Expression of cyclooxygenase-1 and -2 in the left dorsal colon after different durations of ischemia and reperfusion in horses

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Objective—To identify expression and localization of cyclooxygenase (COX)-1 and COX-2 in healthy and ischemic-injured left dorsal colon of horses.

Sample Population—Left dorsal colon tissue samples from 40 horses.

Procedures—Tissue samples that were used in several related studies on ischemia and reperfusion were evaluated. Samples were collected during anesthesia, before induction of ischemia, and following 1 hour of ischemia, 2 hours of ischemia and 30 minutes of reperfusion, and 2 hours of ischemia and 18 hours of reperfusion. Histomorphometric analyses were performed to characterize morphological injury. Immunohistochemical analyses were performed to characterize expression and localization of COX-1 and COX-2.

Results—COX-1 and COX-2 were expressed in control tissues before ischemia was induced, predominantly in cells in the lamina propria. Ischemic injury significantly increased expression of COX-2 in epithelial cells on the colonic surface and in crypts. A similar significant increase of COX-1 expression was seen in the epithelial cells.


Strangulating large colon volvulus is a devastating cause of colic characterized by high morbidity and mortality rates and can account for 11% to 27% of surgical cases of colic in horses. Even with resection, a portion of the severely injured colon is usually left in the horse because volvulus typically occurs at the base of the colon, where the affected portion of the colon cannot be easily accessed via the standard ventral midline surgical approach. Injury is characterized by diffuse loss of mucosal surface epithelium and variable damage to crypt epithelial cells. The integrity of the remaining colonic mucosa can determine outcome. The close correlation between survival and depth of the lesion into crypts can be explained by the transmucosal leakage of endotoxin, bacterial chemotactic peptides, and bacteria and by inadequate repair of the denuded mucosal surface. Mucosal restitution and tightening of paracellular pathways are 2 rapid repair processes critical for recovery of the surface epithelium and are usually complete within hours. Both repair processes can be enhanced by PGs. Because PGs are also responsible for much of the pain and endotoxemia that may occur during the critical perioperative period, most horses with colic are routinely treated with an NSAID, such as flunixin meglumine.

The ability of NSAIDs to interfere with mucosal repair in the porcine and equine jejunum has been explained by reduced formation of PGs, which enhance barrier properties by secretion-induced collapse of

<table>
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<th>ABBREVIATIONS</th>
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<tr>
<td>COX</td>
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<tr>
<td>DSS</td>
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<tr>
<td>HOCl</td>
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<tr>
<td>LDC</td>
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<td>PG</td>
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dilated intercellular spaces. In an in vitro model of ischemia-induced tissue injury in equine jejunum, iluninrix meglumine inhibited recovery of mucosal barrier function. In healthy equine right ventral colon, both indomethacin and iluninrix meglumine increased permeability to mannitol in vitro by a mechanism that was reversed by PGE, indicating that PGs might control tight junction permeability in the equine colon as well as the jejunum. In contrast, in HOCl-injured equine right dorsal colon treated with indomethacin, the addition of PGE did not enhance recovery, suggesting that prostaglandins may act differently in injured versus uninjured equine colon.

Prostaglandin biosynthesis is catalyzed by 2 distinct enzymes, COX-1 and -2. Cyclooxygenase-1 is constitutively expressed in many tissues and functions as a housekeeping enzyme in those tissues. For example, COX-1 has been linked to the production of cytoprotective prostanooids involved in the maintenance of gastrointestinal mucosal barrier function. Cyclooxygenase-2 was initially considered to be a dynamically regulated enzyme inducible by a variety of inflammatory stimuli and responsible for the production of high concentrations of prostanooids involved in proinflammatory pathways. The simple distinction of COX-1 as constitutive and COX-2 as inducible has been questioned on the basis of recent evidence of constitutive COX-2 expression in healthy tissues, including blood vessels, brain, kidney, and intestine. In gastrointestinal tissue, constitutive expression of COX-2 has been identified in human and murine stomach and colon, where it is thought to modulate normal neuromuscular functions.

The importance of constitutive expression of COX-2 is gaining appreciation with regard to the control of gastrointestinal processes in humans and other animals, and the roles of COX-1 and COX-2 in gastrointestinal maintenance and repair may be relevant to many gastrointestinal tract diseases in horses. Agents that do not inhibit COX-1, but suppress the activity of COX-2, cause less gastrointestinal tract damage than nonselective NSAIDs in human patients, but cannot be termed safe with regard to the gastrointestinal tract.

Prostaglandins from COX-2 make an important contribution to mucosal defense, particularly in repair of mucosal injury. Following DSS-induced colitis, COX-2 knockout mice had profound inhibition of epithelial proliferation and cellular organization in rectal crypts, compared with wild-type mice. Addition of PGE prevented the effects of this injury, indicating that COX-2-derived prostanooids can preserve epithelial proliferation and cellular mobilization in crypts during repair. Ischemic-injured porcine ileum treated with the nonselective NSAID indomethacin did not recover to control values of transepithelial resistance, whereas injured tissues treated with either a selective COX-1 or COX-2 inhibitor recovered fully. These findings suggested that the PGs involved in early recovery may be produced by either COX-1 or COX-2. This is supported by a study in knockout mice that lacked either COX-1 or COX-2, because the remaining COX isomorph in those mice could compensate for the absent isomorph by increasing PG production. Despite the potential importance of PGs in tissues subjected to different types of injury, especially in horses with colic caused by ischemia, little is known about the expression and roles of COX-1 and COX-2 in healthy or diseased equine colonic mucosa.

The purpose of the study reported here was to identify the relative expressions and locations of the COX-1 and COX-2 in mucosa from healthy and ischemic-injured left dorsal colon of horses.

**Materials and Methods**

**Animals**—Tissue samples from 40 adult horses that were used in several related studies were included. The horses were of mixed breeds and ages, were of both sexes, and were healthy and free of gastrointestinal tract disease. Horses were fed grass hay (1% to 2% of their body weight/d), and water was provided ad libitum. Horses were adapted to their diet and environment for at least 1 week before use. The Institutional Animal Care and Use Committee of the University of Florida approved the experimental protocols used in this study.

**Anesthesia and surgery**—Horses were sedated with xylazine hydrochloride (0.5 mg/kg of body weight, IV), and a 14-gauge, 13.3-cm catheter was aseptically inserted into the left jugular vein. General anesthesia was induced with a combination of diazepam (0.1 mg/kg, IV) and ketamine (2 mg/kg, IV) and maintained with isoflurane (1% to 2%) vaporized in 100% oxygen. Isotonic polyionic fluids were infused continuously IV at 5 to 10 mL/kg/h. Mean arterial blood pressure was monitored through a 20-gauge, 3.1-cm catheter placed in the facial artery. Mean arterial blood pressure was maintained at ≥ 70 mm Hg. Horses were positioned in dorsal recumbency and prepared for an aseptic ventral midline celiotomy, through which the large colon was exteriorized. Padded clamps were applied proximally and distally to provide transmural compression to a 15-cm segment of the pelvic flexure. The corresponding major mesenteric veins and arteries were temporarily ligated with umbilical tape at each end of the segment. Between sampling times, the large colon was replaced into the abdomen, and the body wall was temporarily closed with size-2 polyglactin 910 in a simple continuous pattern. In all horses, 4-cm2 mucosal biopsy specimens of control, ischemic, and ischemic and reperfused tissues were harvested at different times during anesthesia for histologic examinations and for experiments on tissue recovery in vitro (Table 1). Biopsy sites were closed routinely with 2-0 polydioxanone in a continuous Lembert pattern. Control samples were harvested before induction of ischemia. In 13 horses, ischemia was performed for 1 hour. In 12 horses, 2 contiguous segments were subjected to 1 and 2 hours of ischemia each respectively, and at the end of ischemia, the clamps and ligatures were removed to allow for 30 minutes of reperfusion to each segment. In 8 horses, ischemia was performed for 2 hours, and then the clamps and ligatures were removed to allow for 30 minutes of reperfusion. In 7 horses, ischemia was performed for 2 hours, the clamps and ligatures were removed, the abdominal incision was closed routinely, and then these horses were allowed to recover.
from anesthesia. After recovery, horses were placed in a stall, monitored and scored for signs of pain according to an established scoring system, and administered butorphanol (0.03 mg/kg, IM, q 4 h) to provide analgesia. These horses were anesthetized again to collect tissues at 18 hours of reperfusion. All horses were euthanized during anesthesia after final tissue collection with an overdose of pentobarbital sodium (88 mg/kg, IV).

Histomorphometric analyses—All tissues samples were fixed in neutral-buffered 10% formalin for light microscopy, embedded in paraffin, cut into 5-µm-thick sections, and placed on glass slides. Slides were stained with H&E stain in a routine manner. For the histomorphometric assessment by light microscopy, a computer-based image-analyzing program was used, and 3 fields from each tissue were examined as described. One investigator unaware of the identity of the treatment group performed all histologic evaluations; mucosal height was expressed as the mean vertical distance in micro-meters between tracings of the muscularis mucosae and the epithelial surface. Epithelial height was expressed in micrometers as the mean vertical perpendicular distance between the basement membrane and the cell apex. Epithelial cell width was measured in micrometers in 3 clearly identifiable epithelial cells in 3 sets in each field. The length of mucosal surface denuded of epithelium 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 necrosis and sloughing individually from the epithelial surface. The number of sloughed cells was counted in each field, and the mean number per 0.1-mm mucosal surface length was then calculated.

Immunohistochemical staining for COX-1 and COX-2—Tissues were fixed in neutral-buffered 10% formalin, routinely processed for paraffin embedding, and cut into 3-µm sections. Following placement on silane-coated slides, sections were deparaffinized and rehydrated. Heat antigen retrieval was performed, and slides were subsequently incubated in 1% H2O2. Slides were washed in PBS solution and incubated with normal goat serum for 1 hour. Slides were then incubated for 30 minutes with goat anti-human COX-1 polyclonal antibody or goat anti-human COX-2 polyclonal antibody. This step was not performed on negative control slides. Slides were washed 3 times in PBS solution between 30-minute incubations with biotinylated donkey anti-goat antibody and streptavidin-labeled peroxidase. Sections were counterstained with Mayer hematoxylin stain for 3 minutes, and color was developed by use of 0.1% ammonium hydroxide (2.9mM). Slides were then washed in distilled water, dehydrated, and mounted in an aqueous mounting medium, and coverslips were applied.

For the evaluation of the images obtained by light microscopy, a computer-based image-analyzing program was used, and 3 randomly selected fields of view from each tissue with a length of 866 µm (equal to the length of 1 image viewed with the 10X objective) were examined. The epithelium, upper and lower portions of the lamina propria, and crypts of the colonic mucosa were scored from 0 to 3 for presence of intracellular COX-1 and COX-2 expression (brown cytoplasm with blue counterstaining) by use of a 40X objective lens. Grade 0 was assigned when stained cells were absent or single cells were observed after careful inspection; grades 1, 2, and 3 were assigned if accumulation of

### Table 1—Distribution of horses (n = 40) among various experimental groups from which ischemic or control colonic biopsy specimens were collected at various times.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Horses (n)</th>
<th>Time (h)*</th>
<th>Ischemic biopsy specimens (n)</th>
<th>Control biopsy specimens (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia (1 h)</td>
<td>13</td>
<td>0</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>Ischemia (1 and 2 h) and reperfusion (0.5 h)†</td>
<td>12</td>
<td>0</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1.5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Ischemia (2 h) and reperfusion (0.5 h)</td>
<td>8</td>
<td>0</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Ischemia (2 h) and reperfusion (18 h)</td>
<td>7</td>
<td>0</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.5</td>
<td>5</td>
<td>2</td>
</tr>
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</table>

*Times given for biopsy specimens are from the start of the ischemic period to the time the biopsy specimen was taken and include the ischemic and reperfusion periods when longer than the ischemic period.

†Time is from start of ischemic periods. Ten horses that had 1- and 2-hour ischemic periods, 2 contiguous segments of colon were subjected to ischemia (1 for each ischemic period) and only 1 specimen was taken during ischemia (at the end of the ischemic period).
stained cells was subjectively assessed as mild, moderate, or marked, respectively. With this scoring system, mild accumulation was assigned if staining was present in < 25% of the cell population examined and if the intensity of most of the staining subjectively appeared mild (lighter shade and small portion of cell positively stained), moderate accumulation was assigned if staining was present in 25% to 50% of the cell population and if the intensity of most of the staining subjectively appeared intermediate between the mild and moderate scorings, and marked accumulation was assigned when > 50% of the cell population was stained and most of the staining intensity appeared intense (dark staining and entire cells stained). The mean of the mucosal layer scores was used for statistical evaluation. Specific cells that stained for COX were identified on the basis of obvious morphological features of these cells, when possible; however, no attempt was made to assess the relationship between COX production and cell types. Constitutive expression of an enzyme was defined as evidence of staining for that enzyme in a control (nonischemic) tissue sampled before ischemia was induced.

Statistical analysis—The Kruskal-Wallis test was used to compare histomorphometric measurements and COX-1 and COX-2 distribution in the colonic mucosa during the different ischemia and reperfusion time periods and with controls. Whenever a significant P value for ischemia and reperfusion was identified, appropriate Bonferroni adjusted P values were used for each comparison. Data were expressed as mean ± SD. A statistical software program was used for analyses. Values of P < 0.05 were considered significant.

Results

Histomorphometric analyses—One and 2 hours of ischemia caused edema, purple discoloration of the serosa, and some scattered serosal ecchymoses in the affected segments. The appearance of the serosal surface of the ischemic colon was similar to that of adjacent control segments at 15 minutes after clamps were removed. Following 30 minutes of reperfusion, the mucosal surface was more edematous and diffusely reddened. Following 18 hours of reperfusion, these changes were less severe throughout the injured segment on a subjective assessment. Histologic changes at 1 and 2 hours of ischemia were predominantly suggestive of cell death and detachment in the surface epithelial cells and upper parts of crypts, which confirmed mucosal disruption following injury (Figure 1). Although approximately one-third of the epithelial barrier was disrupted, tissues had some histologic evidence of restitution at 18 hours, with a mucosal surface that was partly or totally covered with short epithelial cells. Mucosal height
was decreased, compared with controls, at all times, but was only significantly decreased following 2 hours of ischemia. Epithelial height was significantly decreased and epithelial width was significantly increased, compared with control values, at all times, and significantly greater decreases in epithelial height were seen following both 2 hours of ischemia and 30 minutes of reperfusion and 2 hours of ischemia and 18 hours of reperfusion, compared with all other times. Percentage of lifted epithelium was significantly greater following 1 and 2 hours of ischemia and 30 minutes of reperfusion and thereafter. The greatest percentage of denuded epithelium was seen at 2 hours of ischemia with 30 minutes of reperfusion.

Table 2—Histomorphometric measurements of colonic mucosal specimens obtained from 40 horses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 20)</th>
<th>1 hour of ischemia (n = 25)</th>
<th>1 hour of ischemia, 30 minutes of reperfusion (n = 6)</th>
<th>2 hours of ischemia (n = 24)</th>
<th>2 hours of ischemia, 2 hours of ischemia, 30 minutes of reperfusion (n = 8)</th>
<th>2 hours of ischemia, 18 hours of ischemia, 30 minutes of reperfusion (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucosal height (µm)</td>
<td>454.62</td>
<td>417.89</td>
<td>382.44</td>
<td>376.72</td>
<td>366.61</td>
<td>376.24</td>
</tr>
<tr>
<td>(86.18)</td>
<td>(65.66)</td>
<td>(72.44)</td>
<td>(80.51)</td>
<td>(82.13)</td>
<td>(109.49)</td>
<td>(107.80)</td>
</tr>
<tr>
<td>Epithelial height (µm)</td>
<td>33.88</td>
<td>28.62</td>
<td>21.60</td>
<td>23.91</td>
<td>17.31</td>
<td>16.75</td>
</tr>
<tr>
<td>(4.66)</td>
<td>(5.79)</td>
<td>(2.98)</td>
<td>(4.84)</td>
<td>(7.65)</td>
<td>(8.71)</td>
<td>(8.71)</td>
</tr>
<tr>
<td>Epithelial width (µm)</td>
<td>5.00</td>
<td>5.15</td>
<td>5.33</td>
<td>5.47</td>
<td>4.89</td>
<td>4.89</td>
</tr>
<tr>
<td>(0.30)</td>
<td>(0.40)</td>
<td>(0.40)</td>
<td>(1.02)</td>
<td>(1.02)</td>
<td>(1.07)</td>
<td>(1.07)</td>
</tr>
<tr>
<td>Denuded epithelium (%)</td>
<td>0.00</td>
<td>6.12</td>
<td>8.70</td>
<td>44.62</td>
<td>46.45</td>
<td>30.87</td>
</tr>
<tr>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(0.00)</td>
<td>(21.04)</td>
<td>(34.28)</td>
<td>(34.49)</td>
<td>(34.49)</td>
</tr>
<tr>
<td>Lifted epithelium (%)</td>
<td>0.08</td>
<td>5.22</td>
<td>2.38</td>
<td>7.05</td>
<td>0.63</td>
<td>4.91</td>
</tr>
<tr>
<td>(0.26)</td>
<td>(0.32)</td>
<td>(0.20)</td>
<td>(6.17)</td>
<td>(0.97)</td>
<td>(6.70)</td>
<td>(6.70)</td>
</tr>
<tr>
<td>Detached epithelium (%)</td>
<td>0.08</td>
<td>5.88</td>
<td>8.29</td>
<td>8.72</td>
<td>12.10</td>
<td>2.91</td>
</tr>
<tr>
<td>(0.36)</td>
<td>(0.83)</td>
<td>(2.65)</td>
<td>(5.81)</td>
<td>(15.27)</td>
<td>(2.40)</td>
<td>(2.40)</td>
</tr>
<tr>
<td>Sloughed cells/0.1 mm</td>
<td>0.12</td>
<td>4.65</td>
<td>7.56</td>
<td>10.99</td>
<td>14.26</td>
<td>12.58</td>
</tr>
<tr>
<td>(0.23)</td>
<td>(0.69)</td>
<td>(5.29)</td>
<td>(6.72)</td>
<td>(9.48)</td>
<td>(23.25)</td>
<td>(23.25)</td>
</tr>
</tbody>
</table>

Data are expressed as least square means (SD).
*a*Significant (P < 0.05) difference, compared with control tissue.
*Significant (P < 0.05) difference, compared with tissue undergoing 1 hour of ischemia.
*Significant (P < 0.05) difference, compared with tissue undergoing 2 hours of ischemia.
*Significant (P < 0.05) difference, compared with tissue undergoing 1 hour of ischemia and 30 minutes of reperfusion.

Figure 2—Photomicrographs of sections of equine colonic mucosa stained immunocytochemically for COX-1 (brown cells). A—Mucosa from section of uninjured colon. B—Mucosa obtained after 1 hour of ischemia. C—Mucosa obtained after 1 hour of ischemia and 30 minutes of reperfusion. D—Mucosa obtained after 2 hours of ischemia. E—Mucosa obtained after 2 hours of ischemia and 30 minutes of reperfusion. F—Mucosa obtained after 2 hours of ischemia and 18 hours of reperfusion. Bar = 0.1 mm.
tion and was significantly greater, compared with controls, 1 hour of ischemia, and 1 hour of ischemia with 30 minutes of reperfusion (Table 2).

Immunohistochemical analyses—Both COX-1 and COX-2 were constitutively expressed predominantly in cells of the lamina propria (Figures 2–4). Ischemic injury substantially upregulated expression and altered distribution of COX-1 and COX-2.

In the upper portion of the lamina propria, myofibroblasts, lymphocytes, and macrophages stained for COX-1. In the lower portion of the lamina propria, macrophages, lymphocytes, endothelial cells, and myofibroblasts stained for COX-1 (Figure 2). Throughout the entire lamina propria, only a small proportion of the population of lymphocytes and macrophages stained for COX-1. Constitutive expression of COX-1 was not evident in surface epithelial cells, but was evident in crypt cells (Figure 4). Following ischemia, no significant changes in COX-1 expression or distribution were seen in any tissue layers examined, except for a modest but significant increase in epithelial cells.

 Constitutive expression of COX-2 was seen in control tissues throughout cells of the lamina propria and crypts (Figure 3). Throughout the lamina propria, COX-2 expression was seen predominantly within lymphocytes and occasionally in macrophages. As with COX-1 expression, only a small percentage of the lymphocyte and macrophage population stained for COX-2. Unlike COX-1, no COX-2 expression was seen in either myofibroblasts or endothelial cells. Cells in the crypts that constitutively expressed COX-2 included epithelial, neuroendocrine, and Paneth cells.

Ischemia significantly changed the expression and distribution of COX-2 in the colonic mucosa (Figure 4). Following 1 hour of ischemia, significant upregulation of COX-2 was seen in surface epithelium. This upregulation was also evident following 2 hours of ischemia and remained following 30 minutes and 18 hours of reperfusion. Significant upregulation of COX-2 was also seen in cells of the crypts following 2 hours of ischemia, the next 30 minutes of reperfusion, and after 18 hours of reperfusion. Expression of COX-2 was seen almost exclusively in crypt epithelium and was distinct from Paneth and neuroendocrine cells. In the upper and lower portions of the lamina propria, significant upregulation of COX-2 expression was seen following 2 hours of ischemia only. In the upper portion of the lamina propria, cells with upregulation were lymphocytes and macrophages. In the lower portion of the lamina propria, in addition to some COX-2–positive lymphocytes and macrophages, COX-2–positive neutrophils (in vessels) and eosinophils were seen following 2 hours of ischemia. The COX-2–positive neutrophils and eosinophils were a small subset of the total population of these cells. Following 18 hours of reperfusion, COX-2 distribution was similar within the lamina propria as at earlier times, except that eosinophils were no
Discussion

Immunohistochemical staining was used in this study to identify tissue localization and relative expression of COX-1 and COX-2 in equine colonic mucosa, before and after different periods of ischemia and reperfusion. Constitutive expression of COX-1 in equine left dorsal colonic mucosa in this study was predominantly in cells of the lamina propria, whereas COX-2 was seen in epithelial cells of the surface and crypts, in addition to cells of the lamina propria. Ischemia and ischemia-reperfusion significantly increased COX-1 and COX-2 expression, although COX-2 upregulation was more prominent throughout the tissue layers examined in this study. The expression of COX-2 was significantly upregulated in the surface and crypt epithelia, whereas COX-1 expression was significantly increased in surface epithelial cells only. Results of the present study confirm our previous western blot data that indicate that ischemia upregulates both COX-1 and COX-2 in equine colonic mucosa, and provide additional information about the mucosal distribution of both isoforms.

We used 1 and 2 hours of ischemia to create a mild to moderate lesion that was reversible and also preserved sufficient tissue integrity to allow detection of any potential additional injury during reperfusion. In 1 group of horses, 30 minutes of reperfusion was used because, in those studies, we wanted to initiate an early reperfusion injury and then place the tissues in Ussing chambers to follow changes in mucosal barrier properties during the repair process that could not be studied in vivo. Eighteen hours was used in another group of horses to allow comparison with similar studies performed with equine small intestine, and because we were interested in the repair process in vivo to suit the specific aims of that study. All control tissues were harvested before ischemia was induced to prevent any remote effects from inflammatory mediators produced after ischemia-reperfusion injury. We used a validated subjective scoring system for evaluation of immunohistochemical staining to examine the localization and expression of COX-1 and COX-2. Although counting of positively stained cells was considered a more objective approach, the variety of staining patterns observed and overlapping of stained cells made this imprecise and cumbersome.

Upregulation of COX-2 was seen following 1 hour of ischemia and throughout all other time periods examined (Figure 4). Similar upregulation of COX-2 was reported in other studies in gastric, colonic, and small intestinal mucosa following injury. Upregulation of COX-1 was seen only in surface epithelial cells following 2 hours of ischemia and following 2 hours of ischemia with 18 hours of reperfusion, similar to what was found in equine small intestine by use of western blotting. The 2 reperfusion periods chosen (30 minutes and 18 hours) did not result in an increase of COX-1 and COX-2 activities, compared with the preceding ischemic periods, with the exception of a significantly greater upregulation of COX-2 in epithelial cells of the crypts following 18 hours of reperfusion. Failure to detect a reperfusion-induced upregulation at 30 minutes could be explained by inadequate time after ischemia for such a response to develop. The upregulation of COX-2 identified in the cells of the crypts after ischemic injury was consistent with what has been found after colonic injury in mice. Several reports have documented that COX-1 is constitutively expressed throughout the gastrointestinal tract and, in the absence of damage or inflammation, is the major source of PG synthesis in these tissues. As reported in the present study, others have also described constitutive expression of both COX isoforms in porcine ileum, equine jejunum, rat colon, and human jejunum. In porcine ileal mucosa, COX-1 protein values, which were constitutively expressed in intestinal crypt cells, were similar in control and injured tissues following 45 minutes of ischemia. In TNBS-induced colitis in rats, COX-1 expression was not significantly altered from control values during a 2-week period following...
induction of colitis, whereas COX-2 was significantly increased during all time points evaluated after injury, with the greatest increase at 72 hours. Ischemic injury significantly upregulated expression of both COX-1 and COX-2 in equine jejunal mucosal scrapings. Therefore, equine colonic mucosa appears to follow the same pattern of COX expression as does equine jejunal mucosa, with a significant upregulation of COX-1 and COX-2 after ischemia and reperfusion (Figures 2–4). However, the magnitude of upregulation of COX-2 appears to be greater in colonic mucosa.

Most investigators conclude that cells with increased expression of COX-2 in the lamina propria are inflammatory cells, such as neutrophils, macrophages, lymphocytes, and mast cells. For example, increased expression of COX-2 in patients with celiac disease was colocalized with CD3 T lymphocytes, and COX-2 and nitric oxide synthase 2 have been colocalized in neutrophils and macrophages in swine with ulcerative colitis. In the present study, COX-2 was constitutively expressed and associated with lymphocytes and macrophages in the lamina propria of healthy equine left dorsal colon. Ischemia increased this expression, and additionally, a small percentage of the neutrophils and eosinophils migrating and infiltrating the lamina propria expressed COX-2. The increase in expression of COX-2 in neutrophils of the lamina propria after 2 hours of ischemic injury could reflect the tissue accumulation of these cells at this time, as has been determined in this model. Neutrophils are key components of normal defense mechanisms and inflammation and play an important role in the pathogenesis of several gastrointestinal tract disorders. In rats and mice with experimentally induced colitis, COX-2–derived PGD3 acted as a stop signal that reduced granulocyte infiltration, and selective COX-2 inhibition significantly increased granulocyte infiltration. Through such an effect, NSAIDs could exacerbate the neutrophil-related inflammatory response, perpetuate neutrophil-related mucosal damage, and impede repair of injured mucosa.

The most important upregulation of COX-2 following ischemia and reperfusion in the present study was in epithelial cells of the mucosal surface and crypts (Figure 4), where it could control epithelial cell function, protection, or signaling. Both COX-1 and COX-2 play an important role in protection of intestine from radiation and DSS-mediated damage, primarily through the production of PGE,

30,39-42 In addition, COX-2–dependent PGE production is essential for colonic epithelial proliferation, migration, and homeostasis in response to injury. In a model of DSS-induced colitis, COX-2 knockout mice had profound inhibition of epithelial proliferation and organization, which was reversed by administration of PGE,

33 Possibly, the marked upregulation of COX-2 in response to ischemic injury in epithelial and crypt cells of the equine colon induces production of PGE, and PGD2, or similar prostanoids that are important in epithelial recovery and repair.

Upregulation of COX-2 in epithelial and crypt cells in equine colon after mucosal injury could have local and systemic effects with potential clinical importance to horses with colic and colitis. Our findings suggest that ischemic colon could be a rich source of PGs after correction of ischemia without complete resection, consistent with evidence that low-flow ischemia and reperfusion in equine colon increase colonic venous concentrations of eicosanoids by a factor of 5. Local vascular responses in the colon, vasodilation and platelet inhibition, or vasoconstriction and platelet aggregation could be determined by the types and proportions of eicosanoids produced. Additional local responses to PGs in the equine colon could be watery secretion into the intestinal lumen mediated by PG-induced inhibition of neutral sodium and chloride absorption by surface epithelial cells and increased chloride secretion by crypt cells. Although we did not examine layers other than the mucosa, COX-2 upregulation has also been observed in muscle layers in rat colon with experimentally induced colitis. Exogenous PGs can have variable effects on smooth muscle contractility in equine large colon, dependent on colon site and smooth muscle orientation.

Equine colonic mucosa subjected to ischemic injury seems to respond in a similar manner that reported in gastrointestinal tract injury in other species. The most profound of these responses is significant upregulation of COX-2 within the surface and crypt epithelia, with minimal change in COX-1 expression. The local pathophysiologic and clinically relevant roles of these findings are unknown and await investigation into the effects of PGs produced in this tissue. Based on available evidence from horses and other species, possible effects range from harmful to beneficial, including an important role of COX-2 in the mucosal repair process. Therefore, the use of NSAIDs that inhibit COX-2 in horses with colic should be tempered by the recognition that this particular COX isoform could be beneficial in recovery from ischemic injury.

References

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