Effects of Carolina rinse solution, dimethyl sulfoxide, and the 21-aminosteroid, U-74389G, on microvascular permeability and morphology of the equine jejunum after low-flow ischemia and reperfusion

Robin M. Dabareiner, DVM, PhD; Nathaniel A. White, DVM, MS; Jack R. Snyder, DVM, PhD; Bernard F. Feldman†, DVM,PhD; Lydia L. Donaldson, VMD, PhD

Objective—To evaluate effects of Carolina rinse solution, dimethyl sulfoxide (DMSO), and 21-aminosteroid, U-74389G, on microvascular permeability and morphology of the equine jejunum after low-flow ischemia and reperfusion.

Animals—20 healthy adult horses.

Procedure—Under anesthesia, full-thickness biopsy specimens of a distal portion of the jejunum were obtained for baseline measurements. In addition to a control segment, 2 jejunal segments were identified as sham-operated or experimental segments. Experimental segments underwent 60 minutes of low-flow ischemia and 3.5 hours of reperfusion. Treatments were as follows: U-74389G (3 mg/kg, IV; 6 horses), DMSO (20 mg/kg, IV; 6) diluted in 1 L of saline (0.9% NaCl) solution, local perfusion (via jejunal artery) of Carolina rinse solution (0.5 mL/kg; 4), and local perfusion of lactated Ringer's solution (0.5 mL/kg; 4).

Results—Jejunal microvascular permeability was significantly lower after treatment with Carolina rinse solution or DMSO, compared with U-74389G or lactated Ringer's solution treatments. After DMSO treatment, serosal- and submucosal-layer edema was significantly increased in experimental segments, compared with control or sham-operated segments; however, edema increases were significantly less than for lactated Ringer's solution or U-74389G treatments. Significant decreases in intestinal wet weight-to-dry weight ratio were found following Carolina rinse solution or DMSO treatments, compared with lactated Ringer's solution or U-74389G treatments. Edema formation and leukocyte infiltration in jejunal segments of horses treated with lactated Ringer's solution or U-74389G were increased, compared with Carolina rinse solution or DMSO treatments.

Conclusions and Clinical Relevance—Carolina rinse solution and DMSO may be protective against ischemia-reperfusion injury in the equine jejunum. (*Am J Vet Res* 2005;66:525–536)

Results of several studies[1-3] indicate that the phenomenon of reperfusion injury occurs in the equine small intestine. Intestinal injury produced by reperfusion of ischemic intestine is more severe than the injury induced by ischemia alone.[2,3] Results of studies[4-8] on laboratory animals and cats suggest that the biochemical reactions initiated at reperfusion involve the formation of cytotoxic oxidants derived from molecular oxygen. Two major sources of these reactive oxygen metabolites in postischemic tissue are the enzyme, xanthine oxidase, and activated neutrophils entering the tissue during reperfusion.[5,6]

Xanthine dehydrogenase is converted to xanthine oxidase in tissues; xanthine oxidase catalyzes the oxidation of purines by use of the oxidized form of nicotinamide adenine dinucleotide as a cofactor. Xanthine oxidase uses oxygen instead of the oxidized nicotinamide adenine dinucleotide as a cofactor, generating superoxide or hydrogen peroxide radicals. Depletion of adenosine triphosphate during ischemia results in increased concentrations of adenosine monophosphate, which is catabolized to hypoxanthine and is a substrate for xanthine oxidase.[5,8] Conversion of xanthine dehydrogenase to xanthine oxidase during ischemia and reperfusion has been documented in the small intestine of horses.[9] Superoxide radicals formed during the reduction of hypoxanthine have been implicated in the capillary permeability changes observed after ischemia and reperfusion of the feline small intestine, as xanthine oxidase inhibitors such as superoxide dismutase and allopurinol attenuate ischemia-induced permeability changes.[4,8]

Another source of reactive oxygen metabolites is the activated neutrophil.[10] Stimulation of neutrophilic oxidative metabolism results in the release of large amounts of proteolytic enzymes, superoxide anion, cytokines, arachidonic metabolites, and eventually hypochlorous acid. Romson et al[11] demonstrated that myocardial infarcts resulting from coronary artery

Received March 19, 2004.
Accepted May 12, 2004.
From the Marion DuPont Scott Equine Medical Center (Dabareiner, White, Feldman, Donaldson), Virginia-Maryland Regional College of Veterinary Medicine, Virginia Polytechnic Institute and State University, Leesburg, VA 20177; and the Departments of Surgical and Radiological Sciences (Snyder), School of Veterinary Medicine, University of California, Davis, CA 95616. Dr. Dabareiner's present address is the Department of Large Animal Medicine and Surgery, College of Veterinary Medicine, Texas A&M University, College Station, TX 77843-8863.
Supported by a donation from Adelaide C. Riggs.
Address correspondence to Dr. Dabareiner.
† Deceased.

occlusion and reperfusion were decreased 50% by pretreatment with either superoxide dismutase or by decreasing neutrophil infiltration with neutrophil antisera. Grisham et al[12] demonstrated similar findings in the feline intestine after ischemia-reperfusion injury. They proposed that ischemia-reperfusion injury results in xanthine oxidase-generated oxidants that stimulate neutrophil activation, thereby exacerbating tissue injury.[12] Additionally, oxygen-derived radicals can react with the double bonds in cellular phospholipid membranes, resulting in a chain reaction of lipid peroxidation.[13] Lipid peroxidative damage to cell membranes disrupts cellular integrity, leading to vacuolation, swelling, and eventual cell death. Vascular endothelial cell injury results in the expression of endothelial adhesion molecules, causing leukocyte adherence and extravasation.[14] Activated neutrophils cause damage to the endothelium and thereby potentiate protein leakage and intravascular thrombi.[8,10,12,14]

Assessing microvascular permeability to plasma proteins is a sensitive index for detecting damage to the microvasculature.[4,9] Significant increases in capillary permeability occur in the equine small intestine following low-flow ischemia and subsequent reperfusion.[15] An increase in capillary permeability occurs concurrently with endothelial cell damage and neutrophil infiltration and may be caused by reactive oxygen metabolite damage to the microvascular cellular membranes. Pharmacologic agents aimed at inhibiting reactive oxygen metabolites or having anti-inflammatory properties may prevent these permeability changes.

Dimethyl sulfoxide (DMSO) is an anti-inflammatory agent that scavenges hydroxyl radicals, inhibits neutrophil chemotaxis, stabilizes lysosomes, and inhibits platelet aggregation and fibroblastic proliferation.[16] Use of DMSO was beneficial in intestinal ischemia-reperfusion studies[17-20] in laboratory animals and cats. Dimethyl sulfoxide prevented leukocyte adherence to mesenteric venules and attenuated the low-flow ischemia-induced increase in microvascular permeability in feline small intestine.[14] Use of DMSO in studies[17-20] on the equine intestine has produced conflicting results. Dimethyl sulfoxide (1 g/kg, IV) failed to decrease morphologic mucosal damage[18] in the equine large intestine after complete ischemia and various reperfusion periods. A tendency for increased mucosal injury was found in the equine large intestine after administration of DMSO after 2.5 of hours low-flow ischemia and 3 hours of reperfusion, compared with controls.[18] Administration of DMSO (1 g/kg) also failed to prevent morphologic mucosal damage in the equine small intestine after 1 hour of arteriovenous occlusion and 1 hour of reperfusion[19] but did limit peritoneal adhesion formation in foals following 70 minutes of arteriovenous occlusion and 60 minutes of reperfusion.[20]

The 21-aminosteroids are a new group of compounds derived from glucocorticoids that are designed to protect cell membranes by inhibiting lipid peroxidation. These agents scavenge superoxide radicals and lipid hydroperoxides that attenuate iron-catalyzed lipid peroxidation and arachidonic acid release.[13] 21-aminosteroids decrease ischemia-reperfusion injury in the stomachs of dogs[21] and small intestines of laboratory animals[22] by inhibiting membrane lipid peroxidation caused by oxygen-derived peroxyl radicals. The 21-aminosteroid, U-74389G, partially attenuated the increase in myeloperoxidase activity, an indicator of neutrophil accumulation, after 2 hours of arteriovenous occlusion followed by 2 hours of reperfusion in the equine large intestine[23] but had no effect on myeloperoxidase activity and did not prevent mucosal damage after total or partial vascular occlusion and reperfusion of the equine small intestine.[24]

In previous equine studies,[23,24] only limited success has been achieved when individual pharmacologic agents aimed at decreasing reactive oxygen metabolite production were used to prevent tissue damage after ischemia-reperfusion injury. Rinse solutions, used to perfuse donor organs prior to transplant, include a combination of substances aimed at improving circulation, preserving endothelium, and scavenging free radicals. Carolina rinse solution (slightly acidic pH of 6.5) is designed to minimize graft reperfusion injury and contains electrolytes similar to plasma; hydroxyethyl starch for oncotic support; allopurinol and glutathione as antioxidants; desferrioxamine as an iron chelator; the calcium channel blocker nicardipine; adenosine for improved microcirculation; and fructose and glucose as adenosine triphosphate substrates.[25] This solution significantly increases transplant graft survival by attenuating endothelial cell injury, inhibiting adherence of neutrophils in postsinusoidal venules, and improving graft microcirculation.[25-27] In a study,[28] mesenteric arterial perfusion of Carolina rinse solution in an isolated ischemic segment of equine jejunum 10 minutes prior to reperfusion prevented capillary permeability changes, decreased serosal neutrophil infiltration, and resulted in fewer peritoneal adhesions at 10 days, compared with control jejunal segments.

In another study,[29] topical and intraluminal administration of Carolina rinse solution prevented microvascular permeability changes and decreased serosal neutrophil infiltration following reperfusion of ischemic but not distended equine jejunum. In a study[30] on an isolated extracorporeal circuit experimental model of ischemia and reperfusion of the equine jejunum, other customized solutions were also beneficial in decreasing morphologic signs of injury. The purpose of the study reported here was to investigate the potential benefits of Carolina rinse solution, DMSO, or U-74389G in attenuating microvascular permeability changes, edema formation, and neutrophil infiltration induced by low-flow ischemia and reperfusion of the equine jejunum.

Materials and Methods

Horses—Twenty healthy adult horses that were of mixed breeding, either sex, 3 to 12 years old (mean, 7 years), and free of gastrointestinal tract and systemic disease were studied. Health was evaluated by physical examination, fecal parasite examination, and CBC determination. Horses were randomly assigned to 1 of 4 groups. Effects of low-flow ischemia (25% of baseline blood flow) and subsequent reperfusion on the microvascular permeability and histologic and ultrastructural morphology were determined after pretreat-

ment with Carolina rinse solution, lactated Ringer's solution, DMSO, and U-74389G. Results were compared with control horses[15] and horses that underwent low-flow ischemia and subsequent reperfusion of the jejunum with no treatment,[15] which has previously been reported from our laboratory.

Anesthesia protocol—All horses were sedated with xylazine hydrochloride[a] (0.2 to 0.5 mg/kg, IV), and anesthesia was induced with guaifenesin[b] (100 mg/kg, IV) and ketamine hydrochloride[c] (2.2 mg/kg, IV). Horses were positioned in dorsal recumbency, and anesthesia was maintained with halothane in oxygen by use of intermittent positive pressure ventilation. Mean blood pressure measured by direct arterial catheterization was maintained at ≥ 70 mm Hg. Lactated Ringer's solution[d] was administered IV at 5 to 10 mL/kg/h to maintain circulating volume and blood pressure. Dobutamine[e] was administered IV at a constant rate throughout the procedure in all 20 horses to maintain a systolic arterial blood pressure between 100 and 120 mm Hg. The P_{CO_2} was maintained at < 55 mm Hg.

Instrumentation—Horses were instrumented as previously described.[15] Briefly, all horses had a ventral midline celiotomy performed by standard methods. After entering the abdomen, a full-thickness biopsy specimen of distal portion of the jejunum was obtained from each horse for baseline measurements. The enterotomy site was sutured with 2-0 polygalactin in a continuous Lembert suture pattern to prevent leakage and contamination of the abdomen. Beginning at the distal portion of the jejunal vascular arcade, two 30-cm-long intestinal segments that were each supplied by a jejunal artery and vein were identified. One segment was designated as experimental, and an adjacent segment was designated as the sham-operated segment. Latex rubber tubing was used to occlude the lumens and extramural vasculature at each end of the intestinal segments. Both jejunal segments were placed on a plastic sheeting overlying a warm water heating pad[f] (37°C). The intestine was kept moist with sterile saline (0.9% NaCl) solution and covered with plastic sheeting to prevent tissue dehydration and evaporation. The sham-operated segment was used to determine the effects of duration of anesthesia and having the intestine outside the abdomen on intestinal edema and intestinal morphology, as has been done in previous studies[3,15] from our laboratory. The experimental segment was instrumented.

The mesenteric artery and vein were isolated in the experimental segment at the level of the incision, and a No. 4 Doppler ultrasonic probe[g] was applied to the jejunal artery to monitor continuous arterial blood flow to the intestinal segment. The jejunal mesenteric vein of the experimental segment was catheterized with a 22-gauge IV catheter[h] approximately 10 cm proximal to the intestinal segment. The catheter was connected to a pressure transducer[i] to monitor venous pressure of the experimental jejunal segment and obtain blood samples. All pressure transducers were positioned at the level of the base of the heart. Five centimeters proximal to the venous catheter, an adjustable vascular clamp[j] was applied to the mesenteric vein. A large lymphatic vessel draining the jejunal experimental segment was cannulated with a 24-gauge IV catheter[k] and used for lymph sample collection. Systemic arterial and local venous pressures were continuously monitored. Time from induction of anesthesia to completion of instrumentation required 60 minutes for all 20 horses.

Experimental design—All horses underwent 60 minutes of low-flow ischemia and 3.5 hours of reperfusion. All treatments were administered 10 minutes prior to reperfusion. Six horses were treated with U-74389G (3 mg/kg, IV),[l] 6 horses were treated with DMSO (20 mg/kg, IV) diluted in 1 L of saline solution, 4 horses were treated by local perfusion of Carolina rinse solution (0.5 mL/kg)[m] into the jejunal artery of the experimental segment, and 4 horses were treated with local perfusion of lactated Ringer's solution (0.5 mL/kg) into the jejunal artery leading to the experimental segment. This group served as a control for the Carolina rinse solution group because both groups sustained arterial perfusion treatment administration methods. Baseline data included the following: mesenteric arterial blood flow (mL/min/kg of intestinal tissue), mesenteric venous pressure (mm Hg), and lymph flow rate (µL/min/kg of intestinal tissue).[15] Lymph samples were collected in calibrated heparinized micropipettes (100 µL capacity)[n] over a 15-minute period to determine lymph flow rate. Mesenteric and systemic plasma samples were obtained from blood samples that were collected in heparinized tubes at each time of lymph sample collection.[o]

After instrumentation and collection of baseline data, the lymph flow rate was allowed to stabilize at baseline venous pressure (0 to 3 mm Hg), which required 45 to 60 minutes. Ischemia (25% of baseline blood flow) was created for 60 minutes by applying an adjustable vascular clamp on the jejunal artery as it exited the abdominal cavity at the incision. Blood flow to the experimental segment was continuously monitored by use of the Doppler ultrasonic flow probes. Lymph and mesenteric blood samples were collected at 15-minute intervals during the ischemic period. U-74389G or DMSO were administered IV via the jugular vein as a 1-L volume over a 10-minute period before the end of the ischemic period. Carolina rinse solution or lactated Ringer's solution was injected into the jejunal artery of the experimental segment through a 24-gauge catheter 10 minutes before the end of the ischemic period. At the end of the 60-minute ischemic period, the vascular clamp was released and the lymph flow rate was allowed to stabilize, which required 40 to 65 minutes. After lymph flow stabilization, lymph and blood samples were collected and mesenteric blood and lymph flow rates were recorded at baseline mesenteric venous pressures. Mesenteric venous pressure was then increased by 10, 20, and 30 mm Hg so that the lymph flow rate would increase to a maximum value. Mesenteric venous pressure was only increased after the lymph flow stabilized (vol/unit of time) at the previous pressure level. Lymph and mesenteric blood samples and lymph flow and mesenteric blood flow rates were obtained at 15-minute intervals until the lymph rate remained constant, which required 45 to 60 minutes at each value of venous pressure.

Tissue preparation—After data collection, the entire experimental, sham-operated, and control segments of intestine were weighed. If any intestinal fluid had accumulated, it was drained from the segment before weighing. Full-thickness jejunal biopsy specimens were taken from the experimental, sham-operated, and control segments. Biopsy specimens were mounted flat on wooden tongue depressors with 25-gauge needles, washed with 0.9% NaCl, and divided. One section was fixed for 48 hours in neutral-buffered 10% formalin, trimmed, embedded in paraffin, cut in 5-µm-thick sections, and stained with H&E. The other section was placed in freshly prepared cacodylate-buffered 3% glutaraldehyde solution (550 mOsm; pH, 7.2). After 24 hours of fixation, samples were transferred to 2% buffered osmium tetroxide solution. Samples were dehydrated, embedded,[p] cut into 500-µm thick sections, stained with toluidine blue and basic fuchsin, and examined by light microscopy. Selected thin sections were stained with uranyl acetate and lead citrate for transmission electron microscopy.[q]

A 10-cm-long section of the experimental, sham-operated, and control segments were weighed immediately and stored at –70°C. These sections were baked at 80°C for 24 hours in an oven, and the dry weight of each sample was recorded. Dry weight data were used to calculate the lymph flow per kilogram of tissue for each horse and to measure **wet weight-to-dry weight ratio (WW:DW)** on full-thickness jejunum as an estimate of jejunal tissue edema. After completion of each experiment, horses were euthanatized with an overdose of sodium pentobarbital IV.

Tissue morphometrics—Intestinal layers (ie, the mucosa, submucosa, and serosa) of experimental jejunal biopsy specimens were viewed by light microscopy with an objective (magnification, 4X) interfaced with a computer.[r] A pictorial image of the intestinal layers was formed by use of a computer-based imaging program[s] and stored on disk cartridges.[t] Mucosal, submucosal, and serosal layer edema was calculated by use of a computerized stereology program that consisted of a 48-point, 24-line grid.[u] Six views of each intestinal layer from each jejunal biopsy specimen were used for morphometric analysis. Comparisons were made for each intestinal layer among treatment groups. Results were expressed as percentages of mean baseline values.

Laboratory analysis—Lymph volumes were measured in calibrated micropipettes.[n] Lymph and plasma samples were centrifuged to remove red blood cells, and protein concentrations were measured by the modified biuret method.

Osmotic reflection coefficient analysis—The microvascular permeability was determined by estimates of the **osmotic reflection coefficient (ORC)** that was determined on the basis of the steady-state relationship between lymphatic flow rate per kilogram of tissue and the **lymph-to-plasma protein concentration ratio (Cl:Cp)**. The Cl:Cp decreases rapidly as the lymph flow rate is increased by venous pressure increases until Cl:Cp reaches a minimal value. The Cl:Cp remains constant at this minimal value despite further increases in lymph flow rates, which is described as the filtration independent lymph flow rate.[28] Previous work has shown that 1 - Cl:Cp at filtration independent lymph flow rates provides an accurate estimate of the ORC.[15,28,31-33] The theoretic basis for this model has been previously described.[31,32]

Interpretation of results—The Cl:Cp was plotted against the lymphatic flow rate per kilogram of intestinal tissue and calculated as a multiple of baseline lymphatic flow. Mean Cl:Cp for the 6 highest lymph flow rates obtained at a mesenteric venous pressure of 30 mm Hg was calculated for each horse. Mean and SE of the pooled means (± SEM) were calculated for each group of horses to determine the Cl:Cp for the flow independent part of the data, which occurred at the maximum lymph flow rate. Comparisons of the minimal value of the Cl:Cp at filtration independent lymph flow rates were made between treatment groups.

Statistical analysis—Differences in the mean and SE of the pooled means of the Cl:Cp among groups were compared by use of the Kruskal-Wallis 1-way nonparametric ANOVA. When a significant difference was detected, pairwise comparisons were made by use of the Mann-Whitney *U* test. Level of significance was set at $P \leq 0.05$.

For each horse, the ratio of intestinal layer thickness of the sham-operated and experimental segments to that of the control (baseline) jejunum was calculated. Thicknesses of intestinal layers (serosa, mucosa, and submucosa) were compared among groups by use of the Kruskal-Wallis 1-way nonparametric ANOVA. When a significant ($P \leq 0.05$) difference was detected, comparisons were made by use of the Mann-Whitney *U* test. The difference in intestinal layer thickness for each experimental segment, compared with the sham-operated segment, was calculated, and the hypothesis that the difference was 0 was tested by use of the Wilcoxon signed rank test with a value of $P \leq 0.05$ considered significant.

Results

Jejunal blood and lymph flow rates—Mean (± SEM) baseline mesenteric blood flow was not significantly different in any of the 20 horses at a baseline mesenteric venous pressure of 0 to 3 mm Hg. Mesenteric blood flow (6.1 ± 1.1 mL/min/kg of tissue) decreased significantly to 25% of baseline flow during the 60-minute ischemic period. Periodic adjustments in the vascular clamps were required to maintain a constant 25% decrease in blood flow for 60 minutes. During the ischemic period, the serosal surface of the jejunal segment turned from pink to mottled grayish purple. During perfusion of the jejunal artery with Carolina rinse solution or lactated Ringer's solution, the jejunal segment became blanched and white. After vascular clamp release, the serosa turned red and was hyperemic and the intestinal wall was grossly thickened with scattered ecchymosis in the mesentery of horses treated with lactated Ringer's solution or U-74389G. The serosa was reddened and had only a slightly increased wall thickness, as determined by gross palpation, in the horses treated with Carolina rinse solution or DMSO. After the vascular clamps were released, blood flow to the experimental segment increased in horses of all treatment groups (**Figure 1**).

Mean (± SEM) baseline jejunal blood flow in the experimental intestinal section of horses treated with Carolina rinse solution was 29 ± 0.03 mL/min/kg of tissue, which increased significantly ($P = 0.001$) to 180 ± 4.24 mL/min/kg at reperfusion and returned to 28 ± 1.13 mL/min/kg after 240 minutes (Figure 1). Horses treated with lactated Ringer's solution had an initial mean baseline blood flow of 27 ± 0.12 mL/min/kg, which increased to 71.4 ± 4.34 mL/min/kg at reperfusion and decreased to 17.1 ± 0.99 mL/min/kg after 240 minutes. Horses treated

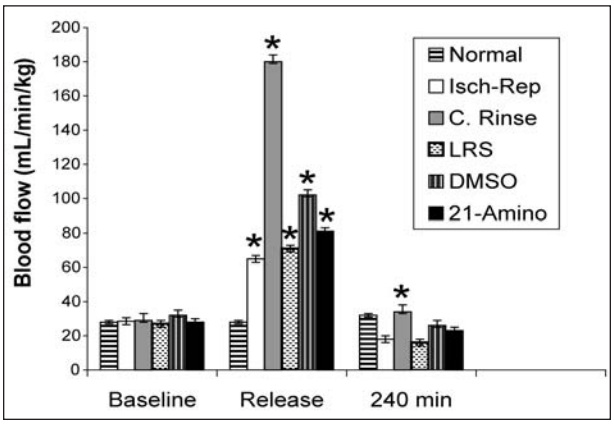

Figure 1—Bar graph of mean (± SEM) jejunal blood flow to experimental jejunal segments of horses in the following treatment groups: control (Normal),[15] ischemia and reperfusion with no treatment (Isch-Rep),[15] Carolina rinse solution (C. Rinse), lactated Ringer's solution (LRS), dimethyl sulfoxide (DMSO), and the 21-aminosteroid, U-74389G (21-Amino). *Significantly ($P < 0.05$) different values within a treatment group from baseline.

with DMSO had a mean baseline jejunal blood flow of 25 ± 1.23 mL/min/kg, which increased significantly to 102 ± 5.34 mL/min/kg at reperfusion and returned to baseline after 240 minutes (26 ± 2.8 mL/min/kg). Horses treated with U-74389G had a baseline jejunal blood flow of 28 mL/min/kg, which increased significantly (P = 0.010) at reperfusion to 81.2 ± 2.4 mL/min/kg and decreased to 23 ± 0.26 mL/min/kg at 240 minutes.

Mean (± SEM) baseline jejunal lymph flow rate was 5.1 ± 1.7 µL/min/kg of tissue for all 20 horses at a mesenteric venous pressure of 0 to 3 mm Hg. Lymph flow rate decreased (0.3 ± 0.3 µL/min/kg) or reached 0 during the ischemic period in all horses and increased (22.1 ± 1.0 µL/min/kg) during the 60-minute reperfusion period before returning to a steady state, which occurred after 30 to 45 minutes. After a constant lymph flow rate was established at a mesenteric venous pressure of 0 to 3 mm Hg, stepwise increases in the venous pressure resulted in incremental increases in lymph flow up to 9 times the baseline lymph flow rate. In all horses, Cl:Cp decreased rapidly as lymph flow increased and became stable at the high lymph flow rates.

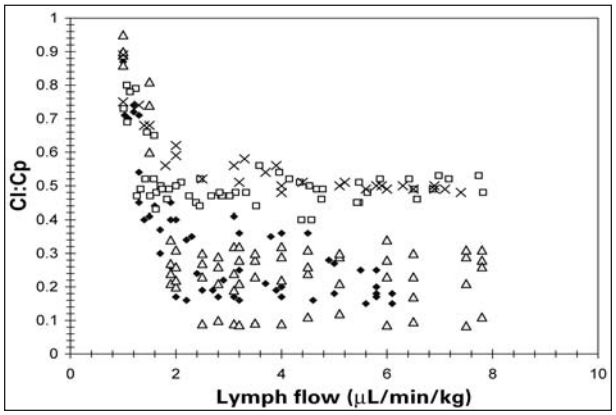

Figure 2—Relationship between lymph-to-plasma protein ratio (Cl:Cp) and lymph flow rate (µL/min/kg of intestinal tissue) of horses in the following treatment groups: control (closed diamond),[15] ischemia and reperfusion with no treatment (open square),[15] Carolina rinse solution (closed triangle), and lactated Ringer's solution (symbol X).

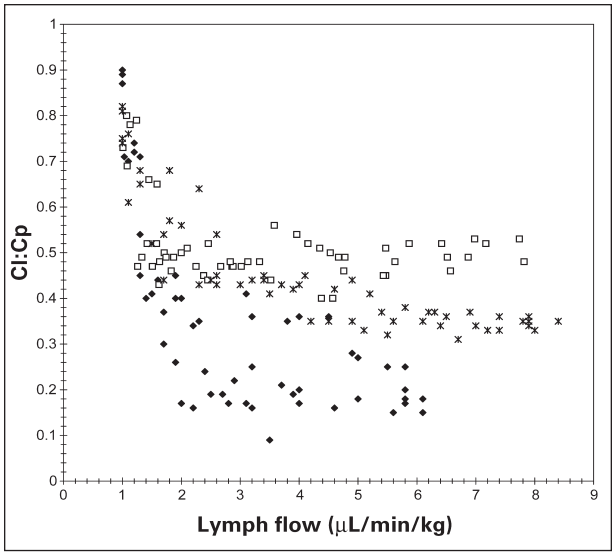

Figure 3—Relationship between Cl:Cp and lymph flow rate of horses in the following treatment groups: DMSO (symbol X), control (closed diamond),[15] and ischemia and reperfusion with no treatment (open square).[15]

Microvascular permeability estimates—Mean (± SEM) Cl:Cp for horses treated with Carolina rinse solution was 0.25 ± 0.04 (ORC, 0.75) after reperfusion, which was significantly (P = 0.001) lower than the mean Cl:Cp for horses treated with lactated Ringer's solution (0.50 ± 0.05; ORC, 0.50; **Figure 2**). Mean Cl:Cp for horses treated with Carolina rinse solution was not significantly different from the Cl:Cp reported for the jejunum of control horses.[15] Mean Cl:Cp for horses treated with lactated Ringer's solution was not significantly different from the Cl:Cp described for horses that underwent ischemia and reperfusion of the jejunum with no treatment.[15] Horses treated with DMSO (20 mg/kg) had a mean Cl:Cp of 0.35 ± 0.02 and an ORC of 0.65, which was significantly (P = 0.006) higher than the Cl:Cp described for control

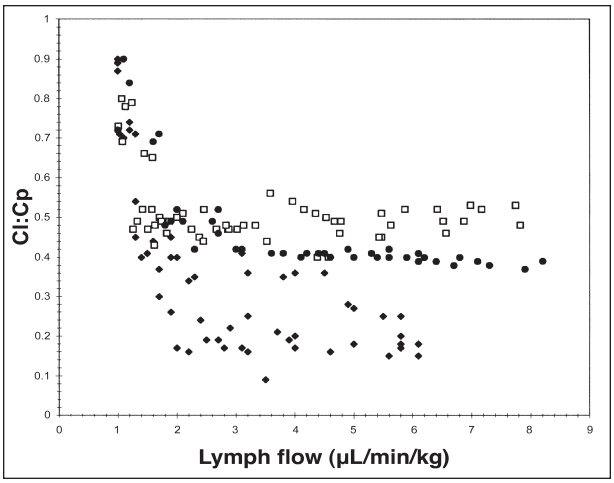

Figure 4—Relationship between Cl:Cp and lymph flow rate of horses in the following treatment groups: U-74389G (closed circle), control horses (closed diamond),[15] and ischemia and reperfusion with no treatment (open square) groups.[15]

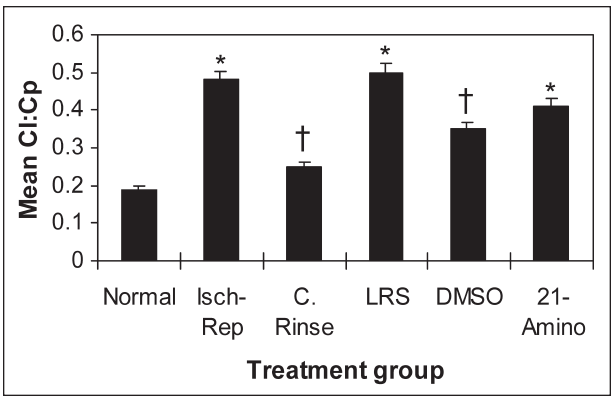

Figure 5—Bar graph of the mean (± SEM) Cl:Cp for horses of all treatment groups. *Significant (P < 0.05) increase in mean Cl:Cp, compared with control horses.[15] †Significant (P < 0.05) decrease in mean Cl:Cp, compared with horses that underwent ischemia and reperfusion with no treatment.[15] *See* Figure 1 for remainder of key.

horses[15] (**Figure 3**) and horses treated with Carolina rinse solution but significantly lower than for horses treated with U-74389G, which had a mean Cl:Cp of 0.42 ± 0.06 and an ORC of 0.58. Horses treated with U-74389G had a mean Cl:Cp that was not significantly different than that of horses treated with lactated Ringer's solution or horses that underwent ischemia and reperfusion with no treatment[15] (**Figure 4**). Overall, horses treated with Carolina rinse solution had a Cl:Cp that was significantly lower than for horses of any other treatment group. Horses treated with DMSO had a significantly lower Cl:Cp, compared with horses treated with U-74389G or lactated Ringer's solution (**Figure 5**).

Morphometric analysis—Comparisons were made of tissue thickness (edema) for mucosal, submucosal, and serosal intestinal layers of the jejunum among control horses[15]; horses that underwent ischemia and reperfusion with no treatment[15]; and horses treated with lactated Ringer's solution, Carolina rinse solution, DMSO, or U-74389G (**Figure 6**). All changes in intestinal layer thickness were calculated relative to the control jejunal segments obtained from each horse.

No significant difference was found over time in the mucosal, submucosal, or serosal thickness in the control jejunal segments from the 20 horses. A significant (P = 0.030) increase in the serosa thickness was found in the sham-operated segments in all horses relative to the control jejunal segments. A significant increase in submucosal and serosal layer edema and a significant decrease in mucosal thickness were found in the experimental segments of horses treated with lactated Ringer's solution, compared with control jejunal segments. Tissue layer thickness of the experimental segment of horses treated with Carolina rinse solution was not significantly different from that of the control jejunal segment. A significant (P = 0.030) increase in serosal and submucosal layer thickness was found in the experimental segment of horses treated with DMSO, compared with control or sham-operated segments; however, the edema increase was significantly less than that observed in the experimental segment of horses treated with lactated Ringer's solution. A decrease in submucosal tissue thickness (edema) was found in the experimental segments of horses treated with U-74389G, compared with horses treated with lactated Ringer's solution, but this difference was not significant (P = 0.080). Thickness of mucosa, submucosal, and serosal layers in the experimental segments of horses treated with lactated Ringer's solution or with U-74389G was not significantly different from that of the horses that underwent ischemia and reperfusion with no treatment.[15] Tissue thickness for all intestinal layers in the experimental segment of horses treated with Carolina rinse solution was not significantly different from that of control horses.[15]

The intestinal WW:DW in the experimental segments, compared with sham-operated or control segments, for each treatment group were determined (**Figure 7**). A significant (P = 0.001) decrease in the WW:DW was found in the experimental segments of horses treated with Carolina rinse solution or DMSO, compared with horses treated with lactated Ringer's solution or U-74389G. The WW:DW for horses treated with Carolina rinse solution or DMSO was not significantly different from that of control horses.[15] No significant difference was found in WW:DW between horses that underwent ischemia and reperfusion with no treatment[15] and horses treated with lactated Ringer's solution or U-74389G.

Light microscopy—Partial serosal layer mesothelial cell loss was the only morphologic alteration observed in the control jejunal segments from all 20 horses. All sham-operated segments were morphologically normal with an increase in submucosal and serosal layer edema and minimal cellular infiltration.

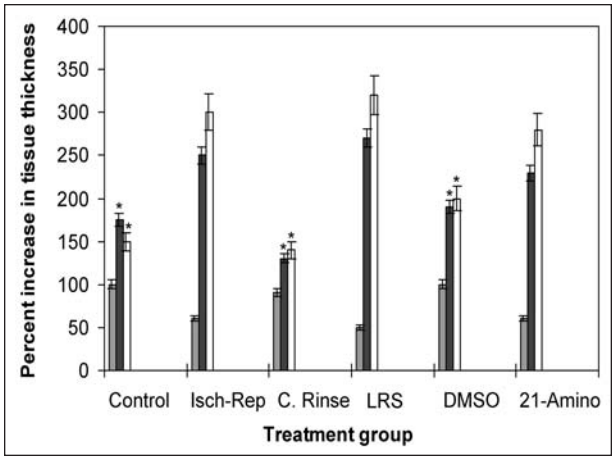

Figure 6—Bar graph of mean (± SEM) percent increase in tissue thickness for the mucosal (gray bar), submucosal (solid bar), and serosal (open bar) layers in experimental jejunal segments of horses in the following treatment groups: control,[15] isch-rep with no treatment,[15] C, Rinse solution, LRS, DMSO, and 21-Amino. *Significantly (P < 0.05) different from horses that underwent ischemia and reperfusion with no treatment.[15] *See* Figure 1 for remainder of key.

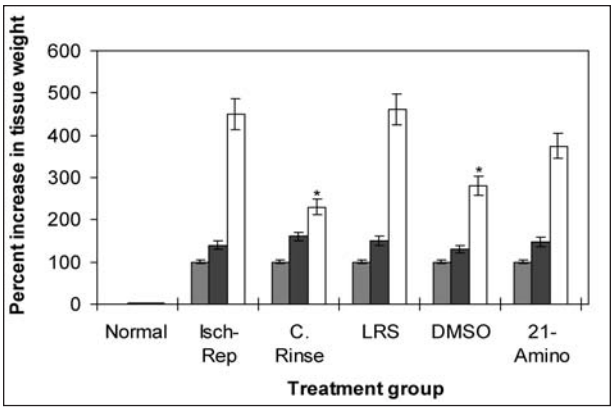

Figure 7—Bar graph of mean (± SEM) intestinal wet weight-to-dry weight ratio (expressed as percent increase in tissue weight) for control (gray bar), sham-operated (solid bar), and experimental (open bar) jejunal segments of horses in the following treatment groups: Normal[15] Isch-Rep with no treatment,[15] C, Rinse solution, LRS, DMSO, and 21-Amino. *Significantly (P < 0.05) less than for other treatment groups. *See* Figure 1 for remainder of key.

Morphologic changes observed in the experimental segments of horses treated with Carolina rinse solution had a morphologically normal mucosa with shortened villi and dilated central lymphatics. Submucosal and serosal layers had mild edema and the serosa had minimal neutrophil infiltration, compared with the adjacent control jejunal segment. Horses treated with lactated Ringer's solution had a morphologically normal mucosa with moderate to severe edema formation in the submucosal and serosal layers (**Figure 8**). The serosa also had a moderate increase in cellular infiltrate (**Figure 9**). Horses treated with DMSO had a morphologically normal mucosal layer and mild submucosal and serosal layer edema with minimally increased cellular infiltrate into the serosa. Horses treated with U-74389G had moderate submucosal and serosal layer edema with a moderate increase in cellular infiltrate in the serosa. The central lacteal of the villus and serosal lymphatics appeared large, and mucosal epithelial separation was observed in the mucosal sections of the experimental segments in some horses treated with U-74389G.

Transmission electron microscopy—Ultrastructure of the mucosa of the horses treated with Carolina rinse solution included an intact microvilli layer with a closely apposed epithelial cell layer and only mild vacuolation or edema (**Figure 10**). Mucosa of horses treated with lactated Ringer's solution appeared disrupted with edema, separating the mucosal epithelial cells with extensive capillary leakage of erythrocytes and leukocytes. Mucosa of

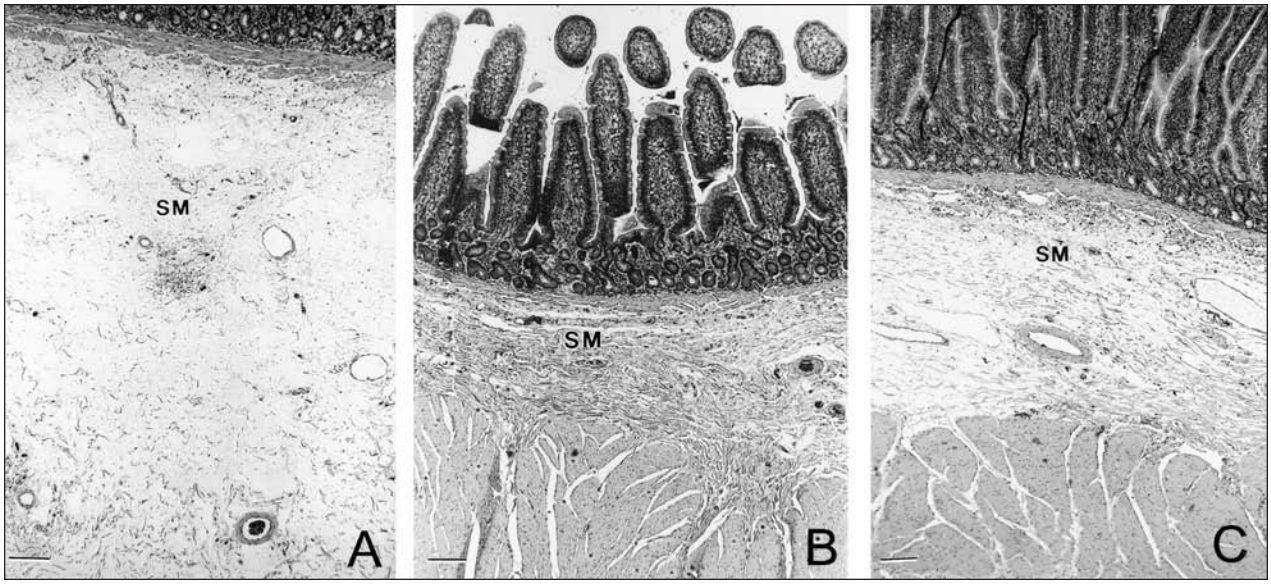

Figure 8—Photomicrographs of sections of jejunal experimental segments with submucosal (SM) layer edema from horses treated with lactated Ringer's solution (A), Carolina rinse solution (B), and dimethyl sulfoxide (C). H&E stain; bars = 66 μm.

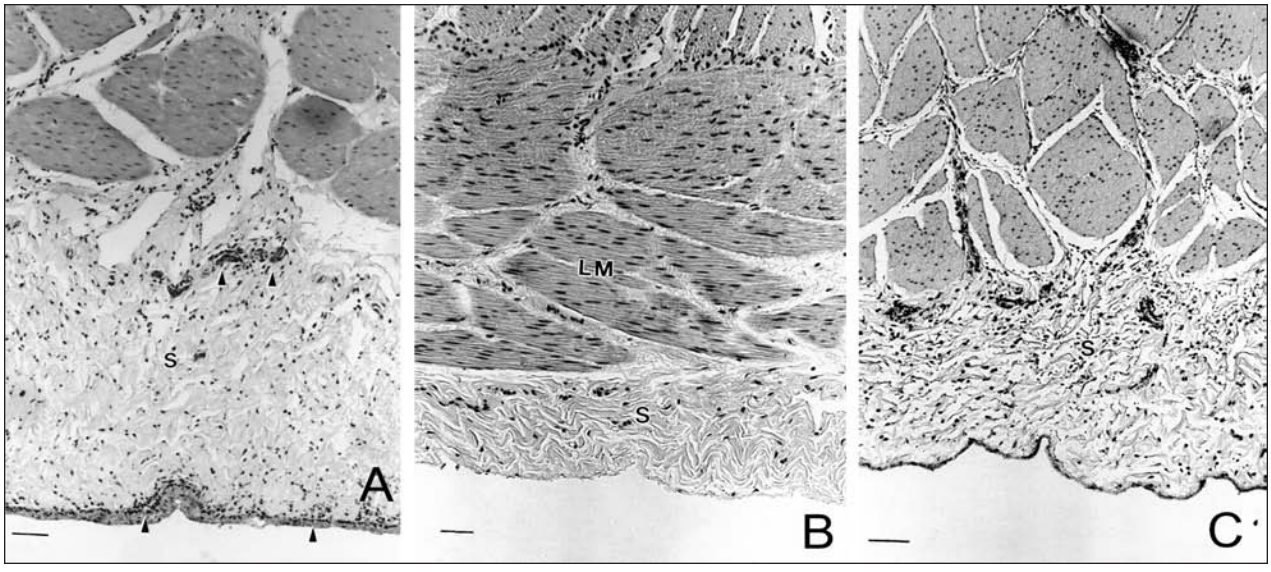

Figure 9—Photomicrographs of sections of the serosa (S) and longitudinal muscle (LM) from the jejunal experimental segments with edema and cellular infiltrate from horses treated with lactated Ringer's solution (A), Carolina rinse solution (B), and dimethyl sulfoxide (C). H&E stain; bars = 50 μm.

horses treated with DMSO had edema separating the epithelial cells and some cellular infiltrate but less than that of horses treated with lactated Ringer's solution. The microvilli layer was intact, but the epithelial cells had vacuolation just under the microvillus layer. Mucosa of horses treated with U-74389G also had an intact microvilli layer, but edema was separating the epithelial cells that appeared disrupted, and leukocyte infiltration was extensive.

Capillaries from the control jejunal segments had tight endothelial cell junctions and mild invagination of the endothelial cell nucleus. Capillaries of horses treated with Carolina rinse solution had tight endothelial cell junctions but had endothelial cell nuclear condensation and swollen mitochondria. Endothelial cells in the capillaries of horses treated with lactated Ringer's solution were damaged with unorganized structure and vacuolation.

Migration of erythrocytes and leukocytes through large gaps between endothelial cell junctions was evident (**Figure 11**). Capillaries of horses treated with DMSO had some separation of endothelial cell junctions, and the endothelial cells had mild swelling with vacuolation and mitochondrial swelling. Capillaries of horses treated with U-74389G had endothelial cells that were breaking away. Large gaps were observed within endothelial cell junctions, and the cells appeared vacuolated and swollen (**Figure 12**).

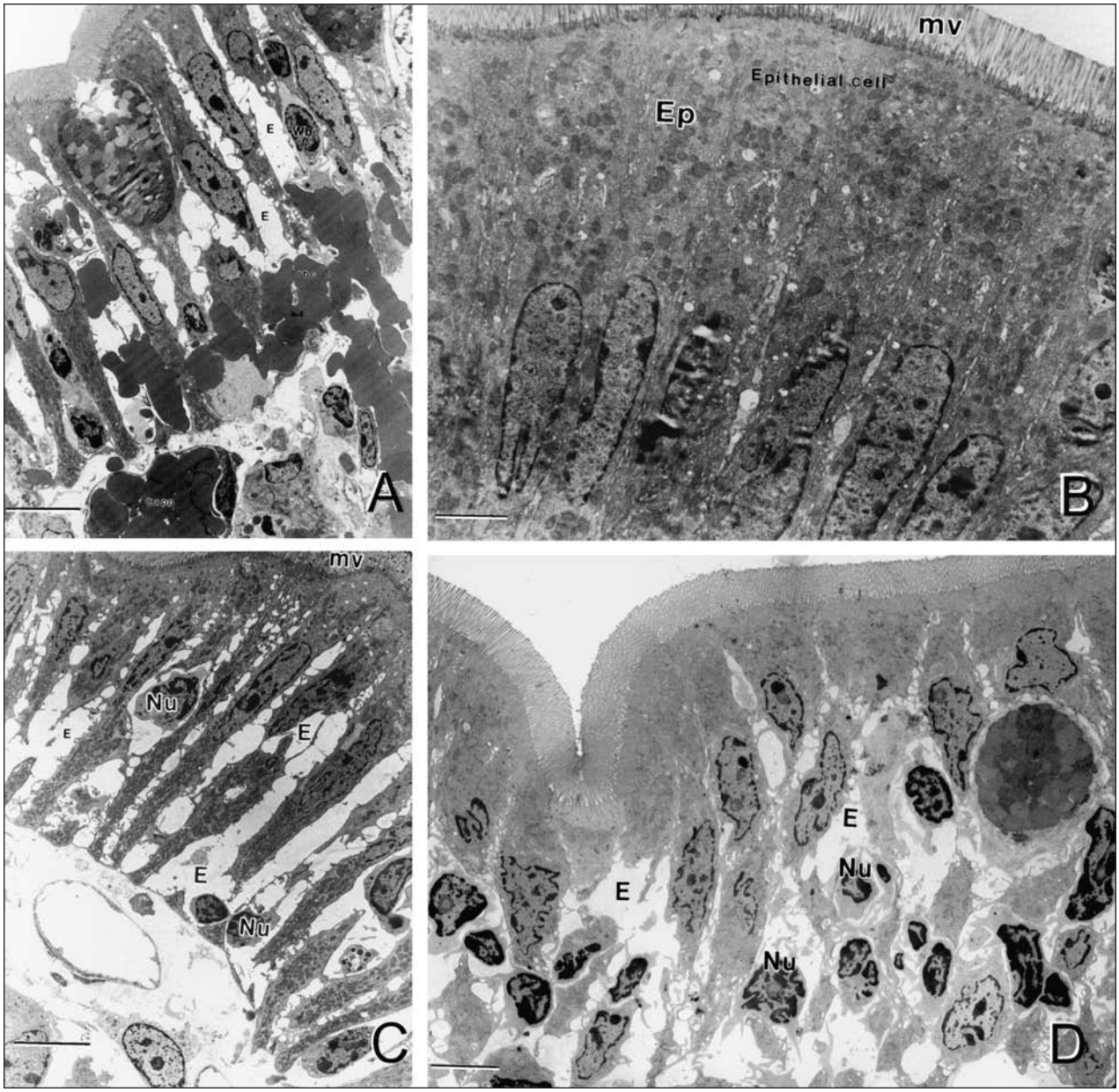

Figure 10—Transmission electron micrographs of jejunal mucosa of horses treated with lactated Ringer's solution (A), Carolina rinse solution (B), dimethyl sulfoxide (C), or U-74389G (D). Intact microvillus (mv) layers are observable. Notice epithelial cell (Ep) separation, edema (E), and neutrophil (Nu) infiltration in the jejunal mucosa of all horses, except for horses treated with Carolina rinse solution. capp = Capillary. rbc = Red blood cell. Wbc = White blood cell. Uranyl acetate and lead citrate stain; bars = 4.0 μm.

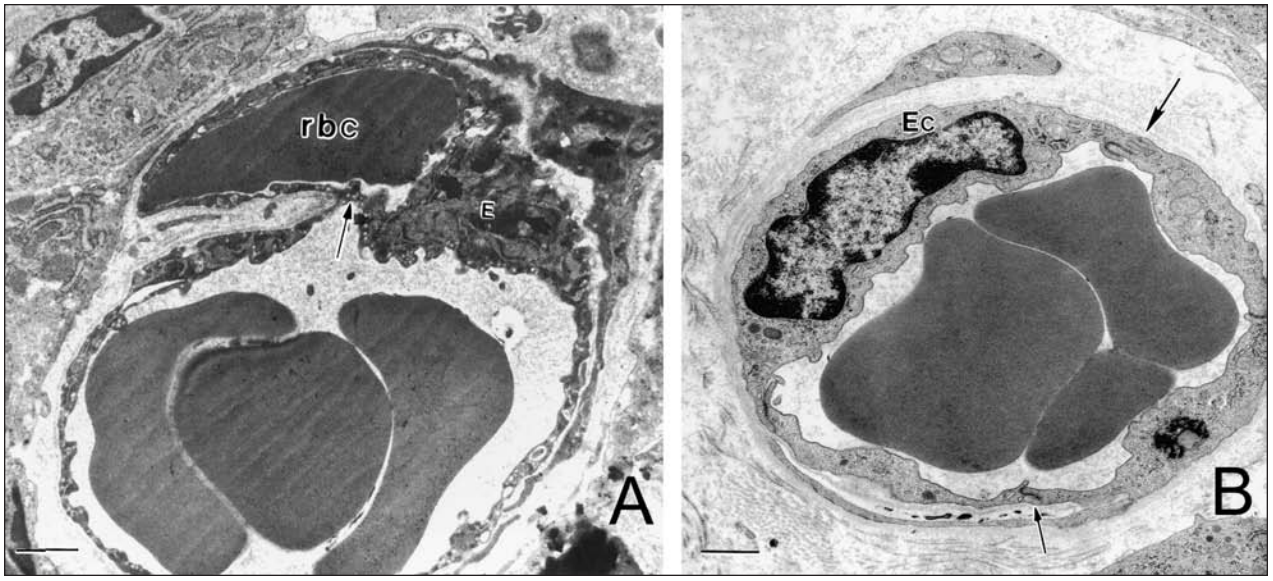

Figure 11—Transmission electron micrographs of a serosal capillary of horses treated with lactated Ringer's solution (A) or Carolina rinse solution (B). Notice the tight endothelial cell gap junction (Ec), endothelial cell (E), and erythrocyte (rbc) migration (arrows). Uranyl acetate and lead citrate stain; bars = 2.0 μm.

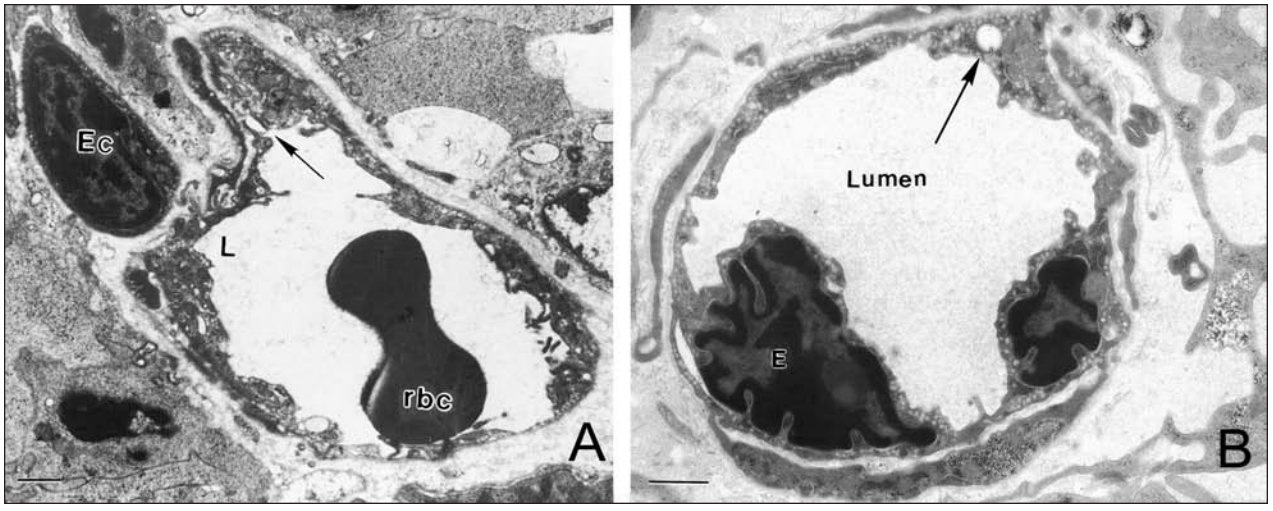

Figure 12—Transmission electron micrographs of a serosal capillary of horses treated with dimethyl sulfoxide (A) or U-74389G(B). Notice the endothelial cell (E) mitochondrial swelling and vacuolation (arrow) and endothelial cell (Ec) disruption and separation of endothelial cell gap junctions (arrow). L = Lumen. Uranyl acetate and lead citrate stain; bars = 2.0 μm.

Discussion

We evaluated the effects of DMSO, U-74389G, and Carolina rinse solution on the microvascular permeability, edema formation, and neutrophil infiltration of the equine small intestine subjected to low-flow ischemia-reperfusion injury. A significant protective effect of Carolina rinse solution and DMSO, but not of U-74389G, was found on ischemia-reperfusion injury of the small intestine.

The mesenteric blood flow leading to the experimental segment increased significantly from baseline in all horses after vascular clamp release. This is termed reactive hyperemia and is characteristic of the small intestine after periods of vascular occlusion.[33] Arterial hypoxia and hypercapnia elicit vasodilation and capillary recruitment. Any condition that causes a decrease in tissue O_2 content or an increase in vasodilator metabolites such as CO_2 or adenosine will cause relaxation of the precapillary sphincters, resulting in an increase in blood flow and capillary recruitment.[33,34] A significantly higher increase in blood flow after vascular clamp release was found in horses treated with Carolina rinse solution or DMSO, compared with horses treated with U-74389G or lactated Ringer's solution. The reactive hyperemia following Carolina rinse solution administration agrees with results of a previous study[15] from our laboratory. Many potential reasons exist for the hyperemic response after administration of these drugs. Adenosine, a potent vasodilator, is a component of Carolina rinse solution and increases mesenteric blood flow and arterial diameter in the small intestine of rats after topical application.[34] Previous work analyzing the ingredients of Carolina rinse solution revealed that the rinse solution lost its

efficacy in preventing microcirculatory disturbances in liver grafts if adenosine was omitted.[26] The calcium channel blocker nifedipine, another component of Carolina rinse solution, has a direct action on the vasculature and caused a decrease in vascular resistance and a 3-fold increase in blood flow in ischemic hearts.[35] These components of the rinse solution could account for the prolonged increase in mesenteric blood flow in this treatment group.

In humans with acute CNS trauma, DMSO improves cerebral blood flow and minimizes infarct size.[36] The mechanism for the effect of DMSO on blood flow is unclear; however, it may relate to the actions of the drug on leukocyte chemotaxis. Results of an in vitro study[37,38] indicate that DMSO inhibits interleukin-8, a potent neutrophil chemotactant, which is produced by monocytes, endothelial cells, and fibroblasts. In myocardial ischemia, coronary artery perfusion increased after pretreatment with neutrophil antisera, which decreased the number of circulating neutrophils.[11] Perhaps the ability of DMSO to limit neutrophil migration and adherence to the vascular endothelium[37-39] is responsible for improving vascular perfusion.

In a study[4] on feline small intestine, reperfusion after 1 hour of intestinal ischemia significantly decreased the ORC of total protein in intestinal capillaries from a normal value of 0.92 to 0.59 as a result of a significant increase in vascular permeability. In a previous study,[15] we demonstrated that 1 hour of low-flow ischemia (25% of baseline blood flow) followed by reperfusion caused a significant decrease in the ORC from normal values of 0.81 to 0.52 in the equine small intestine. In our current study, horses treated with Carolina rinse solution had an ORC of 0.75, which was not significantly different from that reported for control horses.[15] Carolina rinse solution was beneficial in attenuating ischemia-reperfusion-induced capillary permeability changes and edema formation. In our study, the ORC of horses treated with lactated Ringer's solution (which served as controls for horses treated with Carolina rinse solution) was not different from the ORC reported for horses that underwent low-flow ischemia and subsequent reperfusion of the jejunum with no treatment.[15] This finding indicates that flushing the jejunal artery with lactated Ringer's solution has no effect and is in agreement with the findings in another study[25] on the use of Carolina rinse solution in transplanted livers. Results of previous studies[28,29] from our laboratory indicate that the combination of topical and intraluminal administration of Carolina rinse solution was as effective as arterial perfusion in maintaining the ORC in jejunum undergoing ischemia and reperfusion. This application of Carolina rinse solution may be more clinically applicable than arterial perfusion.

Recent evidence indicates that reactive oxygen metabolites and leukocyte activation and adhesion to the vascular endothelium are responsible for the increased microvascular permeability induced by intestinal ischemia-reperfusion injury.[39] Two sources of reactive oxygen metabolites are found in ischemic intestine. One is the conversion of hypoxanthine to xanthine, which produces superoxide anion and hydrogen peroxide. The second is neutrophil nicotinamide adenine dinucleotide phosphate oxidase generation of hypochlorous acid.[5-8,39] This theory is supported by intestinal studies on cats that prevented the ischemia-induced increase in vascular permeability by administration of oxygen radical scavengers such as superoxide dismutase,[8] allopurinol,[6] desferroxamine,[8] DMSO,[16] adenosine,[40] and pharmacologic agents aimed at limiting neutrophil infiltration such as anti-CD18 monoclonal antibody.[7,39] The local perfusion of Carolina rinse solution, and to a lesser extent DMSO (20 mg/kg, IV), prevented the increase in vascular permeability induced by low-flow ischemia and reperfusion in our study. The antioxidant properties of allopurinol, desferrioxamine, and glutathione included within the Carolina rinse solution and hydroxyl radical scavenging ability of DMSO appear to have limited the capillary permeability alterations induced by ischemia and reperfusion in our study.

Neutrophil activation and adhesion to the vascular endothelial cell cause increased microvascular permeability secondary to endothelial cell damage, separation, and protein leakage in intestinal ischemia.[38-40] Hernandez et al[39] reported that the reperfusion-induced increase in intestinal microvascular permeability was significantly attenuated in animals rendered neutropenic with antineutrophil serum or after administration of monoclonal antibodies that prevent granulocyte adherence to the vascular endothelium. Carolina rinse solution prevented reperfusion-induced killing of endothelial cells and improved graft survival from 25% to 60% after orthotopic rat liver transplantation.[25] Improved survival was associated with decreasing the number of adherent neutrophils to the vascular endothelium in postsinusoidal venules, which was associated with preservation of endothelial cell ultrastructure.[25-27] In previous studies[28,29] from our laboratory, mesenteric arterial perfusion and topical Carolina rinse solution decreased serosal neutrophil infiltration. In intravital microscopic intestinal studies,[6,40] allopurinol and adenosine, components of Carolina rinse solution, attenuated leukocyte adherence to venular endothelium during ischemia and reperfusion. In rats, DMSO prevented N-formyl-methionyl-leucyl-phenylalanine–induced leukocyte adherence to the mesenteric venules.[41] Beneficial properties of DMSO are attributed to the ability to inhibit the production of interleukin-8, a potent neutrophil chemotactant.[36] The beneficial affects of Carolina rinse solution and DMSO on vascular permeability may be partially attributed to the antineutrophil properties of these agents.

A common feature of many intestinal lesions found at surgery is edema formation of the affected intestinal segment. Edema formation can be secondary to increased capillary filtration of the microvasculature and leakage of plasma proteins.[33] Mural edema following ischemia-reperfusion injury may lead to further intestinal ischemia by increasing the capillary to interstitium distance, thereby decreasing oxygen and nutrient delivery to tissues.[33] In our study, treatment with Carolina rinse solution, and to a lesser extent DMSO, limited edema formation in the submucosal and serosal layers of the experimental segments of jejunum after

ischemia and reperfusion. Perfusion with lactated Ringer's solution had no effect. Horses treated with U-74389G had a decrease in submucosal edema, but this change was not significant. Similar nonprotective effects have been reported[24] after treatment with U-74389G (3 or 6 mg/kg, IV) following total and partial vascular occlusion for 1 hour followed by 2 hours of reperfusion in the equine jejunum. In our study, the decrease in serosa and submucosal intestinal layers and decrease in WW:DW in horses treated with Carolina rinse solution or DMSO correlated well with the microvascular permeability estimates. All horses in our study had increased serosal edema in the sham-operated segments, which was probably the result of 6 hours of extra-abdominal exposure of the intestinal segment.

In our study, administration of DMSO (20 mg/kg, IV) prior to reperfusion was beneficial in attenuating the ischemia-reperfusion–induced permeability changes and in limiting edema formation in the equine jejunum. An investigation[42] on other species with partial intestinal ischemia revealed the protective effects of DMSO in the small intestine when administered IV prior to reperfusion at a dose of 20 mg/kg. Intravenous administration of DMSO at a dose of 2.5 g/kg significantly decreased ischemia-induced brain edema and improved blood flow in monkeys.[36,43] In rats, DMSO (20% solution) administered IP or IV significantly decreased peritoneal adhesion formation induced by ischemia and reperfusion.[41] Administration of DMSO (3 g/kg, IP) prevented the morphologic alterations observed after jejunal ischemia-reperfusion injury in rats.[44]

Conflicting results exist in the literature as to whether DMSO is beneficial in ischemic intestinal lesions in horses. In an ischemia-reperfusion intestinal model, DMSO (100 mg/kg, IV) administered prior to reperfusion and twice daily for 3 days limited adhesion formation after 10 days in the jejunum of foals.[20] Moore et al[18] administered DMSO (1 g/kg) to horses after 2.5 hours of low-flow ischemia and 3 hours of reperfusion and found no beneficial effect on morphologic alterations in the large intestine. The failure of DMSO to protect the large intestine may have been the result of the high dose of DMSO (1 g/kg) administered or possibly the ischemic model used was too severe in terms of injury for the DMSO to be effective. In our study, the use of the partial ischemic model in the small intestine resulted in minimal morphologic damage but significant alterations in capillary permeability, which is a sensitive indicator of tissue injury. On the basis of the results of our study, it appears that DMSO (20 mg/kg, IV) administered prior to reperfusion is beneficial in low-flow ischemic lesions of the equine small intestine.

In our study, mucosal epithelial separation was observed in half of the experimental segments from horses treated with U-74389G. Mucosal damage was not observed in horses undergoing 1 hour of low-flow ischemia (25% of baseline blood flow) and subsequent reperfusion with no treatment[15] or in horses of other treatment groups. Because the results of other intestinal studies[23,24] in horses with the same dose of U-74389G have not revealed these delirious effects, it is unclear why the horses of our study had mucosal injury. In our study, the administration of U-74389G did not prevent the reperfusion-induced changes in capillary permeability or edema formation in the equine jejunum, which supports the results of previous work[24] that revealed a lack of protection by U-74389G in equine jejunum undergoing ischemia and reperfusion.

In our study, Carolina rinse solution and DMSO provided a protective effect against ischemia and reperfusion of the equine jejunum by attenuating vascular permeability changes and decreasing edema formation and neutrophil infiltration. The anti-inflammatory property that these 2 compounds have in common is the inhibition of neutrophil adherence to the vascular endothelium. Results of our study support the role of neutrophil involvement in ischemia-reperfusion damage of the equine jejunum.

Use of DMSO or Carolina rinse solution attenuated edema formation, vascular permeability changes, and cellular infiltration caused by low-flow ischemia and reperfusion of the jejunum. Clinicians dealing with patients having inflammed or compromised intestine commonly use IV administration of DMSO. Results of our study lend credibility to its continued use. In our study, we used arterial perfusion of the mesenteric artery leading to the experimental jejunal segment as a means of administering Carolina rinse solution. Results of a recent experimental study[29] indicate that combining topical and intraluminal administration of Carolina rinse solution to jejunum undergoing ischemia and reperfusion had similar protective effects of attenuating vascular permeability changes and decreasing cellular infiltration, as did arterial perfusion in our study. Combined topical and intraluminal administration of Carolina rinse solution is less invasive than arterial perfusion and would allow a much larger segment of inflamed intestine to be treated. These findings indicate that combined topical and intraluminal administration of Carolina rinse solution is a potentially clinically applicable treatment to prevent immediate inflammatory changes during reperfusion of ischemic or distended jejunum.

a. Rompun, Mobaye Corp, Shawnee, Kan.
b. GG, Rhone-Poulene Inc, NY.
c. Vetalar, Fort Dodge Laboratories Inc, Fort Dodge, Iowa.
d. LRS, Baxter Healthcare Corp, Deerfield, Ill.
e. Dobutrex, Eli Lilly Industries Inc, Carolina, Puerto Rico.
f. Heatpad, Hamilton Industries, Cincinnati, Ohio.
g. Model TSI, Transonic Systems Inc, Ithaca, NY.
h. J-Catheter, Deseret-Medical Inc, Sandy, Utah.
i. Model CCQ PM-2A, Honeywell Inc, Hayward, Calif.
j. Wolliner vascular clamp, Wolliner Inc, Davis, Calif.
k. Wiretrol II, Drummond Scientific Co, Broomall, Pa.
l. U-74389G, The Upjohn Co, Kalamazoo, Mich.
m. Available from Dr. John Lemasters, University of North Carolina, Chapel Hill, NC.
n. Ultramicro Pipetman, Rainin Instrument Co, Emeryville, Calif.
o. Model 5851, National Appliance Co, Portland, Ore.
p. Poly/Bed 812, Polysciences, Warren Park, Pa.
q. Model 1585, Nissei Sangyo America Ltd, Mountain View, Calif.
r. Macintosh IIfx, Apple Computer Inc, Cupertino, Calif.
s. Image, Davis, Calif.
t. Infinity Optical Disk, Peripheral Land Inc, Freemont, Calif.
u. Morphometrix, Davis, Calif.

References

1. White NA, Moore JN, Trim CM. Mucosal alterations in experimentally induced small intestinal strangulating obstruction in ponies. *Am J Vet Res* 1980;41:193–198.

2. Sullins KE, Stashak TS, Mero KN. Pathologic changes associated with induced small intestinal strangulation obstruction and nonstrangulating infarction in horses. *Am J Vet Res* 1985;46:913–916.

3. Dabareiner RM, Snyder JR, Sullins KE, et al. Evaluation of the microcirculation of the equine jejunum and ascending colon after ischemia and reperfusion. *Am J Vet Res* 1993;54:1683–1692.

4. Granger DN, Sennett M, McElearney P, et al. Effect of local arterial hypotension on cat intestinal capillary permeability. *Gastroenterology* 1980;79:474–480.

5. Granger DN, Rutili G, McCord JM. Superoxide radicals in feline intestinal ischemia. *Gastroenterology* 1981;81:22–29.

6. Parks DA, Granger DN. Ischemia-induced vascular changes: role of xanthine oxidase and hydroxyl radicals. *Am J Physiol* 1983;245:G285–G289.

7. Schoenberg MH, Fredholm BB, Haglund U, et al. Studies on the oxygen radical mechanism involved in the small intestinal reperfusion damage. *Acta Physiol Scand* 1985;124:581–589.

8. Granger DN, McCord JM, Parks DA, et al. Xanthine oxidase inhibitors attenuate ischemia-induced vascular permeability changes in the cat intestine. *Gastroenterology* 1986;90:80–84.

9. Prichard M, Ducharme NG, Wilkins PA, et al. Xanthine oxidase formation during experimental ischemia of the equine small intestine. *Can J Vet Res* 1991;55:310–314.

10. Welbourn CR, Goldman G, Paterson IS. Pathophysiology of ischaemia reperfusion injury: central role of the neutrophil. *Br J Surg* 1991;78:651–655.

11. Romson JL, Hook BG, Kunkel SL, et al. Reduction of the extent of ischemic myocardial injury by neutrophil depletion in the dog. *Circulation* 1983;67:1016–1023.

12. Grisham MB, Hernandez AL, Granger DN. Xanthine oxidase and neutrophil infiltration in intestinal ischemia. *Am J Physiol* 1986;251:G567–G574.

13. Braughler JM, Pregenzer JF. The 21-aminosteroid inhibitors of lipid peroxidation: reactions with lipid peroxyl and phenoxy radicals. *Free Radic Biol Med* 1989;7:125–130.

14. Parks CA, Shah AK, Granger DN. Oxygen radicals: effects on intestinal vasculature permeability. *Am J Physiol* 1984;247:G167–G170.

15. Dabareiner RM, Snyder JR, White NA, et al. Microvascular permeability and endothelial cell morphology associated with low-flow ischemia/reperfusion injury in the equine jejunum. *Am J Vet Res* 1995;56:639–648.

16. Brayton CF. Dimethyl sulfoxide (DMSO): a review. *Cornell Vet* 1986;76:61–90.

17. Reeves MJ, Vansteenhouse J, Stashak TS, et al. Failure to demonstrate reperfusion injury following ischaemia of the equine large colon using dimethyl sulphoxide. *Equine Vet J* 1990;22:126–132.

18. Moore RM, Muir WW, Bertone AL, et al. Effects of dimethyl sulfoxide, allopurinol, 21-aminosteroid U-74389G, and manganese chloride on low-flow ischemia and reperfusion of the large colon in horses. *Am J Vet Res* 1995;56:671–687.

19. Arden WA, Stick JA, Parks AH, et al. Effects of ischemia and dimethyl sulfoxide on equine jejunal vascular resistance, oxygen consumption, intraluminal pressure, and potassium loss. *Am J Vet Res* 1989;50:380–387.

20. Sullins KE, White NA, Lundin CS, et al. Prevention of ischaemia-induced small intestinal adhesions in foals. *Equine Vet J* 2004;36:370–375.

21. Lantz GC, Badylak SF, Hiles MC, et al. Treatment of reperfusion injury in dogs with experimentally induced gastric dilatation-volvulus. *Am J Vet Res* 1992;53:1594–1598.

22. Stone WC, Bjorling DE, Southard JH, et al. Evaluation of intestinal villus height in rats after ischemia and reperfusion by administration of superoxide dismutase, polyethylene glycol-conjugated superoxide dismutase, and two 21-aminosteroids. *Am J Vet Res* 1992;53:2153–2156.

23. Vatistas NJ, Snyder JR, Hildebrand SV, et al. Effects of the 21-aminosteroid U-74389G on ischemia and reperfusion injury of the ascending colon in horses. *Am J Vet Res* 1993;54:2155–2160.

24. Vatistas NJ, Snyder JR, Hildebrand SV, et al. Effects of U-74389G, a novel 21-aminosteroid, on small intestinal ischemia and reperfusion injury in horses. *Am J Vet Res* 1996;57:762–770.

25. Currin RT, Toole JG, Thurman RG, et al. Evidence that Carolina rinse solution protects sinusoidal endothelial cells against reperfusion injury after cold ischemic storage of rat liver. *Transplantation* 1990;50:1076–1078.

26. Gao WS, Hijioka T, Lindert KA, et al. Evidence that adenosine is a key component in Carolina Rinse responsible for reducing graft failure after orthotopic liver transplantation in the rat. *Transplantation* 1991;52:992–998.

27. Post S, Rentsch M, Gonzalez AP, et al. Effects of Carolina rinse and adenosine rinse on microvascular perfusion and intrahepatic leukocyte-endothelium interaction after liver transplantation in the rat. *Transplantation* 1993;55:972–977.

28. Dabareiner RM, White NA, Donaldson L. Evaluation of Carolina rinse solution as a treatment for ischaemia reperfusion of the equine jejunum. *Equine Vet J* 2003;35:642–646.

29. Young BL, White NA, Donaldson LL, et al. Treatment of ischaemic jejunum with topical and intraluminal Carolina rinse. *Equine Vet J* 2002;34:469–474.

30. Van Hoogmoed LM, Snyder JR, Nieto J, et al. In vitro evaluation of a customized solution for use in attenuating effects of ischemia and reperfusion in the equine small intestine. *Am J Vet Res* 2001;62:1679–1686.

31. Taylor AE, Parker JC, Granger DN, et al. Assessment of capillary permeability using lymphatic protein flux: estimation of the osmotic reflection coefficient. In: Effros RM, Schmid-Shonbein H, Ditzel J, eds. *Microcirculation: current physiologic, medical, and surgical concepts*. New York: Academic Press Inc, 1981;19–32.

32. Granger DN, Granger JP, Brace RA, et al. Analysis of the permeability characteristics of cat intestinal capillaries. *Circ Res* 1979;44:335–344.

33. Shepard AP, Granger DN. Metabolic regulation of intestinal circulation. In: Shepard AP, Granger DN, eds. *Physiology of intestinal circulation*. New York: Raven Press, 1984;33–48.

34. Proctor KG. Intestinal arteriolar responses to mucosal and serosal applications of adenosine analogues. *Circ Res* 1987;61:187–193.

35. Crome R, Hearse DJ, Manning AS. Ischemia- and reperfusion-induced arrhythmias: beneficial actions of nifedipine. *J Cardiovasc Pharmacol* 1986;8:1249–1256.

36. Rosenblum W. Dimethyl sulfoxide effects on platelet aggregation and vascular reactivity in pial microcirculation. *Ann N Y Acad Sci* 1983;411:110–119.

37. DeForge LE, Fantone JC, Kenney JS, et al. Oxygen radical scavengers selectively inhibit interleukin 8 production in human whole blood. *J Clin Invest* 1992;90:2123–2129.

38. Sekizuka E, Benoit JN, Grisham MB, et al. Dimethylsulfoxide prevents chemoattractant-induced leukocyte adherence. *Am J Physiol* 1989;256:H594–H597.

39. Hernandez LA, Grisham MB, Twohig B, et al. Role of neutrophils in ischemia-reperfusion-induced microvascular injury. *Am J Physiol* 1987;253:H699–H703.

40. Grisham MB, Hernandez LA, Granger DN. Adenosine inhibits ischemia-reperfusion-induced leukocyte adherence and extravasation. *Am J Physiol* 1989;257:H1334–H1339.

41. Granger DN, Barrowman JA. Microcirculation of the alimentary tract. II. Pathophysiology of edema. *Gastroenterology* 1983;84:1035–1049.

42. Mayer JH III, Anido H, Almond CH, et al. Dimethyl sulfoxide in prevention of intestinal adhesions. *Arch Surg* 1965;91:920–923.

43. DeTorre JC, Surgeon JW. Dexamethazone and DMSO in experimental transorbital cerebral infarction. *Stroke* 1976;7:577–583.

44. Ravid M, Van-Dyk D, Bernheim J. The protective effect of dimethyl sulfoxide in experimental ischemia of the intestine. *Ann N Y Acad Sci* 1983;411:100–104.