Effects of the cyclooxygenase inhibitor meloxicam on recovery of ischemia-injured equine jejunum

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**Objective**—To determine the effect of meloxicam and flunixin meglumine on recovery of ischemia-injured equine jejunum.

**Animals**—18 horses.

**Procedures**—Horses received butorphanol tartrate; were treated IV with saline (0.9% NaCl) solution (SS; 12 mL; n = 6), flunixin meglumine (1.1 mg/kg; 6), or meloxicam (0.6 mg/kg; 6) 1 hour before ischemia was induced for 2 hours in a portion of jejunum; and were allowed to recover for 18 hours. Flunixin and SS treatments were repeated after 12 hours; all 3 treatments were administered immediately prior to euthanasia. Selected clinical variables, postoperative pain scores, and meloxicam pharmacokinetic data were evaluated. After euthanasia, assessment of epithelial barrier function, histologic evaluation, and western blot analysis of ischemia-injured and control jejunal mucosa samples from the 3 groups were performed.

**Results**—Meloxicam- or flunixin-treated horses had improved postoperative pain scores and clinical variables, compared with SS-treated horses. Recovery of transepithelial barrier function in ischemia-injured jejunum was inhibited by flunixin but permitted similarly by meloxicam and SS treatments. Eighteen hours after cessation of ischemia, numbers of neutrophils in ischemia-injured tissue were higher in horses treated with meloxicam or flunixin than SS. Plasma meloxicam concentrations were similar to those reported previously, but clearance was slower. Changes in expression of proteins associated with inflammatory responses to ischemic injury and with different drug treatments occurred, suggesting cyclooxygenase-independent effects.

**Conclusions and Clinical Relevance**—Although further assessment is needed, these data have suggested that IV administration of meloxicam may be a useful alternative to flunixin meglumine for postoperative treatment of horses with colic. (Am J Vet Res 2007;68:614–624)

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After old age–associated causes, colic is the leading cause of death in horses,1,2 Many of these deaths are attributable to the results of increased intestinal absorption of endotoxin through a compromised intestinal epithelial barrier.3 Many of the systemic effects and clinical signs of endotoxia are mediated by PGs, which are elaborated from arachidonic acid by the constitutively expressed COX-1 and inducible COX-2 enzymes. Therefore, NSAIDs such as flunixin meglumine are frequently administered to horses with colic to inhibit COX and ameliorate the detrimental effects of PGs. However, PGs are critical for recovery of barrier function in ischemia-injured intestine.4,5 Treatment of ischemia-injured equine jejunum with the nonselective COX inhibitor flunixin meglumine retards recovery of intestinal barrier function.6,8 Previous evaluations of the COX-2 selective inhibitors deracoxib and etodolac in horses have not resulted in identification of an alternative NSAID to flunixin meglumine that is practical to use in clinical cases of colic in horses.6,8

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**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>PG</td>
<td>Prostaglandin</td>
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<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
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<tr>
<td>NSAID</td>
<td>Nonsteroidal anti-inflammatory drug</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen-activated protein kinase</td>
</tr>
<tr>
<td>PPARγ</td>
<td>Peroxisome proliferator-activated receptor gamma</td>
</tr>
<tr>
<td>PD</td>
<td>Spontaneous potential difference</td>
</tr>
<tr>
<td>ISc</td>
<td>Short-circuit current</td>
</tr>
<tr>
<td>TER</td>
<td>Transepithelial electrical resistance</td>
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<tr>
<td>FITC-LPS</td>
<td>Lipopolysaccharide from Escherichia coli serotype O111:B4 labeled with fluorescein isothiocyanate</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-pressure liquid chromatography</td>
</tr>
<tr>
<td>p38 MAPK</td>
<td>p38 mitogen-activated protein kinase</td>
</tr>
<tr>
<td>Phospho-p38</td>
<td>Phosphorylated p38 mitogen-activated protein kinase</td>
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Meloxicam is a preferential COX-2 inhibitor in the enolic acid class of NSAIDs and has a favorable gastrointestinal adverse effect profile in humans, compared with the adverse effect profile of nonselective COX inhibitors. The European Agency for the Evaluation of Medicinal Products recently approved meloxicam for oral and IV use in horses at a dose of 0.6 mg/kg every 24 hours. The purpose of the study reported here was to determine and compare the effects of meloxicam and flunixin meglumine on recovery of ischemia-injured equine jejunum. Our hypothesis was that administration of the preferential COX-2 inhibitor meloxicam to horses that underwent jejunal ischemia would permit sufficient local PG production to facilitate recovery of intestinal barrier function, while ameliorating clinical signs of pain and endotoxia attributable to the detrimental systemic effects of PGs. Furthermore, we speculated that the NSAIDs flunixin meglumine and meloxicam would affect expression or activity of other proteins associated with the inflammatory response to ischemia-reperfusion, given the emerging interest in COX-independent actions of COX inhibitors on MAPKs and PPARα.

Materials and Methods

Animals and surgical procedures—All procedures were approved by the North Carolina State University Animal Care and Use Committee. Eighteen horses (3 to 20 years old) with no previous history of systemic disease, colic, or abdominal surgery were included in the study. The horses weighed 350 to 700 kg. The horses had previously undergone a 4-week quarantine period including vaccination, anthelmintic treatment, and observation. An IV catheter was placed in the left jugular vein prophylactically. Five minutes prior to premedication and approximately 1 hour before initiation of ischemia, horses in group 1 received saline (0.9% NaCl) solution (12 mL, IV), which was repeated every 12 hours and immediately prior to euthanasia; horses in group 2 received flunixin meglumine (1.1 mg/kg, IV), which was repeated every 12 hours and immediately prior to euthanasia; and horses in group 3 received meloxicam (0.6 mg/kg, IV), which was repeated immediately prior to euthanasia (approx 21 hours later). The dose of meloxicam was determined on the basis of a previously published pharmacokinetic study in horses and the dose licensed by the European Agency for the Evaluation of Medicinal Products. A venous blood sample (10 mL) was collected for pharmacokinetic analysis at 5 and 30 minutes and 3, 9, and 19 hours after meloxicam was administered. Each horse was premedicated with xylazine (1.1 mg/kg, IV), and induction of anesthesia was achieved with diazepam (0.1 mg/kg, IV) and ketamine (2.2 mg/kg, IV). After orotracheal intubation, each horse was maintained at a surgical plane of anesthesia with isoflurane vaporized in 100% O2. All horses received butorphanol tartrate (0.05 mg/kg, IV) immediately after positioning in dorsal recumbency. A midline celiotomy was performed with an aseptic technique, and the distal portion of the jejunum was located. The contents of the distal portions of the jejunum and ileum were milked manually into the cecum, after which two 30-cm loops of jejunum were isolated by use of Doyen intestinal forceps. The loops were approximately 1 m apart, and the first was 60 cm oral to the antimesenteric band of the ileum. One loop in each horse was used as control tissue, and the other underwent ischemia for 2 hours. The ischemia was induced by clamping local mesenteric arteries and veins with Penrose drains to avoid excessive trauma to the vessels. At the cessation of ischemia, the loops were reperfused and a full-thickness wedge biopsy specimen was obtained from the control and ischemic loops. The biopsy sites and celiotomy were closed routinely, and horses were allowed to recover from anesthesia.

After surgery, horses underwent clinical examination every 2 to 4 hours (including assessment of heart and respiratory rates, rectal temperature, gastrointestinal borborygmi, and mucus membrane color and recording of feed and water consumption, defecation, and urination), and all horses received butorphanol tartrate (0.05 mg/kg, IM, q 4 h). In addition, horses were monitored 2, 8, and 16 hours after the cessation of ischemia for signs of pain and a pain score was assigned by use of a modification of a previously established behavioral pain scoring system (Appendix). Any horse that developed gross signs of pain was administered additional analgesia. Horses had access to water ad libitum immediately after surgery and were offered small amounts of timothy hay. Horses were euthanatized with pentobarbital sodium (100 mg/kg, IV) 18 hours after the cessation of ischemia. After euthanasia, ischemia-injured and control jejunal tissues were harvested for in vitro experiments and immediately placed in oxygenated (95% O2 and 5% CO2) equine Ringer’s solution (NaCl, 114 mM; KCl, 5 mM; CaCl2, 1.25 mM; MgCl2, 1.10 mM; NaHCO3, 25 mM; NaH2PO4, 0.3 mM; and Na2HPO4, 1.65 mM).

Ussing chamber studies—Harvested jejunum was incised along the antimesenteric surface, and mucosa was stripped from the seromuscular layer in oxygenated equine Ringer’s solution and mounted in Ussing chambers (aperture, 3.14 cm2) as described previously. Mucosa was bathed on both mucosal and serosal sides in 10 mL of oxygenated equine Ringer’s solution maintained at 37°C by the use of circulating water-jacketed reservoirs. The serosal bathing solution additionally contained 10 mmol of mannitol/L. The spontaneous PD was measured by use of Ringer-agar bridges connected to calomel electrodes, and the PD was short-circuited through silver-silver chloride electrodes with a voltage clamp that corrected for fluid resistance. Resistance (Ω·cm2) was calculated from the spontaneous PD and Isc. If the spontaneous PD was between −1 mV and 1 mV, tissues were current clamped at ±100 µA for 5 seconds and PD recorded. The Isc and PD were recorded every 15 minutes for 2 hours. Data were entered into spreadsheets that calculated TER from Isc and PD by use of Ohm’s law. Results expressed as the mean TER ± SEM over a 1-hour period were used for insulin and FGF-2–LPS flux assessments after a 30-minute equilibration period.

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**Inulin flux**—Radiolabeled methylated inulin (14C-methylated inulin; 10 μCi/mL) in 3% inulin was placed on the mucosal side of control and ischemia-injured tissues mounted in Ussing chambers following an initial 30-minute equilibration period. One 60-minute flux was subsequently conducted from 30 to 90 minutes of the experimental period on samples obtained from the serosal side of the bathing solution; β emission (counts/min) was measured in a scintillation counter. Mucosal-to-serosal flux (Jm) of inulin was calculated by use of standard equations.13,14

**FITC-LPS flux**—Lipopolysaccharide from *Escherichia coli* serotype O111:B4 labeled with fluorescein isothiocyanate (3 μg of FITC/mg of LPS) was placed on the mucosal side of control and ischemia-injured tissues mounted in Ussing chambers (2.5 × 10⁻³ g of FITC-LPS/mL of Ringer’s solution) following an initial 30-minute equilibration period. One 60-minute flux was subsequently conducted from 30 to 90 minutes of the experimental period on 200-μL samples obtained in triplicate from the serosal side of the bathing solution. Fluorescence of samples and a standard curve (created for each experiment) were measured in a fluorescent 96-well plate reader at an excitation wavelength of 485 nm and emission wavelength of 538 nm. Mucosal-to-serosal flux of LPS was calculated to account for variation in amount of FITC labeling between batches of different FITC-LPS by correcting the FITC-LPS concentrations obtained from the standard curve to total amount (mg) of LPS that passed from the mucosal to serosal sides for each 1-cm² area of the Ussing chamber and subtracting the concentration of LPS present at the beginning of the 1-hour flux period from that present at the end of the 1-hour flux period.

**Histologic examination**—Samples of mucosa from control and ischemia-injured jejunum were obtained via wedge biopsy procedures at the cessation of ischemia and at the time of euthanasia (18 hours after cessation of ischemia). Three-micrometer-thick sections were cut at 300-μm intervals from each mucosal sample and processed for routine H&E staining and light microscopy. Sections were independently examined by 2 observers (DL and SAB). Three well-oriented villi were identified in each section; the height of each villus (as an indicator of the degree of villus contraction) and the width at the midpoint of each villus were obtained by use of a light microscope with an ocular micrometer. For height measurements, the base of the villus was defined as the intersection between adjacent villi at the opening of the crypt. For villi in which the height of 1 side of the villus was markedly different from the other side, a mean height was recorded.

In addition, the height of the epithelial-covered portion of each villus was measured. The surface area of the villus was calculated by use of a modified formula for the surface area of a cylinder as follows:

\[
\text{Villus surface area} = (2\pi \times 1/2 \times d \times h)
\]

where \(\pi = 3.14\), \(d = \) villus diameter at midpoint, and \(h = \) villus height. The percentage of denuded villous surface area that remained denuded was calculated from the total surface area of the villus and the surface area of the villus covered by epithelium. The percentage of denuded villous surface area was used as an index of epithelial restitution. Number of epithelial neutrophils were counted in a 10-μm² grid reticle on 5 different villus tips and expressed as mean count per millimeter².

**Meloxicam sample analysis**—Plasma was separated from blood samples obtained immediately prior to drug administration and 5 and 30 minutes and 3, 9, and 19 hours after meloxicam administration. Meloxicam concentrations in plasma were analyzed via HPLC by use of a method developed in our laboratory. The HPLC system² had a variable wavelength UV detector set at 363 nm. Sample separation was achieved by use of a reverse-phase column (4.6 X 150 mm). The mobile phase was 60% 0.05M sodium acetate buffer and 40% HPLC grade acetonitrile; glacial acetic acid was added to decrease the pH of the mobile phase to 3.7. Meloxicam sodium standard (93.8% pure) was dissolved in deionized water to a total concentration of 1 mg of meloxicam base/mL. Further dilutions were made in deionized water to create spiking solutions for use in pooled blank equine plasma to prepare standard curves prior to each run. All samples were subjected to solid-phase extraction by use of 1-mL hydrophilic-lipophilic-balanced cartridges in a vacuum manifold. Cartridges were initially conditioned with 1 mL of HPLC-grade methanol and 1 mL of deionized water. Plasma (1 mL) was extracted through the cartridge, which was then washed with a 95.5 (vol/vol) mixture of deionized water and methanol. The sample was then eluted into clean borosilicate glass tubes with 1 mL of methanol and evaporated under compressed room air at 40°C for 25 minutes. The sample was reconstituted with 200 μL of mobile phase for injection onto the HPLC system. The injection volume was 25 μL and the retention time was approximately 4.5 minutes.

**Pharmacokinetic analysis**—The data were analyzed by use of a computer program.6 Noncompartmental analyses were performed to determine pharmacokinetic parameters including the plasma concentration at time zero, elimination half-life, clearance, apparent volume of distribution, and area under the curve extrapolated to infinity.

**Gel electrophoresis and western blot analysis**—Mucosal scrapings from control and ischemia-injured mucosa of 3 representative horses from each drug treatment group obtained 18 hours after the cessation of ischemia (ie, at the time of euthanasia) were snap frozen and stored at −80°C. One-gram tissue aliquots were thawed to 4°C and added to 3 mL of chilled radioimmunoprecipitation assay buffer (0.15M NaCl, 50mM Tris [pH, 7.2], 0.5% deoxycholic acid, 1% Triton X-100, 0.1% SDS, and 1% nonylphenylpolyethylene glycol), including protease inhibitors (phenylmethyl sulfonyl fluoride, sodium orthovanadate, and aprotinin). The mixture was homogenized on ice and then centrifuged twice at 10,000 × g for 10 minutes at 4°C; the supernatant was collected. Protein analysis of extract was performed by use of the Lowry assay to determine protein concentration of each sample. Equal (normalized) concentrations of protein extracts were mixed with SDS-PAGE sample buffer and reduc-
ing agent and boiled for 5 minutes at 100°C. Lysates were loaded on 4% to 12% gradient precast Bis-Tris polyacrylamide gels, and electrophoresis was carried out according to standard protocols. Proteins were transferred to a nitrocellulose membrane by use of an electrophoretic transfer apparatus according to the manufacturer's protocol. Membranes were boiled for 5 minutes in PBS solution and then blocked for 16 hours at 4°C in Tris-buffered 150mM NaCl solution and 3% dry powdered milk. Membranes were washed and incubated for 2 hours in primary antibody (1:500 dilution of anti-human COX-1 antibody, 1:500 dilution of anti-human COX-2 antibody, or 1:500 anti-rabbit total p38 MAPK antibody) or incubated overnight at 4°C for detection of the activated form of p38 MAPK, phospho-p38 (1:1,000 dilution). The membranes were washed and then incubated with horseradish peroxidase-conjugated secondary antibody at a dilution of 1:15,000 (COX-1 and COX-2), 1:2,500 (β-actin), 1:1,000 (PPARγ and phospho-p38), or 1:5,000 (total p38 MAPK). After additional washes, the membranes were developed for visualization of proteins by addition of enhanced chemiluminescence reagent. A membrane that was initially probed for phospho-p38 was stripped by use of standard procedures and reprobed for total p38 MAPK so that the ratio of phospho-p38 to total p38 MAPK concentration could be calculated and compared via densitometry on the same membrane. The same membrane was subsequently stripped a second time and reprobed for β-actin to confirm equal protein loading of lanes. Densitometry was performed by use of appropriate software. Data were expressed as mean ± SEM densitometry units for control or ischemia-injured tissue for each of the 3 drug treatment groups (saline solution, flunixin, and meloxicam). In addition, densitometry of ischemia-injured tissue was compared with densitometry of control tissue from the same horse and expressed as the mean ± SEM percentage change in ischemia-injured tissue for each drug treatment group. The phospho-p38-to-total p38 MAPK concentration ratio was calculated for each tissue from each horse; the ratio for control tissue from saline solution–treated horses was corrected to a value of 1, and the ratios for the other tissue groups were corrected to this value. The change in ratio for ischemia-injured tissue, compared with control tissue for the same horse, was then calculated and expressed as a mean ± SEM change in ratio.

Statistical analysis—Heart and respiratory rates were analyzed by use of a 2-way ANOVA for the effects of drug treatment and time. Pain scores were analyzed by use of an ANOVA on ranks for the effect of treatment and time. Mucosal neutrophil counts and villus height measurements were evaluated by use of a 2-way ANOVA for the effects of drug treatment and loop (control vs ischemia-injured). Percentage of villus denudation was evaluated by use of an ANOVA on ranks for the effect of drug treatment and loop. Transepithelial electrical resistance, inulin and FITC-LPS fluxes, and densitometry data were analyzed by use of a 1-way ANOVA. Post hoc analyses were performed with Tukey tests for parametric data. For all tests, significance was set at a value of P < 0.05.

Results

Clinical variables and pain scores—No horse was deemed by the investigators to require additional analgesia or have gross signs of pain; thus, data from all 18 horses were available for analysis. There was no significant difference in total preoperative pain scores among the 3 groups of horses. Horses treated with saline solution alone had significantly higher total pain scores at all time points after surgery, compared with the preoperative pain score of meloxicam-treated horses; at 2 and 8 hours after cessation of ischemia, compared with the preoperative pain score of flunixin-treated horses; and at 2 hours after cessation of ischemia, compared with the preoperative pain score of saline solution–treated horses. There was no significant difference in total pain scores between horses administered flunixin and those administered meloxicam (Figure 1).

Before surgery, heart and respiratory rates did not differ significantly among treatment groups. Both heart and respiratory rates were significantly increased at all time points after surgery in saline solution–treated horses, compared with preoperative values in this group, except for respiratory rate at 8 hours (Figure 2). Compared with preoperative values, heart rate in horses treated with flunixin was significantly increased at 8 hours after surgery, whereas heart rate was not significantly increased in horses treated with meloxicam at any time point after surgery. Compared with findings in saline solution–treated horses, heart rates were lower in flunixin-treated horses at 16 hours after surgery and lower in meloxicam-treated horses at 8 and 16 hours after surgery. At all time points after surgery, respiratory rate was significantly lower in horses treated with meloxicam, compared with those treated with saline solution. In horses treated with flunixin, respiratory rate was lower than that in saline solution–treated horses at 16 hours after surgery. Respiratory rate at 16 hours after surgery was significantly lower in horses treated with meloxicam, compared with the value in horses treated with flunixin.

TER—In horses treated with saline solution, TER of ischemia-injured jejunum was significantly (P < 0.001) increased, compared with control uninjured tissue (94 ± 4.4 Ω·cm² vs 55 ± 3.3 Ω·cm²). In horses treated with flunixin, the recovery in TER of ischemia-injured jejunum was blocked and was not significantly different from control tissue (46 ± 2.1 Ω·cm² vs 47 ± 4.8 Ω·cm²). Meloxicam treatment resulted in increased (P < 0.001) TER in ischemia-injured tissue, compared with control tissue (104 ± 7.0 Ω·cm² vs 55 ± 2.0 Ω·cm²). The extent of TER recovery in meloxicam-treated horses was not significantly different from that detected in ischemia-injured tissue from horses treated with saline solution; however, these 2 values were both significantly (P < 0.001) greater than the TER of ischemia-injured jejunum in flunixin-treated horses and the TER of control tissue in all 3 groups. Exposure of control or ischemia-injured jejunum to FITC-LPS for measurement of mucosal-serosal flux did not change TER, compared with findings regarding jejunal specimens that were not treated with LPS (data not shown).

Inulin and FITC-LPS fluxes—Compared with meloxicam-treated horses, the mucosal-serosal inulin...
flux across ischemia-injured tissue was significantly (P = 0.002) greater in flunixin-treated horses (1.65 ± 7.4 × 10⁻¹ mg·h⁻¹·cm⁻² and 3.86 ± 1.1 × 10⁻³ mg·h⁻¹·cm⁻², respectively). Although the mucosal-to-serosal inulin flux across ischemia-injured tissue was greater in flunixin-treated horses, compared with saline solution–treated horses (1.84 ± 3.8 × 10⁻¹ mg·h⁻¹·cm⁻²), this difference was not significant (P = 0.07). The FITC-LPS flux in ischemia-injured jejunum (0.34 ± 5.8 × 10⁻¹ mg·h⁻¹·cm⁻²) from horses treated with flunixin was significantly (P = 0.002) greater than values in control tissue in all 3 treatment groups (0.0151 ± 3.8 × 10⁻¹ mg·h⁻¹·cm⁻², 0.0129 ± 2.8 × 10⁻³ mg·h⁻¹·cm⁻², and 0.0118 ± 1.7 × 10⁻³ mg·h⁻¹·cm⁻² in saline solution–, flunixin–, and meloxicam-treated horses, respectively). There were no other significant differences among groups.

Histologic findings—At the cessation of the 2-hour period of ischemia, numbers of neutrophils in ischemia-injured mucosa were significantly (P < 0.05) increased in each treatment group, compared with the respective control mucosa (Figure 3). There was no difference in numbers of neutrophils among the control mucosa from the 3 treatment groups or among ischemia-injured mucosa from the 3 treatment groups. Eighteen hours after the cessation of the ischemic period, numbers of mucosal neutrophils were still increased in all ischemia-injured tissues in all 3 treatment groups, compared with the respective control tissues; however, there was an additional increase in numbers of neutrophils in ischemia-injured tissue of horses treated with flunixin or meloxicam, and these values were each significantly (P < 0.05) greater than the value for ischemia-injured tissue in saline solution–treated horses.

In saline solution–, flunixin–, and meloxicam-treated horses after 2 hours of ischemia, there was no villus denudation in control tissue and 41.6 ± 4.8% and 44.9 ± 5.6% in ischemia-injured tissue, respectively. The percentages of villus denudation in the ischemia-injured tissues of flunixin- and meloxicam-treated horses were significantly (P < 0.001) greater than the respective control tissue values. In saline solution–, flunixin–, and meloxicam-treated horses after 2 hours of ischemia, there was significant villus contraction, as identified by a reduction in villus height between control (358 ± 13 µm, 306 ± 19 µm, and 354 ± 16 µm, respectively) and ischemia-injured tissue (178 ± 10 µm, 178 ± 6 µm, and 215 ± 12 µm, respectively). There was no effect of drug treatment on degree of epithelial denudation or villus contraction at the end of the 2-hour period of ischemia. In saline solution–, flunixin–, and meloxicam-treated horses at 18 hours after cessation of ischemia, epithelial denudation was 0%,

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Figure 1—Total pain scores assigned to horses treated* with saline (0.9% NaCl) solution (S), flunixin meglumine (F), or meloxicam (M) before and at intervals after a 2-hour period of surgically induced jejunal ischemia. Pain scores were assigned before (B-lsc) and at 2, 8, and 16 hours after cessation of ischemia (2h-Alsc, 8h-Alsc, and 16h-Alsc, respectively). Lines within the box represent the median pain score; the upper and lower limit of the box represent the 75th and 25th percentiles, respectively; the upper and lower limit of the error bars represent the 90th and 10th percentiles, respectively; and the dots represent outliers.

*Five minutes prior to premedication and approximately 1 hour before initiation of ischemia, 6 horses received saline solution (12 mL, IV), which was repeated every 12 hours for the duration of the study; 6 horses received flunixin meglumine (1.1 mg/kg, IV), which was repeated every 12 hours for the duration of the study and immediately prior to euthanasia (approx 21 hours later); and 6 horses received meloxicam (0.6 mg/kg, IV), which was repeated immediately prior to euthanasia (approx 21 hours later).

Figure 2—Mean ± SEM heart rate (A) and respiratory rate (B) in horses treated* with saline solution (black bars; n = 6), flunixin meglumine (light gray bars; 6), or meloxicam (dark gray bars; 6) before and at 2, 8, and 16 hours after cessation of a 2-hour period of surgically induced jejunal ischemia. †Value significantly (P < 0.05) greater than the preoperative value in each of the 3 treatment groups. †Value significantly (P < 0.05) greater than the preoperative value for horses treated with meloxicam or flunixin. §Value significantly (P < 0.05) greater than the preoperative value for horses treated with meloxicam.
0.4 ± 0.4%, and 0% in control tissue and 7.15 ± 1.6%, 9.9 ± 2.2%, and 0.6 ± 0.3% in ischemia-injured tissue, respectively. There were no significant differences among groups. However, at 18 hours after cessation of ischemia, there was evidence of epithelial restitution in ischemia-injured jejunum because a lower percentage of each villus was denuded, compared with the extent of denudation present immediately after the end of the 2-hour period of ischemia. At 18 hours after cessation of ischemia, there was still significant villus contraction, as identified by a reduction in villus height between control (262 ± 10 µm, 274 ± 10 µm, and 279 ± 6 µm, respectively) and ischemia-injured tissue (99 ± 6 µm, 58 ± 4 µm, and 106 ± 5 µm, respectively). There were no differences among groups.

Meloxicam pharmacokinetic parameters—For the HPLC assay, intra-assay accuracy and precision were calculated at 10, 1, and 0.1 µg/mL. Five samples of each concentration were analyzed. Overall, accuracy of the HPLC assay was within 4.04 ± 2.66% of the true value, and precision was within 4.04 ± 0.23% of the mean. Mean recovery for drug in plasma at these concentrations was 81 ± 6.6%. The limit of detection was 0.019 µg/mL, and the limit of quantification was 0.025 µg/mL. Calibration curves were linear between the concentrations of 10 and 0.025 µg/mL, with a coefficient of determination (r²) > 0.99 and calibration samples within ±15% of the true concentration.

Pharmacokinetic parameters obtained for meloxicam (Table 1) were compared with other reported values. Drug clearance was much lower in the present study, compared with previously reported values. Plasma meloxicam concentrations in the study horses were typical of those achieved following IV administration in this species.

Western blot analysis—In the western blot analyses of mucosal scrapings from control and ischemia-injured mucosa of 3 representative horses from each drug treatment group obtained 18 hours after the cessation of ischemia (ie, at the time of euthanasia), there was no difference in β-actin expression across all lanes.

![Figure 3](image)

Table 1—Summary of noncompartmental pharmacokinetic parameters derived following IV administration of meloxicam (0.6 mg/kg) to 6 horses prior to undergoing a 2-hour period of surgically induced jejunal ischemia and comparison of those parameters with other available data. 

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
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<th>Comparison</th>
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<tr>
<td>C₀ (µg/mL)</td>
<td>9.78 ± 1.0</td>
<td>9.23¹</td>
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<tr>
<td>AUC₀₋∞ (h·µg/mL)</td>
<td>33.44 ± 10.58</td>
<td>14.53²</td>
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<tr>
<td>AUMC₀₋∞ (h²·µg/mL)</td>
<td>151.61 ± 85.39</td>
<td>ND</td>
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<tr>
<td>λ₁ (h⁻¹)</td>
<td>0.18 ± 0.04</td>
<td>ND</td>
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<tr>
<td>t½λ₁ (h)</td>
<td>4.07 ± 1.07</td>
<td>2.7⁴</td>
</tr>
<tr>
<td>Cl (mL/kg/min)</td>
<td>0.33 ± 0.14</td>
<td>0.8²</td>
</tr>
<tr>
<td>Vdₓ∞ (L/kg)</td>
<td>0.11 ± 0.04</td>
<td>0.58⁴</td>
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C₀ = Maximum concentration. AUC₀₋∞ = Area under the concentration-time curve. AUMC₀₋∞ = Area under the first moment-time curve. λ₁ = Slope of the terminal phase. t½λ₁ = Half-life of terminal phase. Cl = Systemic clearance. Vdₓ∞ = Apparent volume of distribution. ND = No data.

![Figure 4](image)
(including stripped blots [data not shown]), confirming equal protein loading. For saline solution–treated horses, COX-1, PPARγ, and total p38 MAPK expression was significantly increased in ischemia-injured mucosa, compared with control mucosa, at 18 hours after cessation of ischemia (Figures 4–6).

In ischemia-injured mucosa from horses treated with flunixin, COX-1 expression was significantly increased, compared with findings in control tissue, and PPARγ expression was significantly increased, compared with findings in all control groups and all other ischemia-injured groups. However, compared with the control tissue, the percentage increase in PPARγ expression in ischemia-injured mucosa from horses treated with flunixin was similar to that detected in tissues from saline solution–treated horses. Total p38 MAPK expression in ischemia-injured mucosa of flunixin-treated horses was increased, compared with control tissue; the percentage increase in total p38 MAPK expression in ischemia-injured mucosa (compared with control tissue) was less than the difference detected between tissues from saline solution–treated horses. The increase in phospho-p38–to–total p38 MAPK ratio in ischemia-injured tissues of flunixin-treated horses (compared with control tissue) was significantly smaller than the increase in ratio for saline solution–treated horses.

In meloxicam-treated horses, COX-1 was upregulated in ischemia-injured mucosa relative to expression in control mucosa. Expression of COX-2 in meloxicam-treated horses was not significantly increased with ischemia (compared with the control tissue), but the percentage increase in ischemia-injured tissue, relative to the control tissue, was significantly less than the relative increase in COX-2 expression in ischemia-injured tissues in saline solution–treated horses. The expression of PPARγ in both control and ischemia-injured mucosa from meloxicam-treated horses was increased, compared with expression in control mucosa from saline solution–treated horses. Relative to the respective control tissues, the magnitude of the increase in PPARγ expression in ischemia-injured mucosa in meloxicam-treated horses was significantly less than the increase in PPARγ expression in ischemia-injured mucosa in either saline solution– or flunixin-treated horses. Total p38 MAPK expression was increased in ischemia-injured mucosa from meloxicam-treated horses, compared with control tissue; however, the magnitude of this increase was significantly less in the meloxicam-treated horses than it was in horses treated with saline solution or flunixin. Furthermore, the ratio of phospho-p38 to total p38 MAPK concentration in ischemia-injured tissue of meloxicam-treated horses, compared with control tissue, was similar to that detected in flunixin-treated horses and significantly less than that detected in saline solution–treated horses.

**Discussion**

Compared with findings in horses treated with saline solution, the effects of treatment with meloxicam on postoperative pain scores, heart rate, and respiratory rate were comparable or greater than the effect of treatment with flunixin meglumine, which suggests that meloxicam deserves further evaluation as an analgesic for pain caused by colic and endotoxemia in horses.

Results of the present study have confirmed our previous findings that flunixin administration retards recovery of TER in ischemia-injured jejunal mucosa, compared with TER recovery in horses treated with saline solution, at 18 hours after cessation of an episode of ischemia. This indicated that mucosal barrier function was impaired. In contrast, meloxicam administration did not impede recovery of isch-
that of the respective control jejunal tissue, but the TER of flunixin-treated ischemia-injured jejenum was similar to that of the control tissue. These data suggest that PGs are critical for this apparently increased recovery response in ischemia-injured jejenum; seemingly, this increased response of TER is critical for recovery of barrier function because LPS flux was increased in ischemia-injured jejenum from horses treated with flunixin but not in ischemia-injured jejenum from other groups of horses.

There are several possible explanations for the increased recovery of TER in ischemia-injured jejunal mucosa. First, in normal mucosa, paracellular permeability is not uniform along the whole villus-crypt axis. Paracellular permeability is lower on the villus, compared with the crypt, which is relatively leaky. Prostaglandins mediate recovery of barrier function in ischemic-injury via collapse of the lateral intercellular space. The presence of PGs in ischemia-injured equine jejenum in horses not administered nonselective NSAIDs may contribute to collapse of the lateral intercellular space throughout the whole of the villus-crypt axis, contributing to increases in TER (over that of control tissue) because of increased collapse of the lateral intercellular space and enhanced barrier function within the crypts. Second, the increased recovery of TER in ischemia-injured jejenum is not associated with reductions in insulin or LPS flux (compared with values in control mucosa), even though LPS and insulin fluxes increase when the increased recovery of intestinal barrier function is impaired, as occurs with flunixin treatment. The interepithelial tight junction is composed of a variety of proteins. Interactions of these proteins allow the formation of various pores within the junction, which are size and charge restrictive. Thus, mediators of inflammation can differentially regulate paracellular permeability and paracellular flux of molecules of variable size and charge, leading to the possibility that PGs selectively reduce passage of small electrolytes that contribute to TER in ischemia-injured tissue, and reduce the movement of large molecules such as insulin or LPS used in flux studies through larger pores. Inhibition of PG synthesis may therefore abolish the selectivity of these pores, resulting in an increased movement of electrolytes and subsequent reduction in TER and in an increased movement of larger molecules that are typically used for flux studies. However, elucidation of the exact mechanism of the increased TER in ischemia-injured equine jejenum at 18 hours after cessation of ischemia awaits further mechanistic study.

The increase in LPS flux that occurred in ischemia-injured jejenum from horses treated with flunixin is of particular concern. Administration of the passage of LPS across the intestinal mucosa is critical to reduce the proinflammatory action of LPS on the systemic inflammatory response and diminish the deleterious effects.

Figure 6—Results of densitometry analysis for total p38 MAPK (A and B) and the ratio of phospho-p38 to total p38 MAPK (C and D) in control uninjured (Con) and ischemia-injured (Isc) jejunal mucosa from 3 representative horses at 18 hours after cessation of a 2-hour period of surgically induced jejunal ischemia. Horses were treated* with saline solution, flunixin meglumine, or meloxicam before and at intervals after the period of ischemia. Data are represented as mean ± SEM densitometry units (A), mean ± SEM percentage change in densitometry units between control uninjured and ischemia-injured tissue within each horse (B), mean ± SEM change in the ratio of phospho-p38 to total p38 MAPK between control uninjured and ischemia-injured tissue in the 3 drug treatment groups (C), and the change in the ratio of phospho-p38 to total p38 MAPK between ischemia-injured and control uninjured tissue within each of the 3 drug treatment groups (D). †Value significantly (P < 0.05) different from control tissue from meloxicam- and flunixin-treated horses. §Value significantly (P < 0.05) different from that of the other 2 groups. See Figures 1 and 3 for key.

emia-injured jejenum; the extent of recovery of TER in ischemia-injured mucosa was similar to that detected in horses treated with saline solution, indicating that meloxicam- and saline solution–treated horses had enhanced intestinal barrier function of ischemia-injured intestinal loops, compared with flunixin-treated horses. We have previously determined that PGs are required for recovery of barrier function (as measured by TER) in ischemia-injured porcine ileum. Data from the present study have suggested that the COX-2 preferential inhibitor meloxicam may permit sufficient COX-1 activity for PG-mediated recovery of intestinal barrier function, while inhibiting the detrimental effects of COX-2–elaborated PGs on clinical signs of endotoxemia and pain.

Ischemia-injured equine jejunal mucosa does not always have TER greater than that of control uninjured jejenum in the postoperative period. From previous studies, TER of ischemia-injured mucosa from saline solution–treated horses is less than that of control jejenum immediately after cessation of jejunal ischemia in horses but increases significantly within 2 hours of in vitro recovery; this recovery response is absent in flunixin-treated ischemia-injured jejenum. At 18 hours after cessation of ischemia in the present study, TER of ischemia-injured mucosa from saline solution– or meloxicam-treated horses was greater than
of LPS on enteroctye migration and activation and on epithelial restitution. Furthermore, LPS can itself induce failure of epithelial barrier function through opening of tight junctions. It is likely that in horses with intestinal ischemia that are treated with flunixin, increased LPS flux across the intestinal barrier contributes to sustained epithelial cell dysfunction and further exacerbates the initial inhibition of recovery of barrier function caused by inhibition of COX and inhibition of endogenous PG production.

It is interesting that treatment of the horses in the present study with either meloxicam or flunixin resulted in a further increase in accumulation of mucosal neutrophils in ischemia-injured mucosa, compared with findings after saline solution treatment, even though numbers of neutrophils in control mucosa of all 3 treatment groups were not different. In another study, the numbers of mucosal neutrophils in ischemia-injured tissues from horses treated with saline solution, flunixin, or etodolac were not different at 18 hours after cessation of ischemia. The difference in results between the 2 studies is likely related to the earlier administration of flunixin or meloxicam treatment (ie, prior to induction of anesthesia) in the present study. Results of several studies have suggested that NSAID administration before injury may increase free radical formation during injury, and doses of NSAIDs that block PGE2 production may be insufficient to block production of neutrophil chemoattractants, thereby resulting in increased numbers of infiltrating neutrophils. These factors are unlikely to be important in clinical situations because NSAIDs are rarely administered to horses before strangulating obstructions develop. Furthermore, epithelial barrier function in meloxicam-treated horses appeared to recover in a similar manner to that detected in saline solution–treated horses, and the detrimental effects of flunixin treatment were similar to those reported previously, despite this increase in neutrophil influx.

The low volume of distribution of meloxicam in plasma in our study was comparable to the value determined in another study and was likely attributable to extensive plasma protein binding of meloxicam. The half-life of the terminal phase in the present study was considerably shorter than that identified previously in a study but was comparable to values determined in 2 other studies. The plasma concentration required to achieve 50% of maximum response (EC50) of meloxicam in horses with experimentally induced arthritis in the right carpal joint is 0.2 µg/mL. In the present study, evaluation of pain scores, heart rate, and respiratory rate in horses with ischemia-injured jejunal tissue suggested that once-daily IV treatment with 0.6 mg of meloxicam/kg was sufficient to provide adequate analgesia, and mean plasma meloxicam concentrations remained > 0.2 µg/mL during the 19-hour pharmacokinetic component of the study. However, meloxicam clearance was much slower in the present study than the previously reported values. Therefore, drug dose and frequency may need to be reevaluated in clinical settings where factors such as dehydration, concurrent drug administration, anesthesia, and the systemic inflammatory response are important influences on glomerular filtration rate and, therefore, drug clearance. Only after these studies have been completed can the use of meloxicam in the treatment of colic be recommended.

In a previous study, there was no significant increase in COX-1 or COX-2 expression in ischemia-injured equine jejunum at 18 hours after cessation of ischemia, except in horses treated with etodolac. At an earlier time point in that study (ie, immediately after cessation of ischemia), COX-1 and COX-2 expressions were increased in ischemia-injured jejunum, compared with control tissues, and both COX-1 and COX-2 were expressed in control unjured jejunum. In the present study, there was increased expression of COX-1 in ischemia-injured jejunal tissue, compared with findings in control mucosa, at 18 hours after cessation of ischemia in all 3 treatment groups. The increase in expression in COX-1 was not surprising because of the increase in neutrophilic infiltration into the mucosa in ischemia-injured mucosa detected in our study; on the basis of results of previous studies, the increase in COX-1 expression is likely attributable to increased numbers of neutrophils rather than increased tissue expression, though this requires confirmation in horses. Although COX-1 expression is not typically upregulated in inflammatory conditions, there is evidence to suggest that expression of COX-1 increases in the mononuclear cells of the lamina propria of gastric mucosa with increasing severity of mucosal ulceration. The neutrophilic infiltration in ischemia-injured mucosa, compared with control mucosa, may have also accounted for the increased expression of PPARγ and total p38 MAPK detected in saline solution–treated horses in the present study. Furthermore, given that p38 MAPK is phosphorylated in response to chemotaxis, it was not surprising that the ratio of phospho-p38 to total p38 MAPK concentration increased in ischemia-injured mucosa of saline solution–treated horses, compared with control uninjured tissues. Meloxicam reduced the upregulation of COX-2 expression associated with ischemia, compared with that detected in saline solution–treated horses. This may be attributable to alternate mechanisms of action of NSAIDs because several NSAIDs are PPARγ ligands and activation of PPARγ is important for the transcriptional regulation of COX-2. The nuclear hormone receptor PPARγ functions as a transcription factor and is known to be an important mediator of inflammatory gene transcription. Regulation of PPARγ expression in ischemia-reperfusion conditions has not been well studied; however, PPARγ expression is downregulated in alveolar macrophages of humans with allergic asthma, suggesting that expression may be responsive to change in inflammatory diseases. The role of PPARγ signaling in equine intestine deserves further attention, particularly because meloxicam appears to inhibit the upregulation of its expression during ischemic episodes. Expression of the MAPKs has also been not well studied in ischemia, although some of the members of this group, ERK 1/2, JNK, and p38 MAPK, are activated in response to ischemia and other stresses and are critical for epithelial barrier function, stabilization of COX-2 mRNA, and inflammation. In rodents with gastric ischemia-reperfusion injury, MAPK 1 is downregulated approximately 5-fold immediately after cessation of ischemia but is upregulated approximately 2-fold after 60 minutes of reperfusion, and these findings deserve further evaluation in horses.
interesting that flunixin and especially meloxicam reduce the upregulation of total p38 MAPK expression that occurs during ischemia and subsequent reperfusion. The fact that both of these NSAIDs also inhibit phosphorylation of p38 MAPK may point to an alternative anti-inflammatory mechanism for the NSAIDs commonly used in horses, as has been identified with other NSAIDs in experimental situations.10

The data obtained in the present study have indicated that meloxicam may be a useful alternative to flunixin meglumine for treatment of horses with colic, through its ability to provide adequate analgesia and beneficial effects on clinical variables while permitting recovery of intestinal barrier function. The results of our study also suggest that the commonly used NSAIDs in horses should be evaluated for COX-independent mechanisms of action.


References


Appendix

Behavioral pain scoring system used to assess signs of pain in horses that were treated with saline (0.9% NaCl) solution, flunixin meglumine, or meloxicam before and at intervals after a 2-hour period of surgically induced jejunal ischemia. Scores from each category are added to give a total subjective pain score (modified from Pritchett et al12).

<table>
<thead>
<tr>
<th>Behavioral category</th>
<th>Behavioral score</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Gross signs of pain</td>
<td>None</td>
</tr>
<tr>
<td>Head position</td>
<td>Above withers*</td>
</tr>
<tr>
<td>Ear position</td>
<td>Forward; frequent movement</td>
</tr>
<tr>
<td>Location</td>
<td>At door, watching environment</td>
</tr>
<tr>
<td>Spontaneous locomotion</td>
<td>Moves freely</td>
</tr>
<tr>
<td>Response to another horse</td>
<td>Ears forward; head up; moves to door</td>
</tr>
<tr>
<td>Response to open door</td>
<td>Moves to door</td>
</tr>
<tr>
<td>Response to approach</td>
<td>Moves to observer; ears forward</td>
</tr>
<tr>
<td>Lifting feet</td>
<td>Freely when prompted</td>
</tr>
</tbody>
</table>

*Highest point of vertebral column between scapulae.
NA = Not applicable.