Effects of acetylcysteine and migration of resident eosinophils in an in vitro model of mucosal injury and restitution in equine right dorsal colon

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Objectives—To evaluate the in vitro protective effects of acetylcysteine and response of resident mucosal eosinophils in oxidant-induced injury to tissues of right dorsal colon of horses.

Animals—9 adult horses.

Procedure—Gastrointestinal mucosa was damaged in vitro with 3 mM hypochlorous acid (HOCl) with and without prior exposure to 6 mM acetylcysteine. Control tissues were not exposed to HOCl or acetylcysteine. Control and damaged tissues were incubated in Krebs-Ringer-bicarbonate solution and tissue permeability to radiolabeled mannitol was also used to assess mucosal barrier integrity. Tissues were examined by light microscopy before and after HOCl exposure and during and after incubation.

Results—Exposure to HOCl caused tissue damage and decreased tissue resistance. Restitution did occur during the incubation period. Eosinophils were located near the muscularis mucosae in freshly harvested tissues and migrated towards the luminal surface in response to HOCl-induced injury. Compared with tissues treated with HOCl without acetylcysteine, pretreatment with acetylcysteine prevented HOCl-induced tissue damage, changes in resistance, and histologically detectable eosinophil migration. The permeability to mannitol increased to the same extent in tissues treated with HOCl alone or with acetylcysteine and HOCl.

Conclusions and Clinical Relevance—Eosinophils migrated toward the mucosal surface in equine colon in response to oxidant-induced damage in vitro. This novel finding could be relevant to inflammation in equine colon and a pathophysiologic feature of many colonic diseases. Acetylcysteine protected the mucosa against oxidant-induced injury and may be useful as a treatment option for various gastrointestinal tract disorders in horses. (Am J Vet Res 2003;64:1205–1212)
Eosinophils can generate large amounts of leukotriene C₄, which increases vascular permeability, mucus secretion, and smooth muscle contraction. Additionally, activated eosinophils generate a large number of cytokines, indicating that they have the potential to sustain or augment the immune response, inflammatory reaction, and tissue repair. Eosinophils can also initiate antigen-specific immune responses by acting as antigen-presenting cells. In diseased intestinal tissue, eosinophils can be found in all strata of the tissue, and there is a correlation between degree of eosinophil accumulation and disease severity. However, there is currently only a limited understanding of the biological and pathologic significance of eosinophils in the gastrointestinal tract.

Eotaxin is a chemokine considered to be responsible for eosinophil recruitment into the gastrointestinal tract, and its mRNA is strongly expressed in equine jejunum and colon. This could explain why the gastrointestinal tract mucosa of clinically normal horses has large numbers of resident eosinophils. In addition, accumulation of eosinophils has been described in models of acute colitis and of ischemia and reperfusion injury in horses. Clinical cases of eosinophilic gastroenteritis have been reported in horses. The cause of eosinophil accumulation is unknown in these cases but has been attributed to immune-mediated processes, such as food allergy or parasite infection.

The purpose of the study reported here was to evaluate in vitro the protective effects of acetylcysteine and investigate the response of resident mucosal eosinophils in oxidant-induced injury to tissues of the right dorsal colon in horses. This study was designed to test our hypotheses that acetylcysteine can protect colonic mucosa against oxidant-induced injury in vitro, that resident gastrointestinal eosinophils will migrate towards the luminal surface in response to oxidant-induced injury in vitro, and that this migration can be inhibited by blocking tissue damage with acetylcysteine. We chose to study these mechanisms in equine right dorsal colon because this segment of the gastrointestinal tract is affected in 3 important diseases of the horse: large colon strangulation, infectious colitis, and right dorsal colitis.

Materials and Methods

Horses and tissue collection—The Institutional Animal Care and Use Committee of the University of Illinois approved the following procedures. Nine adult horses donated to the University of Illinois Large Animal Clinic were euthanized with pentobarbital sodium (88 mg/kg, IV) for reasons other than gastrointestinal tract diseases. A full-thickness segment of tissue was immediately removed from the lateral aspect of the right dorsal colon (close to its attachment to the base of the cecum) and rinsed of intestinal contents. The tissue was cut into full-thickness segments, and each segment was transported to the laboratory in cold (4°C) Krebs-Ringer-bicarbonate (KRB) solution: this solution contained 112mM NaCl, 25mM NaHCO₃, 10mM glucose, 5mM KCl, 3mM Na acetate, 2.5mM CaCl₂, 1.2mM MgSO₄, 1.2mM KH₂PO₄, and 0.01mM mannitol.

Preincubation conditions—Each horse provided enough tissue for both treatment groups and the control group. Full-thickness segments of right dorsal colon were subjected to 1 of 2 preincubation protocols involving either acetylcysteine with hypochlorous acid (HOCl) or HOCl alone. Tissues treated with acetylcysteine and HOCl were first incubated with 6mM acetylcysteine in KRB solution for 10 minutes; after this pretreatment, tissues were exposed to 3mM HOCl and 6mM acetylcysteine in KRB solution for an additional 10 minutes. Tissues treated with HOCl alone were exposed to 3mM HOCl in KRB solution for 10 minutes. During these preincubation steps, flasks containing tissues were placed in a water bath at 37°C, and the contents were perfused with a gas mixture of 95% O₂ and 5% CO₂. After preincubation, these tissues were rinsed with an excess of KRB solution to remove any remaining HOCl or acetylcysteine. Control tissues were kept in KRB solution (without additions) and not subjected to preincubation steps.

Each preincubated and control tissue segment was pinned on a rubber surface with the mucosal surface visible and submerged in a fresh batch of KRB solution at 20 to 22°C (room temperature). While the solution was constantly perfused with 95% O₂ and 5% CO₂, sharp dissection was used to remove mucosal sheets that were mounted in Ussing chambers with KRB solution as the bathing solution. These chambers had an aperture of 1.13 cm², and each tissue surface was bathed with 7 mL of solution. The bathing solution was maintained at a pH of 7.4 by constant perfusion with 95% O₂ and 5% CO₂ and at 37°C by circulating it with a gas lift through water-jacketed reservoirs.

Incubation conditions and resistance measurements—Throughout the incubation period in the Ussing chambers, the short circuit current (Isc) was recorded on voltage clamps via Ag-AgCl₂ electrodes that were connected to 4% agar bridges in KRB solution. The potential difference (PD) was recorded through calomel electrodes connected to similar agar bridges placed within 1 to 2 mm of the tissue surface. When the tissue was not in place, the PD was nullified by an offsetting voltage to establish a correction for fluid resistance. With the tissue mounted, the transepithelial PD was automatically and continuously nullified by the passage of an external current (ie, the Isc) across the tissue by use of an automatic voltage-current clamp amplifier, except for 10-second periods at 15-minute intervals during which spontaneous tissue PD was measured. The transmural electrical resistance was calculated according to Ohm’s law, whereby resistance is equal to spontaneous tissue PD divided by Isc. The effect of the fluid resistance on Isc was automatically corrected. Resistance (Ω·cm⁻²) was used as a measure of integrity of the colonic mucosa and permeability of the paracellular pathway.

Mannitol permeability—Radiolabeled (³H) mannitol was added to the mucosal side of each tissue segment at the beginning of the experiment. For scintillation counting, fluid samples (250 µL each) were collected from both sides of the tissue after a 60-minute equilibration period and towards the end of the experiment. The transmucosal flux of mannitol was expressed as percentage of the scintillation counts detected on the mucosal side of the tissue that moved to the serosal side of the tissue per hour. Mannitol permeability (described by the transmucosal flux of mannitol) was determined as an additional measure of integrity of the colonic mucosa and permeability of the paracellular pathway.

Histologic examination—At times 0 (immediately before incubation), 30 minutes, and 1, 2, 3, and 4 hours after incubation, tissues were fixed in neutral-buffered 10% formalin. Tissues were subsequently embedded in paraffin and cut into 5-µm-thick sections. Slides were prepared with these sections and
stained with H&E in a routine manner for all histomorphometric measurements of tissue damage. Slides were stained with Luna's eosinophil stain to determine eosinophil migration. To perform Luna's eosinophil stain, slides are desiccated in xylene, stained with Biebrich scarlet-hematoxylin, differentiated in 1% acid alcohol, and subsequently stained with lithium carbonate. On microscopic examination, eosinophil granules and erythrocytes appear stained reddish-orange, Charcot-Leyden crystals appear red, and all nuclear elements appear blue. One investigator (AKR) who was unaware of treatment group allocation of the specimens performed all histologic evaluations.

**Histomorphometric measurements**—A computer-based program was used for histomorphometric analysis of the images obtained by light microscopy. For each measurement, 3 high power fields of each tissue were evaluated. Mucosal height was measured as the mean vertical distance between tracings of the muscularis mucosa and the lumen surface. Epithelial height was the mean vertical distance between tracings of the basement membrane and the cell apex. The mean width per cell was calculated from the width of 3 groups of 5 adjacent clearly identifiable epithelial cells in each field of view. The length of mucosal surface denuded of epithelium was measured and expressed as a percentage of the total mucosal surface length. If ≥ 5 epithelial cells 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, this was defined as lifted epithelium. The length of epithelium affected by lifting was expressed as a percentage of the total surface length of the mucosa in the section. Detached cells were defined as cells that appeared morphologically almost normal but were separated from the basement membrane in groups of ≥ 5 cells and completely detached from adjacent epithelium. The length of epithelium affected by detachment was measured and expressed as a percentage of the total surface length of the mucosa in the section. Sloughed cells were defined as cells undergoing necrotic cell death and sloughing individually from the epithelial surface. The number of sloughed cells was counted for each field; the mean number of sloughed cells per 0.1 mm of surface length was calculated.

**Eosinophil migration**—The mucosa was divided into 5 zones; at intervals of one-fourth of the mean distance from the muscularis mucosa to the luminal surface, zones M1 to M4 were delineated, and the luminal surface of epithelial cells was designated zone M5 (Fig 1). Absolute numbers of eosinophils were counted in each zone in 3 high power fields for each tissue, and the mean number of eosinophils per zone was calculated. Mean numbers were then used to calculate the percentage of eosinophils in each of the 5 zones with regard to the total eosinophil count in all zones.

**Statistical analyses**—Data were expressed as mean ± SD. Values of P < 0.05 were considered significant. A statistical software program was used for analyses. 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 identified, appropriate Bonferroni adjusted P values were used for each family of comparisons.

For histomorphometric measurements, the following comparisons were made: values for both treatment groups after 0 and 240 minutes of incubation were compared with fresh tissue; in all groups, values for tissues at 240 minutes of incubation were compared with values in the same group at 0 minutes; values for tissues treated with acetylcysteine and HOCl at all times were compared to values for tissues treated with HOCl alone at the same time.

**Results**

**Resistance**—Preincubation with HOCl significantly decreased tissue resistance, compared with that measured in control tissues throughout the 4 hours of incubation (Fig 2). Resistance of tissues treated with acetylcysteine and HOCl was similar to that of control tissues and significantly higher than resistance of tissues treated with HOCl alone during the first 3 hours.

![Figure 1](image1.png)  
**Figure 1**—Photomicrograph of a section of tissue from the right dorsal colon of a horse, which serves to illustrate zones M1 to M5 that were used for counting eosinophilic granulocytes. Notice that the epithelium is attached to the basement membrane and consists of tall and narrow epithelial cells. With Luna’s eosinophil stain, eosinophils (arrowheads) are stained red and nuclear components appear blue. ht = Mucosal height (measured as the mean vertical distance between tracings of the muscularis mucosa and the lumen surface).

![Figure 2](image2.png)  
**Figure 2**—Mean ± SD tissue resistance of tissues (from the right dorsal colon of 9 horses) preincubated with 8mM acetylcysteine and 3mM hypochlorous acid (HOCl; circles) or 3mM HOCl alone (squares) and control tissues (triangles) during incubation (240 minutes) in Krebs-Ringerbicarbonate (KRB) solution. Control tissues were not preincubated but were incubated in KRB solution. *Significant (P < 0.05) difference between tissues preincubated with HOCl alone and tissues preincubated with acetylcysteine and HOCl at that time point. †Significant (P < 0.05) difference between tissues preincubated with HOCl alone and control tissues at that time point.
Table 1—Histologic measurements (mean ± SD) of tissues (from the right dorsal colon of 9 horses) preincubated with acetylcysteine and HOCl or HOCl alone and control tissues after 0, 30, 60, 120, 180, and 240 minutes of incubation in KRB solution.

<table>
<thead>
<tr>
<th>Tissue treatment</th>
<th>Time (min)</th>
<th>Mucosal height (µm)</th>
<th>Epithelial height (µm)</th>
<th>Epithelial cell width (µm)</th>
<th>Denuded mucosa (%)</th>
<th>Lifted epithelium (%)</th>
<th>Detached epithelium (%)</th>
<th>Sloughed epithelial cells/0.1 mm</th>
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<tr>
<td>Control</td>
<td>0</td>
<td>392.9 ± 43.6</td>
<td>29.8 ± 4.7</td>
<td>5.4 ± 0.8</td>
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<td>0 ± 0</td>
<td>0.1 ± 0.2</td>
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<td></td>
<td>30</td>
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<td>29.1 ± 0.6</td>
<td>5.9 ± 0.9</td>
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<td></td>
<td>60</td>
<td>349.8 ± 57.5</td>
<td>24.6 ± 5.9</td>
<td>6.3 ± 0.9</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0.3 ± 0.6</td>
<td>0 ± 0.2</td>
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<tr>
<td></td>
<td>120</td>
<td>311.3 ± 33.7</td>
<td>28.6 ± 6.7</td>
<td>6.1 ± 1.3</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0.2 ± 0.4</td>
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<tr>
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<td>180</td>
<td>302.7 ± 79.2</td>
<td>23.2 ± 3.1</td>
<td>6.3 ± 0.7</td>
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<td>0 ± 0</td>
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<td></td>
<td>240</td>
<td>263.3 ± 72.3</td>
<td>20.3 ± 7.3</td>
<td>6.7 ± 1.3</td>
<td>2.4 ± 7.9</td>
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<td>HOCl alone</td>
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<td>31.1 ± 10.1</td>
<td>6.4 ± 1.2</td>
<td>12.2 ± 18.8a</td>
<td>19.3 ± 19.7</td>
<td>9.9 ± 21.9</td>
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<td>296.1 ± 99.4</td>
<td>28.1 ± 8.8</td>
<td>6.9 ± 1.4</td>
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<td>5.8 ± 0.8</td>
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<td>and HOCl</td>
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<td>31.7 ± 14.3</td>
<td>6.1 ± 1.2</td>
<td>2.6 ± 8.6c</td>
<td>2.2 ± 5.7</td>
<td>0 ± 0</td>
<td>1.1 ± 2.5</td>
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<tr>
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<td>339.2 ± 106.5c</td>
<td>28.1 ± 11.8</td>
<td>6.4 ± 2.1</td>
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<td>0 ± 0</td>
<td>0 ± 0</td>
<td>0.8 ± 1.7</td>
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<td>340.8 ± 113.8</td>
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<td>8.2 ± 19.5</td>
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<td>240</td>
<td>322.4 ± 50.8c</td>
<td>21.6 ± 11.6e</td>
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<td>11.5 ± 24.2c</td>
<td>2.6 ± 5.0</td>
<td>2.5 ± 7.5</td>
<td>2.4 ± 2.9</td>
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*Value significantly different from that of fresh tissues. *Value significantly different from that at 0 minutes within the same treatment group.

Figure 3—Photomicrographs of sections of tissues (from the right dorsal colon of 9 horses) preincubated with acetylcysteine and HOCl (left image) and HOCl alone (right image) at 0 (A), 60 (B), and 240 minutes (C) of incubation in KRB solution. Arrows in the left image indicate the most superficial eosinophils detected. In tissues preincubated with acetylcysteine and HOCl, the most superficial eosinophil is located in the middle portion of the mucosa, whereas in tissues exposed to HOCl alone, eosinophils are on or near the mucosal surface. Luna’s eosinophil stain. A—Notice that immediately after exposure to HOCl, groups of epithelial cells are lifted off the basement membrane (right image); this change is not seen in tissue exposed to acetylcysteine and HOCl (left image). B—After 60 minutes, groups of epithelial cells are completely detached from the mucosa in tissues preincubated with HOCl alone (right image), whereas epithelial cells in tissues preincubated with acetylcysteine and HOCl are firmly attached to the basement membrane (left image). C—In both treatment groups, the mucosal surface in tissues is covered with short and wide epithelial cells; sloughed epithelial cells are seen on the mucosal surface. Compared with tissues preincubated with acetylcysteine and HOCl, mucosa exposed to HOCl alone is thinner and has groups of detached epithelial cells on the surface (right image).

of incubation. Towards the end of the incubation period, resistance in both tissues treated with acetylcysteine and HOCl and control tissues decreased but did not reach the values for tissues treated with HOCl alone. The resistance of the tissues treated with HOCl alone increased during the incubation period.
Permeability to mannitol—Tissue permeability to mannitol was increased (P < 0.05) in HOCl-treated (0.63 ± 0.13%/h) and tissues treated with acetylcysteine and HOCl (0.69 ± 0.13%/h), compared with values obtained in control tissues (0.17 ± 0.14%/h).

Histomorphometric measurements—Preincubation with HOCl decreased mucosal (P < 0.001) and epithelial cell height (P < 0.001; Table 1) after 240 minutes of incubation. Immediately after preincubation with HOCl, there was an increase of denuded mucosal surface (P = 0.001) and of lifted epithelial cells (P < 0.001; Fig 3), which was later followed by an increase in detached (P < 0.001) and sloughed (P < 0.001) epithelial cells. Compared with findings in HOCl-treated tissues, tissues exposed to acetylcysteine and HOCl had increased mucosal (P = 0.001) and epithelial cell heights (P = 0.001), as well as decreased amounts of denuded mucosal surface (P < 0.001 to 0.006) and decreased lifted (P < 0.001) and detached epithelial cells (P > 0.001). The only significant difference between tissues exposed to acetylcysteine and HOCl and fresh tissues was slightly greater proportion of sloughed epithelial cells in the former after 240 minutes (P < 0.001; Fig 3).

Eosinophil migration—In control tissues, more than 80% of tissue eosinophils were located in the basilar 2 zones (zones M1 and M2) at all times, and no eosinophils were seen in the luminal zone (zone M3) at any time (Table 2). Preincubation with HOCl resulted in a progressive relative increase in eosinophils in the 2 superficial zones (zones M4 to M5) of the mucosa (Fig 3), with the increase of eosinophils on the mucosal surface (zone M5) becoming significant (P = 0.004) after 30 minutes and remaining significant throughout the remainder of the experiment. Tissues treated with acetylcysteine and HOCl had proportionally fewer eosinophils in the superficial 2 zones (zones M4 and M5) than tissues treated with HOCl alone at all times (P < 0.001 to 0.003). No significant difference was found in eosinophil distribution between tissues treated with acetylcysteine and HOCl and control tissues or in the change of eosinophil distribution over the time of incubation in either of these experimental sample groups (Fig 3).

Discussion

The model used in the study reported here produced consistent mucosal damage during preincubation with HOCl, and restitution occurred during the 4-hour incubation period. The initial resistance of tissues treated with HOCl alone was low as a result of cell damage, but tissue resistance increased during the incubation period, which indicated recovery of some barrier function. The resistance of control tissues and tissues treated with acetylcysteine and HOCl were comparable, although resistance of the latter was generally lower at the end of incubation. Resistances in both tissue groups decreased slightly towards the end of the experiment, which indicated progressive cell death. Results of the histologic examination of sections supported the findings that preincubation with HOCl damaged the tissue, and restitution occurred during incubation; with time, denuded mucosal surfaces became covered with attenuated (flat and wide) epithelial cells. The damage in tissues treated with acetylcysteine and HOCl was significantly less severe; this treatment also blocked the eosinophil migration that was detected in tissues damaged by HOCl alone.

Eosinophils in undamaged right dorsal colon mucosa were located primarily in the abluminal half (zones 1 and 2) of the lamina propria near the muscularis mucosae. Damage of the tissue with HOCl resulted in remarkably rapid migration of these cells towards
the mucosal surface, and a significant accumulation of eosinophils on the luminal surface was detected within 30 minutes of the injury. Eosinophils appeared to be degranulating in tissues treated with HOCl alone to a greater extent than they were in other tissues, but this process was not statistically evaluated. To the authors’ knowledge, eosinophil migration in vitro has not been described previously. Our model of mucosal damage may be useful for in vitro studies of pathophysiologic processes and regulation of eosinophil trafficking in the gastrointestinal tract mucosa. Induction of eosinophil migration in vitro in response to oxidant-induced tissue injury is especially interesting, because this model lacks any of the factors that are usually associated with eosinophil accumulation in the intestine, such as activation of the immune system by food allergens or parasites. These findings would suggest that resident eosinophils in the colonic mucosa may have a wide range of functions.

Eosinophils are resident cells of the gastrointestinal tract mucosa and can therefore respond quickly to local stimuli. By comparison, neutrophilic granulocytes are located primarily in the blood stream and have to be recruited to the tissue, which involves the processes of chemotaxis, adherence to the endothelium, and extravasation. We postulate that eosinophils may be involved in a rapid local inflammatory response to nonspecific tissue injury in the gastrointestinal tract, a task for which they seem well equipped. The distributions of eosinophils within various strata of the lamina propria and mucosa may also be useful as a possible histologic marker for the severity of tissue injury. The normal distribution of eosinophils in different portions of the gastrointestinal tract of horses and the pattern of distribution in various disease processes have not been established.

In the model used in the study reported here, the protective effects of acetylcysteine were attributed to the antioxidant properties of this drug. In addition to being a powerful free radical scavenger, acetylcysteine can provide protection against oxidant injury by increasing intracellular glutathione concentration. Eosinophil migration was possibly inhibited by acetylcysteine via reduced tissue injury, although a direct effect of acetylcysteine on eosinophil migration cannot be excluded. In our study, acetylcysteine was applied to the tissue before exposure to HOCl to ensure high tissue concentration; the benefits (if any) of acetylcysteine application after initiation of tissue damage are not known. However, it is possible that acetylcysteine could protect against progression of damage associated with free radicals produced by inflammation and reperfusion injury even if administered after these processes had started.

In the treatment of reperfusion injury in vivo, a possible beneficial effect of acetylcysteine is inhibition of platelet thrombus formation, because platelet activation with capillary obstruction may play an important role in reperfusion injury in horses. Obstruction of microvasculature by platelets has been documented in naturally occurring large colon strangulations and experimental models of ischemia and reperfusion injury of the ascending colon of ponies and horses. Occlusion of blood supply would exacerbate mucosal ischemia and possibly limit the recovery of epithelial cells. Therefore, treatment of reperfusion injury should include the reestablishment of microvascular patency.

The finding that increased tissue permeability to mannitol was similar in the HOCl and acetylcysteine-HOCl treatment groups was unexpected. However, acetylcysteine does have mucolytic properties that may have disrupted the mucus gel layer on the mucosal surface. The mucus gel layer has been postulated to be the main barrier against macromolecule permeability of the intestinal wall. The increased permeability to mannitol in the tissues treated with HOCl alone was probably caused by severe epithelial damage, whereas in the tissues treated with acetylcysteine and HOCl could be explained predominantly by removal of mucus. The mucolytic effect of acetylcysteine probably did not affect resistance, because the ions involved in electrophysiologic measurements are at constant concentrations throughout the tissue milieu and aqueous component of the mucus layer. Small ions may also cross mucus layers more readily than mannitol molecules because of their small size and the high water content of mucus.

The mucolytic effect of acetylcysteine does not appear to be associated with any damaging effects on gastrointestinal tract mucosa. In our study, we did not have sufficient chambers to evaluate as a separate group any potential tissue damage inflicted by acetylcysteine alone; however, there is no report of direct damage to mucosal epithelium caused by acetylcysteine. In both in vivo and in vitro models involving direct exposure of gastrointestinal tract mucosa to acetylcysteine, neither macroscopic, histologic, nor electrophysiologic findings indicate a detrimental effect of this drug to the mucosa at concentrations 10 to 100 times greater than that used in the study of this report. Our data indicated that tissues treated with acetylcysteine and HOCl had resistance values close to those of control tissues, no detachment of epithelial cells, and no migration of eosinophils to the luminal surface; this suggests that acetylcysteine markedly protected tissues against HOCl-induced damage and any acetylcysteine-induced damage was slight. Under conditions in which the mucus layer could be protecting epithelium against a noxious agent, systemic delivery to the diseased large colon by IV, IM, or PO routes of administration could be preferable to intraluminal application. The first 3 routes of application are used presently in human medicine. In Europe, acetylcysteine is administered PO as a mucolytic agent in horses and intratracheal, IM, or IV injections are used to a lesser extent. The beneficial and detrimental effects of treatment with acetylcysteine in vivo remain to be fully elucidated. Nevertheless, results of the study reported here indicate that acetylcysteine protects the equine colonic mucosa against oxidant-induced injury in vitro; furthermore, eosinophils migrated towards the damaged mucosal surface in vitro, and acetylcysteine prevented this migration. These findings suggest that acetylcysteine may be of use in the treatment of ischemic and inflammatory diseases of the equine colon, such as large colon volvulus and colitis.
References


42. Luna LG. Histopathologic methods and color atlas of special stains and tissue artifacts. Gaithersburg, Md: American Histolab Inc Publications Division, 1992;255–256.


