Anti-inflammatory effects of intravenously administered lidocaine hydrochloride on ischemia-injured jejunum in horses

Vanessa L. Cook, VetMB, PhD; Jennifer Jones Shults, DVM; Marsha R. McDowell, DVM; Nigel B. Campbell, BVetMed, PhD; Jennifer L. Davis, DVM, PhD; John F. Marshall, BVMS; Anthony T. Blikslager, DVM, PhD

Objective—To investigate effects of lidocaine hydrochloride administered IV on mucosal inflammation in ischemia-injured jejunum of horses treated with flunixin meglumine.

Animals—24 horses.

Procedures—Horses received saline (0.9% NaCl) solution (SS; 1 mL/50 kg, IV [1 dose]), flunixin meglumine (1 mg/kg, IV, q 12 h), lidocaine (bolus [1.3 mg/kg] and constant rate infusion [0.05 mg/kg/min], IV, during and after recovery from surgery), or both flunixin and lidocaine (n = 6/group). During surgery, blood flow was occluded for 2 hours in 2 sections of jejunum in each horse. Uninjured and ischemia-injured jejunal specimens were collected after the ischemic period and after euthanasia 18 hours later for histologic assessment and determination of cyclooxygenase (COX) expression (via western blot procedures). Plasma samples collected prior to (baseline) and 8 hours after the ischemic period were analyzed for prostanooid concentrations.

Results—Immediately after the ischemic period, COX-2 expression in horses treated with lidocaine alone was significantly less than expression in horses treated with SS or flunixin alone. Eighteen hours after the ischemic period, mucosal neutrophil counts in horses treated with flunixin alone were significantly higher than counts in other treatment groups. Compared with baseline plasma concentrations, posts ischemia prostaglandin E₂, metabolite and thromboxane B₂ concentrations increased in horses treated with SS and in horses treated with SS or lidocaine alone, respectively.

Conclusions and Clinical Relevance—In horses with ischemia-injured jejunum, lidocaine administered IV reduced plasma prostaglandin E₂, metabolite concentration and mucosal COX-2 expression. Coadministration of lidocaine with flunixin ameliorated the flunixin-induced increase in mucosal neutrophil counts. (Am J Vet Res 2009;70:1259–1268)

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From the Departments of Clinical Sciences (Cook, Jones Shults, McDowell, Davis, Marshall, Blikslager) and Molecular Biomedical Sciences (Campbell), College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606. Dr. Cook’s present address is Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824. Supported by the Morris Animal Foundation (Cook) and the Merck-Merial Veterinary Scholars Summer Research Program (Jones Shults, McDowell).
Address correspondence to Dr. Cook (vcook@cvm.msu.edu).

Abbreviations

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<tr>
<th>Abbreviation</th>
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<tr>
<td>COX</td>
<td>Cyclooxygenase</td>
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<td>CRI</td>
<td>Constant rate infusion</td>
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<td>PG</td>
<td>Prostaglandin</td>
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<td>PGE₂</td>
<td>Prostaglandin E₂</td>
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<td>PGEM</td>
<td>Prostaglandin E₂ metabolite</td>
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<td>SS</td>
<td>Saline (0.9% NaCl) solution</td>
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<td>TXB₂</td>
<td>Thromboxane B₂</td>
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In a survey of diplomates of the American Colleges of Veterinary Internal Medicine and Veterinary Surgeons, respondents considered compromise of the gastrointestinal tract a major cause of endotoxemia in horses, and flunixin meglumine was the NSAID most frequently administered to affected horses. Flunixin alleviates many of the clinical signs of endotoxemia, including elevated heart and respiratory rates, fever, and signs of abdominal pain. The effects of flunixin are primarily achieved through inhibition of inflammatory PGs that are produced as a result of COX activity. However, treatment with flunixin inhibits recovery of ischemia-injured jejunum because PGs inhibited by flunixin are also essential for mucosal repair. Therefore, an ideal treatment for horses with compromised intestinal mucosae would be one that ameliorates the inflammation and pain associated with endotoxemia, while concurrently allowing recovery of injured portions of the intestinal tract.

When IV administration of lidocaine hydrochloride is combined with flunixin meglumine treatment
in horses with experimentally induced ischemia of the jejunum, the inhibitory effects of flunixin on recovery of ischemia-injured mucosa are avoided and effective analgesia is provided. Intravenous administration of lidocaine is widely used in equine hospitals as a treatment for horses with postoperative ileus. In a double-blinded, prospective study, humans undergoing elective colorectal surgery who received IV treatment with lidocaine had faster return of intestinal sounds and shorter periods of hospitalization, compared with findings in patients who received treatment with SS.

The effects of IV administration of lidocaine on cardiac and cerebral ischemia and reperfusion in other mammalian species have been evaluated experimentally. In dogs with experimentally induced cardiac ischemia, IV administration of lidocaine improved cardiac contractility, reduced the size of myocardial infarcts, and reduced the formation of a lipid peroxidation product, compared with findings in dogs that received a control treatment. Similar benefits have been detected in rats with experimentally induced cerebral ischemia. Lidocaine administered IV as a bolus followed by CRI reduced infarct size and improved neurologic outcome, compared with findings in rats that did not receive lidocaine. A similar benefit was detected in rats when administration of lidocaine was delayed until 45 minutes after the onset of ischemia.

The mechanism by which lidocaine exerts its beneficial effects in animals with ischemic injury is not completely understood. Incubation of neutrophils from humans and rats with lidocaine reduces neutrophil respiratory burst, adhesion, and phagocytosis. However, the concentration of lidocaine required to achieve these effects in vitro is higher than the concentration that is attained via systemic administration. Another study revealed that priming of human neutrophils induced by platelet-activating factor is inhibited by local anesthetics at concentrations equivalent to those attained following systemic administration. Therefore, the anti-inflammatory effects of lidocaine detected in vitro could indeed occur in vivo at the lower concentrations attained after systemic administration.

In horses, neutrophilic infiltration of intestinal tissues following ischemic injury has been detected and is thought to contribute to intestinal injury after ischemic events. In horses with experimentally induced ischemia of the jejunum, treatment with flunixin meglumine increases mucosal inflammation (as assessed by an increase in numbers of neutrophils in the mucosa of ischemia-injured jejunum), compared with findings following treatment with SS. Therefore, lidocaine could inhibit activation and infiltration of neutrophils into ischemia-injured intestinal tissues in horses treated with flunixin.

Because the detrimental effects of flunixin meglumine on mucosal recovery are attributable to inhibition of COX-1, an alternative theory for the beneficial effects of lidocaine is that lidocaine upregulates COX-1 gene expression and subsequent COX-1 protein synthesis, which allows sufficient production of PGs for mucosal healing. However, a previous study revealed that local administration of lidocaine prior to tooth extraction had no effect on COX-1 gene expression and production of COX-1–associated TXB₂. Alternatively, lidocaine may reduce COX-2 gene expression. In that same study, the effect of lidocaine on COX-2 gene expression was also evaluated; however, a negative control could not be included, so it was not possible to determine whether there was an effect.

The objective of the study reported here was to investigate the effect of lidocaine hydrochloride administered IV on mucosal inflammation after ischemic injury of the jejunum in horses treated with flunixin meglumine. We hypothesized that IV administration of lidocaine would reduce mucosal inflammation after ischemic injury in horses treated with flunixin. To evaluate this, we analyzed histologic indices of mucosal healing and inflammation, expressions of COX-1 and COX-2 proteins in ischemia-injured jejunum, and plasma concentrations of COX-1– and COX-2–associated prostanoids.

**Materials and Methods**

**Horses**—The samples used in this study were obtained from horses used in a previous study. All procedures were approved by the North Carolina State University Institutional Animal Care and Use Committee. Twenty-four horses of both sexes that ranged in age from 3 to 24 years old and in body weight from 382 to 605 kg were used. Horses had no history of gastrointestinal tract problems and were quarantined for 2 weeks prior to surgery. Results of physical examinations performed prior to surgery were within reference limits. Horses were housed in individual stalls with hay and water available at all times.

**Treatments**—Each horse was randomly assigned to 1 of 4 treatment groups (n = 6 horses/group). The 4 treatments were IV administration of SS (1 mL/50 kg [1 dose]), flunixin meglumine (1 mg/kg, q 12 h), lidocaine hydrochloride (1.3 mg/kg bolus administered over a 15-minute period followed by CRI [0.05 mg/kg/min]), or a combination of both flunixin and lidocaine at the aforementioned doses. For horses that received lidocaine (alone or with flunixin), the CRI was stopped at the end of surgery; after the horses had recovered from anesthesia (approx 2 hours after the end of surgery), lidocaine was administered IV (1.3 mg/kg) as a bolus over a 15-minute period followed by CRI (0.05 mg/kg/min) until the end of the study.

**Surgical procedures**—Immediately before surgery, each horse was premedicated with xylazine hydrochloride (1.1 mg/kg, IV) and a catheter was placed in the left jugular vein. One dose of cefetiofur sodium (2.2 mg/kg, IV) was administered prophylactically. In horses assigned to receive flunixin meglumine or SS, treatment was administered immediately prior to anesthesia. Anesthesia was induced with diazepam (0.1 mg/kg, IV) and ketamine hydrochloride (3 mg/kg, IV); following endotracheal intubation, anesthesia was maintained with isoflurane vaporized in 100% oxygen. In horses assigned to receive lidocaine, the bolus was administered immediately after the start of inhalational anesthesia and the CRI was started subsequently. Butorphanol (0.05 mg/kg, IV) was administered to all horses immediately after induction of anesthesia.
anesthesia and every 6 hours thereafter (0.05 mg/kg, IM) to provide analgesia. Mean arterial pressure was maintained at >70 mm Hg during anesthesia.

Surgery was performed by use of aseptic technique and a midline celiotomy. Two 30-cm segments of jejunum were isolated by use of Doyen forceps. The forceps were adjusted to the tightest setting to prevent collateral blood flow. The local blood supply to these 2 segments was occluded by use of Kelly hemostats. Penrose drains were placed between the hemostats and the tissues to minimize damage to the blood vessels. The blood vessels were occluded for 2 hours, after which all the forceps and hemostats were removed.

At the end of the ischemic period, an approximately 3-cm-long, full-thickness, wedge-shaped biopsy specimen was obtained from the antimesenteric border of ischemia-injured jejunum; the biopsy site was in the central portion of one of the ischemia-injured segments. A similar biopsy specimen was obtained from a segment of uninjured, nonmanipulated (control) jejunum located approximately 20 cm orad to the proximal border of an ischemia-injured segment. Half of each specimen was pinned to a card to maintain morphology and placed in neutral-buffered 10% formalin for 24 hours prior to processing for histologic, morphometric, and immunohistochemical evaluations. The mucosa was separated from the other half of each specimen, frozen in liquid nitrogen, and stored at –80°C until used for western blot analysis. The biopsy sites were closed via a single-layer inverting suture pattern, and then the abdomen was closed routinely. For horses receiving lidocaine, the CRI was temporarily discontinued during recovery from anesthesia.

**Post surgical procedures**—After recovery from anesthesia, each horse was returned to its stall and a physical examination (determination of a behavioral pain score; measurement of heart rate, rectal temperature, and respiratory rate; and evaluation of borborygmi, urination, defecation, appetite, and water intake) was performed every 4 hours. Results of these findings have been previously reported.8 Free access to water was allowed, and small handfuls of hay were offered. Horses receiving lidocaine treatment were given an additional bolus of lidocaine (1.3 mg/kg, IV) over 15 minutes when they were returned to their stalls, approximately 2 hours after intraoperative administration of lidocaine had been discontinued; the CRI of lidocaine (0.05 mg/kg/min, IV) was then restarted. Horses receiving treatment with flunixin meglumine were given an additional dose (1 mg/kg, IV) 12 hours after the initial treatment.

Eighteen hours after the end of the ischemic period, each horse was euthanatized by use of pentobarbital sodium (100 mg/kg, IV) and immediately necropsied for collection of samples. The ischemia-injured segment of jejunum that had not been previously biopsied was identified by the marks caused by the Doyen forceps at both ends of the segment and by mild discoloration of the serosa and mesentery. A segment of uninjured jejunum approximately 30 cm from the biopsy site of the control specimen obtained during surgery was selected. Mucosal scrapings from both the ischemia-injured and control jejunal segments were collected, frozen in liquid nitrogen, and stored at –80°C until used for western blot analyses. Full-thickness sections of both ischemia-injured and uninjured control tissue were collected, pinned to a card to maintain morphology, and placed in neutral-buffered 10% formalin for 24 hours prior to processing for histologic and immunohistochemical evaluations.

**Histologic, morphometric, and immunohistochemical evaluations**—Specimens of ischemia-injured and uninjured control jejunal obtained at the end of and 18 hours after the ischemic period were removed from neutral-buffered 10% formalin after 24 hours of fixation and transferred to 70% ethanol for processing for histologic, morphometric, and immunohistochemical evaluation. After paraffin embedding, each tissue block was oriented parallel to the axis of the crypt villus, 5-µm-thick sections were obtained at 300-µm intervals, and the sections were stained with H&E stain.

Two or 3 sections from each jejunal segment from each horse were independently evaluated by 3 observers (VLC, JJS, and MRM) who were unaware of the origins of the tissue specimens. Three villi from each section, which were well aligned on the crypt-villus axis, were histologically evaluated for epithelial injury; the extent of injury was graded from 0 to 5 by use of a previously described scale.27 The overall grade of epithelial injury for each jejunal segment was calculated as the mean grade of all evaluated villi from the examined sections. The observers additionally performed morphometric analyses on the selected villi. The height of a selected villus from the crypt villus junction and diameter at the midpoint, with the villus aligned longitudinally along the crypt-villus axis, were measured by use of a micrometer in the eyepiece of a light microscope. The length along which the villus was denuded of epithelium was also recorded for each measured villus. The surface area of each measured villus was calculated by use of a modified formula for calculating the surface area of a cylinder as follows:

\[
\text{Villus surface area} = (2\pi \times \frac{1}{2} \times |\frac{4}{\pi}d|h)
\]

where \(d\) = villus diameter and \(h\) = villus height. The villus surface area that was denuded of epithelium was calculated by subtracting the surface area of the villus covered by epithelium from the total surface area of the villus. This value was then expressed as a percentage of the total villus surface area as follows:

\[
\text{Percentage denudation} = \frac{([\text{Total villus surface area} - \text{villus surface area covered with epithelium}]/\text{total villus surface area}) \times 100}
\]

For each horse, epithelial restitution was evaluated for samples from control and ischemia-injured segments of jejunum by comparing the percentage denudation between samples collected at the end of and 18 hours after the ischemic period. To evaluate neutrophil infiltration of the mucosa, immunohistochemical techniques were used to detect myeloperoxidase, a marker for mature granulocytes.28 Additional sections from the aforementioned paraffin-embed-
ded specimens were acquired by use of the same procedure and deparaffinized prior to incubation with antibody. Sections of bone marrow with myeloid cells were used as a positive control to optimize antibody concentration and incubation time. One section (from each jejunal segment) was incubated with rabbit anti-human myeloperoxidase antibody (1:10 dilution) overnight (approx 12 hours) at 4°C. The number of neutrophils that had infiltrated the intestinal villi were counted independently by 2 investigators (VLC and JFM) who were unaware of the origins of the tissue specimens. In each section, numbers of neutrophils in 5 villi were counted by use of a 10-μm grid; for each segment of jejunal tissue, the mean number of neutrophils per square millimeter was calculated from the data for the examined sections.

Western blot analyses of COX-1 and COX-2 protein expressions—Analyses were performed on samples from uninjured control and ischemia-injured jejunal segments obtained at the end of and 18 hours after the ischemic period from 3 randomly selected horses in each treatment group. Frozen samples were thawed to 4°C, and protein was extracted by adding 1 mL of chilled modified radioimmunoprecipitation buffer (0.15M NaCl, 50mM sodium Tris [pH, 7.2], 0.9% deoxycholic acid, 1% Triton X-100, 0.1% SDS, and 1% octylphenoxy polyethoxethanol) to the samples along with the protease inhibitors phenylmethylsulfonyl fluoride, sodium orthovanadate, and aprotinin. The mixture was homogenized on ice and centrifuged at 4°C at 22,000 × g for 10 minutes. The supernatant was retained and centrifuged again at 4°C at 5,590 × g for 10 minutes and the final supernatant retained for analysis. The protein concentration of extracted samples was determined by use of the biuret reaction.

Equal amounts of protein from each sample were mixed with a 4× sample buffer and a 20× reducing agent and boiled for 5 minutes at 100°C. Denatured protein (35 μg) was added to each well of an 18-well 4% to 12% Bis-Tris precast polyacrylamide gel, and electrophoresis was performed according to standard protocols. Proteins were transferred to a polyvinylidene membrane, which had been activated with methanol, by the use of an electroblotting transfer apparatus.

Membranes were boiled in PBS solution for 5 minutes to increase antigen retrieval and blocked for 2 hours at room temperature (approx 21°C) in Tris-buffered 150mM NaCl with 0.05% Tween 20 and 5% powdered milk. Membranes were incubated overnight at 4°C with primary antibody (polyclonal goat anti–COX-1 antibody [1:200 dilution] or anti–COX-2 antibody [1:200 dilution] and polyclonal rabbit anti-β actin antibody [1:20,000 dilution]). Following 3 washes, the membranes were incubated for 1 hour at room temperature with horseradish peroxidase–conjugated secondary antibody. After an additional 3 washes, enhanced lumino substrate for horseradish peroxidase was added to the membranes to make the protein bands detectable. Densitometric analysis was performed on scanned images by the use of specialized software.

Although samples of control and ischemia-injured jejunum from each horse were processed on the same gel, samples from all 12 selected horses could not be processed on 1 gel. Therefore, to control for differences in protein transfer and image development among immunoblots, the percentage difference in densitometric units between samples from ischemia-injured and control jejunum at each time point for each horse was calculated by use of an equation as follows:

\[
\text{Percentage difference in densitometric units} = \frac{(\text{ischemia-injured tissue value} - \text{control tissue value})}{\text{control tissue value}} \times 100
\]

For statistical analysis, these percentage difference values were used rather than the number of densitometric units.

Prostanoid concentrations—Plasma was harvested from blood samples obtained prior to induction of anesthesia (before administration of any treatments [baseline samples]) and 8 hours after the end of the ischemic period (postischemia samples). Prostanoid analyses were performed 8 hours after the end of the ischemic period because the greatest differences in behavioral pain scores among the groups were detected at that time point. The plasma samples were frozen and stored at −80°C until evaluated for prostanoid concentrations. Prostaglandin E₂ concentration was evaluated by converting all unstable metabolites to the stable 13,14-dihydro-15-keto prostaglandin A₃ (ie, PGEM) for quantification by use of competitive enzyme immunoassay. Thromboxane A₂ concentration was determined via measurement of its stable metabolite TXB₂, by use of competitive enzyme immunoassay.

Among individual horses, there can be wide variation in plasma concentrations of prostanoids. Therefore, to more accurately examine the effect of different treatments, the data for prostanoid concentrations were examined by calculating the percentage change between the baseline concentration and the postischemia concentration for each horse. The percentage change between postischemia and baseline concentrations was calculated as follows:

\[
\text{Percentage change} = \left( \frac{\text{Postischemia concentration} - \text{baseline concentration}}{\text{baseline concentration}} \right) \times 100
\]

Statistical analysis—Grade of epithelial injury was compared by use of a Kruskal-Wallis 1-way ANOVA on ranks. Percentage denudation of villus surface area, a histologic index of repair, was analyzed by use of a 1-way ANOVA for effect of treatment. Percentage denudation of villus surface area was also analyzed within each treatment group via a 1-way repeated-measures ANOVA for the effect of ischemia. Percentage change in prostanoid concentrations was analyzed by use of a 1-way ANOVA for the effect of treatment among the groups, followed by a post hoc pairwise multiple comparison procedure (ie, Fisher least significance difference method). A value of \(P < 0.05\) was considered significant.

Results

Intraoperative and postoperative monitoring—Intraoperative mean arterial pressure was maintained...
between 70 and 110 mm Hg in all horses. Dobutamine was used when necessary and titrated to effect. None of the horses had overt signs of pain, and none required additional analgesia at any time during the study. Heart rate and rectal temperature were significantly increased after surgery in ischemia-injured groups compared with preoperative values, but no differences among groups were detected. These findings have been previously reported.\(^9\)

**Histologic and morphometric examination**—After 2 hours of ischemic injury (ie, at the end of the ischemic period), the median grade of epithelial injury was 3 in biopsy specimens obtained from ischemic segments of jejunum and 0 in specimens obtained from uninjured control segments of jejunum in all treatment groups. Eighteen hours after the end of the ischemic period, the median (25th to 75th percentile) grade of specimens from ischemia-injured jejunum was 0 (0 to 0.5), 0.75 (0.5 to 1.5), 1.25 (0.5 to 1.5), and 1.25 (1 to 1.5) in horses treated with SS, flunixin meglumine, lidocaine, or flunixin and lidocaine combined, respectively. There was no significant difference in grade of epithelial injury among treatment groups.

At the end of the ischemic period, denudation of villi was not detected in biopsy specimens obtained from uninjured control jejunal segments in any treatment group, whereas denudation in biopsy specimens obtained from ischemia-injured jejunal segments was evident. The mean ± SEM percentage denudation in ischemia-injured jejunal specimens was 38.1 ± 2.8% across all treatment groups, but there was no significant difference in percentage denudation among treatment groups. Restitution of villus epithelium in ischemia-injured jejunum was mostly complete at 18 hours after the end of the ischemic period; the percentage denudation was significantly reduced, compared with values after 2 hours of ischemic injury. In specimens obtained from ischemia-injured jejunal segments 18 hours after the end of the ischemic period, mean percentage denudation was 8.7 ± 8.3%, 9.5 ± 8.0%, 14.8 ± 15.4%, and 9.8 ± 7.3% from horses treated with SS, flunixin, lidocaine, or flunixin and lidocaine combined, respectively, with no significant difference among treatment groups.

**Immunohistochemical evaluation of mucosal neutrophils**—Mucosal neutrophil counts were lowest in biopsy specimens obtained from segments of uninjured control jejunum at the end of the ischemic period, with no significant difference among treatment groups. In each treatment group, neutrophil counts were increased in specimens obtained from injured jejunum after 2 hours of ischemia but did not differ significantly from neutrophil counts in control specimens collected at the same time point. At the end of the ischemic period, neutrophil counts in ischemia-injured jejunal specimens did not differ significantly among treatment groups (Figure 1).

In each treatment group, mucosal neutrophil counts were highest in specimens obtained from ischemia-injured jejunal segments at 18 hours after the end of the ischemic period and were significantly increased, compared with counts in specimens of both control and ischemia-injured jejunum obtained at the end of the ischemic period (all values of \(P < 0.008\); Figure 1). The largest number of mucosal neutrophils was detected in specimens obtained from segments of ischemia-injured jejunum in horses treated with flunixin meglumine alone. The neutrophil counts in those specimens were significantly greater than the counts in ischemia-injured specimens obtained from horses in all other treatment groups (SS, \(P < 0.001\); lidocaine and flunixin combined, \(P < 0.001\); lidocaine alone, \(P = 0.015\)). At 18 hours after the end of the ischemic period, significantly more mucosal neutrophils were detected in specimens of ischemia-injured jejunum than were detected in specimens of control jejunum from horses treated with SS (\(P = 0.007\)), flunixin alone (\(P < 0.001\)), and lidocaine alone (\(P = 0.006\)). However, there was no difference between mucosal neutrophil counts in specimens of ischemia-injured jejunum and counts in specimens of control jejunum at that time point for horses treated with flunixin and lidocaine combined.

**Western blot analyses of COX-1 and COX-2 protein expression**—Densitometric analysis revealed that there was no significant difference in \(\beta\) actin concentrations among the analyzed samples, which confirmed that equal amounts of protein were added to each lane. A band at approximately 70 kDa, consistent with the molecular weight of COX-1 protein, was easily detected in each lane that contained protein from samples of

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**Figure 1**—Mean ± SEM numbers of neutrophils (reported per square millimeter) detected immunohistochemically in the mucosa of jejunal tissue samples obtained from 24 horses that received 1 of 4 treatments and in which ischemia was experimentally induced in 2 segments of the jejunum. Horses (n = 6/group) were treated IV with SS (1 mL/50 kg [1 dose]), flunixin meglumine (1 mg/kg, q 12 h; F), lidocaine hydrochloride (1.3 mg/kg bolus and then CRI [0.05 mg/kg/min] during and after surgery; L), or flunixin and lidocaine combined (L+F). Tissue samples were collected from portions of uninjured (control) jejunum at the end of the 2-hour period of ischemia (black bars), ischemia-injured jejunum at the end of the 2-hour period of ischemia (light gray bars), control jejunum 18 hours after the ischemic period (dark gray bars), and ischemia-injured jejunum 18 hours after the ischemic period (white bars). *For this variable, value is significantly increased, compared with values in all other treatment groups (all values of \(P < 0.015\)). †Within a treatment group, value for this variable is significantly increased, compared with values in control jejunum at 18 hours after the ischemic period (SS, \(P = 0.007\); F, \(P < 0.001\); L, \(P = 0.006\)). ‡Within a treatment group, value for this variable is significantly increased, compared with values in control and ischemia-injured jejunum at the end of the 2-hour ischemic period (SS, \(P ≤ 0.004\); F, \(P ≤ 0.001\); L, \(P ≤ 0.001\); L+F, \(P ≤ 0.008\)).
control jejunum obtained at the end of or 18 hours after the ischemic period, indicative of constitutive expression of COX-1 protein (Figure 2). Multiple bands of various molecular weights were detected when the antibody against COX-2 protein was used. The band at approximately 70 kDa, which was a doublet in samples collected at 18 hours after the ischemic period, was assumed to be COX-2 protein. As with COX-1 protein, COX-2 protein was also detected in control samples obtained at the end of the ischemic period, which suggested that COX-2 protein is also constitutively expressed.

After 2 hours of ischemia, the amount of COX-1 protein in mucosal samples obtained from injured jejunum was virtually unchanged from that of control samples within each treatment group and the percentage difference did not differ significantly among treatment groups; the mean ± SEM value was −3.54 ± 6.6% for SS, 1.14 ± 1.0% for flunixin alone, −6.39 ± 3.9% for lidocaine alone, and −1.04 ± 1.4% for lidocaine and flunixin combined (Figure 3). In all treatment groups at 18 hours after the end of the ischemic period, the percentage differences in COX-1 protein expression in ischemia-injured tissue, compared with findings in control tissue, were positive values and all were significantly (all values of P ≤ 0.01) greater in magnitude than the values immediately after the ischemic period. There were no differences in the values or magnitudes for the percentage differences in COX-1 protein expression among treatment groups at 18 hours after the end of the ischemic period.

After 2 hours of ischemic injury, the amount of COX-2 protein in mucosal samples obtained from injured jejunum was decreased, compared with the amount in control samples, in horses treated with lidocaine alone (Figures 2 and 3). At that time point, the percentage difference in COX-2 protein expression between injured and control tissues for horses treated with lidocaine alone was significantly different from values for horses treated with flunixin meglumine alone (P = 0.003) or SS (P = 0.02). At the end of the ischemic period, the percentage difference in COX-2 protein expression in mucosal samples obtained from ischemia-injured jejunum in horses treated with flunixin and lidocaine combined had a larger negative value than the value in horses treated with flunixin alone; however, these values did not differ significantly (P = 0.058) in magnitude.

As indicated by the percentage change values, the amount of COX-2 protein was increased in mucosal samples obtained from ischemia-injured jejunum, compared with amounts obtained from control samples, in all treatment groups at 18 hours after the end of the ischemic period. However, at this time point, the percentage difference in COX-2 protein expression did not differ among the groups (Figure 3). In all treatment groups, the percentage difference in COX-2 protein expression at 18 hours after the end of the ischemic period was significantly (all values of P ≤ 0.02) different from the value immediately after the ischemic period in the horses treated with lidocaine alone. In horses treated with SS or lidocaine and flunixin combined, the percentage difference in COX-2 protein expression at 18 hours after the end of the ischemic period was significantly (SS, P = 0.06; lidocaine and flunixin, P = 0.048) different from the value immediately after the ischemic period in horses treated with lidocaine and flunixin combined.

Prostanoid concentrations—A wide variation in plasma concentrations of prostanoids was detected among individual horses, similar to results that we previously described. Because of this wide variation, the data for prostanoid concentrations were examined by calculating the percentage change between the preoperative concentration (baseline value) and the concentration 8 hours after the end of the ischemic period (postischemia value) for each horse to more accurately examine the effects of treatment.

In horses treated with SS, plasma PGEM concentration increased between the baseline and postischemia time points and the mean ± SEM percentage change...
In 1 horse in the group treated with flunixin alone, the transepithelial resistance of ischemia-injured jejunal mucosa obtained from horses treated with flunixin is lower than the resistance of ischemia-injured mucosa obtained from horses treated with SS. This decrease in transepithelial resistance associated with flunixin treatment indicates a loss of mucosal barrier function and could be attributable to loss of epithelial cells, failure of epithelial restitution, or failure of closure of paracellular spaces. In the study reported here, there was no difference among treatment groups in grade of epithelial injury or percentage denudation of villus surface area. This suggests that the low transepithelial resistance in ischemia-injured mucosa obtained from horses treated with flunixin is most likely attributable to failure of closure of paracellular spaces. In ischemia-injured jejunum harvested from pigs, COX-1–derived PGs, acting via the second messengers cAMP and Ca²⁺, stimulate closure of paracellular space by stimulating Cl⁻ secretion in the intestinal crypts and inhibiting Na⁺ absorption in the villus tips. Additionally, flunixin meglumine, the most commonly used analgesic, inhibits mucosal recovery. However, when lidocaine hydrochloride is coadministered with flunixin, ischemia-injured jejunum can recover. In the study reported here, we have provided evidence that when lidocaine hydrochloride is coadministered with flunixin, ischemia-injured mucosa from horses treated with flunixin is associated with a reduction in mucosal inflammation.
the end of the ischemic period and were significantly greater than the counts detected immediately after the ischemic period (ie, after 2 hours of ischemic injury), which is in agreement with results from previous studies.57 Infiltration of neutrophils into the mucosa of the small intestine disrupts mucosal repair when the neutrophils traverse through the paracellular spaces between restituting epithelial cells.57 The largest amount of neutrophilic infiltration was detected in ischemia-injured mucosa from horses treated with flunixin alone in our study. Interestingly, when lidocaine was coadministered with flunixin, neutrophil counts in samples of ischemia-injured jejunum mucosa were not significantly different from numbers in samples of control mucosa at 18 hours after the end of the ischemic period. It is possible that lidocaine directly inhibits adhesion and migration of neutrophils. Via this mechanism, the amount of neutrophilic infiltration would be expected to be lower in horses treated with lidocaine alone, compared with the amount of infiltration in those treated with SS. However, the amount of neutrophilic infiltration in ischemia-injured mucosa in the horses treated with SS and lidocaine alone did not differ significantly in the present study. Although treatment with lidocaine did counteract the proinflammatory effect that flunixin has on neutrophilic infiltration in ischemia-injured jejunal mucosa, the mechanism for this positive effect of lidocaine is unknown.

Cyclooxygenase-2 is traditionally considered an inducible enzyme, but it may also have some constitutive roles. In particular, COX-2 contributes to healing of gastric ulcers by promoting epithelial cell proliferation and migration. In the horses of the study reported here, we detected COX-2 protein in uninjured jejunal mucosa. This finding suggests that COX-2 is constitutively expressed, which agrees with the results of a previous study.29 The constitutive role of COX-2 protein in the small intestine is unknown. However, in rats with ischemia-injured ileum, COX-2 is associated with neutrophilic infiltration of and injury to the mucosa and reduced intestinal motility—effects that are prevented in rats that receive a selective COX-2 inhibitor before or after ischemic injury.40 The same study also revealed that COX-2 mRNA expression in the ileal tissues increased after just 30 minutes of ischemia. A similar change would perhaps account for the increase in COX-2 protein expression detected in jejunal tissue after 2 hours of ischemia in horses treated with SS or flunixin meglumine alone, compared with findings in control jejunal tissue, in the study reported here. Interestingly, we found that in horses treated with lidocaine, COX-2 protein expression was decreased in ischemia-injured mucosa after 2 hours of ischemia, compared with findings in control mucosa, suggesting an anti-inflammatory effect of lidocaine. When lidocaine was coadministered with flunixin, COX-2 protein expression in jejunum tissue after 2 hours of ischemia was also decreased (albeit nonsignificantly), compared with findings in control tissue. The effects of lidocaine on COX-2 protein expression may explain, in part, how the effects of flunixin on mucosal healing are overcome when these 2 drugs are coadministered.

In the study reported here, western blot analysis was used to assess mucosal concentrations of COX proteins. To our knowledge, a specific antibody against equine COX-2 protein for use in western blot analyses has not been developed. The antibody we used had cross-reactivity with other proteins, as evidenced by the multiple bands detected at different molecular weights. However, only 1 band that was represented by a doublet and had a molecular weight of approximately 70 kDa was detected. Stimulation of COX-2 protein synthesis and associated glycosylation of the protein can result in formation of a doublet that is detectable via western blot analysis.41 Because COX-2 protein production is determined by intracellular signaling pathways that regulate its transcription,42 it may be more accurate in future studies to perform real-time quantitative PCR assays to assess effects of treatment on COX-2. This method has been used to quantify COX-2 mRNA in laminae of equine hooves.43

Plasma prostaglandin concentrations are frequently used to assess COX activity.44 The activity of COX-1 is the only source of thromboxane A2 in platelets from humans45; therefore, the concentration of TXB2, the stable metabolite of thromboxane A2, can be used as a specific indicator of COX-1 activity. In the study reported here, treatment of horses with flunixin meglumine alone inhibited the increase in plasma TXB2 concentration attributable to ischemic injury, which is consistent with the action of flunixin as a nonselective COX inhibitor. When lidocaine was coadministered with flunixin, TXB2 production at 8 hours after ischemic injury remained inhibited. This suggests that lidocaine has no effect on COX-1 expression. These results were also in agreement with the results of western blot analyses of COX-1 protein at 18 hours after the end of the ischemic period. At that time point, the percentage difference in COX-1 protein expression in ischemia-injured mucosa (compared with the control mucosa value) was not different between horses treated with flunixin and those treated with lidocaine and flunixin combined. To our knowledge, there are few studies in which the effects of local anesthetics on COX enzyme concentration, gene expression, or activity have been examined. However, when bupivacaine or lidocaine was used to provide local anesthesia for humans undergoing molar tooth extraction, there was no effect of either local anesthetic on COX-1 gene expression or TXB2 concentration in the inflammatory transudate.26

Plasma PGEM concentration can be used to assess COX-2 activity because the PGE2 synthase enzyme is preferentially colocalized with COX-2 in the cell.46 In the study reported here, treatment of horses with the nonselective COX inhibitor, flunixin meglumine, prevented the ischemia-induced increase in plasma PGEM concentration that was detected in horses treated with SS, which is indicative of COX-2 inhibition. However, the ischemia-induced increase in plasma PGEM concentration was also prevented in horses treated with lidocaine alone; in horses treated with both drugs, PGEM values at 8 hours after the end of the ischemic period were actually decreased, compared with baseline values. These results were in agreement with the results of the COX-2 western blot analyses and indicated an inhibitory effect of lidocaine on COX-2 expression.
In the present study, IV administration of lidocaine to horses with ischemia-injured jejunum had anti-inflammatory effects in the injured intestinal mucosa and resulted in changes in plasma prostanoid concentrations. This effect was not mediated through changes in the amount of epithelial injury, changes in the degree of epithelial restitution, or alterations in COX-1 protein expression in the ischemia-injured mucosa. However, treatment with lidocaine did result in reduced neutrophil counts and COX-2 protein expression in ischemia-injured mucosa and decreased plasma PGEM concentration, compared with findings in horses that did not receive lidocaine. What remains unclear is whether lidocaine inhibits neutrophil activation and subsequent tissue injury and COX-2 expression or whether reduced mucosal COX-2 expression results in a diminished chemotactic stimulus for neutrophil migration. Further studies are needed to fully elucidate this effect.

b. BCA Protein Assay Kit, Pierce, Rockford, Ill.
c. Criterion XT Precast Gel, Bio-Rad Laboratories, Hercules, Calif.
d. Immobilon P Transfer Membrane, Millipore Corp, Bedford, Mass.
e. COX-1 antibody, SantaCruz Biotechnology Inc, Santa Cruz, Calif.
f. COX-2 antibody, SantaCruz Biotechnology Inc, Santa Cruz, Calif.
g. β-actin antibody, AbCam, Cambridge, Mass.
h. ECL western blotting substrate, Pierce, Rockford, Ill.
i. SigmaScan Pro5, Systat Software Inc, San Jose, Calif.
j. Prostaglandin E metabolite EIA Kit, Cayman Chemical, Ann Arbor, Mich.
k. Thromboxane B, EIA Kit, Cayman Chemical, Ann Arbor, Mich.

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