to HOCl resulted in an immediate increase in percentage of lifted epithelial cells, compared with fresh tissues ($P < 0.001$). Four hours of incubation of tissues exposed to HOCl resulted in lower mucosal height ($P = 0.008$), lower epithelial cell height ($P < 0.001$), and greater epithelial cell width ($P < 0.001$) than in tissue immediately after HOCl exposure. Also, there was a lower percentage of lifted epithelial cells ($P < 0.001$), higher percentage of detached epithelial cells ($P = 0.023$), and a greater number of sloughed epithelial cells ($P < 0.001$), compared with tissues immediately after HOCl exposure. When all treated tissues after 4 hours of incubation were compared with nontreated HOCl-injured tissues 4 hours after incubation, the most consistent finding was a lower mucosal height in treatment groups (not significantly different for butyrate or control tissues [$P < 0.001$ to 0.004 for all other tissues]). In the same comparison, epithelial cell height was greater and epithelial cell width narrower in the treatment groups; however, these differences were not significant except for epithelial cell width in phenylbutazone-treated tissues ($P = 0.003$). Control tissues had a lower percentage ($P = 0.028$) of detached epithelial cells, compared with HOCl-injured tissues after 4 hours of incubation.

Discussion

In our model of oxidant-injured right dorsal colon, mucosal injury was induced with exposure to HOCl and partial epithelial restitution occurred during the 4-hour incubation period. Both transepithelial electrical resistance measurements and histologic findings revealed that restitution occurred, as evident by an increase in resistance with incubation time and covering of the denuded mucosal surface with wide and flat epithelial cells. However, epithelial restitution was not complete; transepithelial electrical resistance did not reach values measured in control tissues, and histologic changes were still detected after the 4-hour incubation period. The greater mannitol permeability after HOCl exposure was an additional measure of severity of mucosal injury. The time required for complete repair of equine colon in vitro is not known; however, the tissue preparation used in our study would not be expected to survive for a sufficient period of time to allow for complete repair. When treated tissues were compared with tissues exposed only to HOCl, the largest number of differences was found in resistance measurements, followed by measurements of mannitol permeability. Both measurements revealed that glutamine had a beneficial effect on restitution of mucosal integrity. Neither measurement revealed adverse effects caused by any of the other substances. Comparison between treatment groups by use of histomorphometry was less rewarding. Mucosal height was the only parameter consistently different between treatment groups and tissues exposed to HOCl only. Changes in other parameters that would indicate a faster or more efficient restitution in treated tissues more clearly (ie, epithelial cell height and width) were not significant. It is possible that larger numbers of tissue samples in the treatment groups would have made histologic evaluation more rewarding. Tissue injury in our model was severe, and repair was likely to be less efficient in vitro than in vivo. Also, functional determinants of mucosal barrier integrity (ie, resistance) tend to lag behind morphologic repair.[3] Our model was limited in its ability to mimic situations in vivo. Mucosal injury was induced in vitro, and HOCl-induced injury does not necessarily reflect the nature of injury encountered by right dorsal colon in vivo. Also, the severity of the injury induced may not reflect the severity of injury most commonly encountered in vivo, and the colon may respond differently and more effectively to a similar injury in vivo. However, in our model, injury was easy to induce and of comparable extent in all tissues, and our measurements revealed differences in mucosal restitution between control and treatment groups. The advantages of this model included its ease and the ability to closely and continuously monitor restitution processes and eliminate as many variables between individuals as possible.

Glutamine was the only substance that significantly increased the efficiency of restitution. This result is comparable to the beneficial effects of glutamine found in previous studies[19-21] and may suggest a potential use for glutamine in the treatment of horses with gastrointestinal tract disease. Butyrate did not influence mucosal restitution in our model; this finding is similar to those of other in vitro studies[20,f] that used rat colonic mucosa. In humans, the preferred epithelial nutrient in the proximal colon is glutamine, whereas in the distal colon it is n-butyrate.[34] Beneficial effects of butyrate on intestinal epithelial cell proliferation and restitution have been detected in vivo[25,26] and in vitro (in the in vitro study, a cell culture medium rather than an electrolyte solution was used).[24] The beneficial effects of butyrate may depend on substances not present in an electrolyte solution; treatment of horses with gastrointestinal tract disease may still be indicated. Absorption of butyrate by rat colon appears to be pH-dependent,[35] and a lower pH of the bathing solution than the one used in our study may have resulted in induction of beneficial effects. However, pH manipulations of bathing solution can complicate ion fluxes and electrophysiologic measurements.

The lack of detrimental effects of phenylbutazone and indomethacin on mucosal restitution was an interesting finding. Phenylbutazone was more toxic to colonic mucosa than ketoprofen or flunixin meglumine when administered IV in high doses, but only a small number of horses were studied.[8] After administration IV or PO, phenylbutazone is metabolized to its active form, oxphenbutazone. This metabolic step is not expected to occur in vitro and may explain differences in results between in vitro and in vivo studies.

Nonsteroidal anti-inflammatory drugs can induce apoptosis of equine colonic epithelial cells,[12] and induction of apoptosis in our model may have improved the efficiency of restitution via more efficient removal of injured cells, thereby counteracting potential detrimental effects of phenylbutazone. The addition of PGE_2 did not change measurements in tissues treated with indomethacin; this may indicate that inhibition of PGE_2 synthesis alone is not involved in epithelial restitution. In the pelvic flexure of the colon in horses, in vitro restitution of bile-injured mucosa was not influenced by treatment with flunixin meglumine or the specific COX-2 inhibitor etodolac, despite significantly decreased prostanoid production.[13] In healthy pelvic flexure of the colon in vitro, equivalent doses of nonspecific COX inhibitors decreased transepithelial electrical resistance, whereas specific COX-2 inhibitors did not.[13] Therefore, COX-1 and COX-2 may act differently in injured and noninjured gastrointestinal mucosa.

In our study, we were able to confirm only one of our hypotheses: that glutamine improves the efficiency of mucosal restitution in our model of oxidant-injured right dorsal colon. Phenylbutazone and indomethacin did not impair mucosal restitution; PGE_2 did not affect measurements; and butyrate did not affect mucosal restitution. These findings may be relevant for the proposed preparation of customized rinse solutions for protection of equine intestinal mucosa. A solution that could be protective for equine jejunal mucosa may not protect equine colonic mucosa,[36] largely because prostaglandins seem to play a different role in mucosal restitution in equine colon, compared with jejunum.[11]

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[h]EVC4000 Multichannel Voltage/Current Clamp (potentiometer), World Precision Instruments, Sarasota, Fla.

[i]EVC4000 Multichannel Voltage/Current Clamp (amplifier), World Precision Instruments, Sarasota, Fla.

[j]New England Nuclear, Boston, Mass.

[k]TriCarb 1600 TR, Packard Instrument Co, Downers Grove, Ill.

[l]Image Pro Express 4.5, Media Cybernetics Inc, Calif.

[m]SAS 8e, SAS Institute Inc, Cary, NC.

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Correction: Distribution of mRNA coding for subtypes of adrenergic receptors in the gastrointestional tract of dairy cows

In the article "Distribution of mRNA that codes for subtypes of adrenergic receptors in the gastrointestinal tract of dairy cows," published August 2004 (2004;65:1142-1150), the following item was incorrect.

In Table 2 on page 1145, the capital letters for the location abomasal corpus in column β_1 were incorrectly listed as F, P, T, W, Z, and U. The correct letters are F, P, T, W, Z, BB, and CC.