Intestinal Ischemia-Reperfusion Injury in Horses: Pathogenesis and Therapeutics

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Abstract: This article discusses the potential role of oxidative injury to the intestinal tract of horses and the therapeutic approaches that have been investigated to decrease cellular damage secondary to ischemia-reperfusion (IR) injury. Equine colic is a major concern for horse owners and veterinary practitioners. Strangulating and obstructive lesions of the small and large intestines commonly require intervention in patients via exploratory celiotomy. However, the application of information from experimentally induced IR injury in horses to clinical cases of naturally occurring equine colic is not clear. Thus, while the exact mechanisms and clinical significance of intestinal IR are being defined and may be matters of academic debate, a review of the available information may provide knowledge of potential underlying pathophysiologic mechanisms contributing to intestinal injury in equine colic. This information may allow clinicians to offer additional therapeutic strategies for horses with strangulating obstruction of the small or large intestine. Further clinical study of the therapeutic options for horses with naturally occurring disease is warranted.

Colic is an important cause of morbidity and mortality in horses and a significant health concern in the equine industry. While numerous underlying causes of equine colic exist, this article discusses ischemia-reperfusion (IR) injury. IR injury involves the detrimental effects of ischemia on the intestines along with the paradoxical effects of reintroduction of oxygen. Reactive oxygen species (ROS) and reactive nitrogen species have been associated with injurious effects to the intestines during reperfusion. Various studies in horses have documented deleterious effects of IR injury; however, the pathogenesis of IR injury is incompletely understood. Furthermore, while experimental models may serve as a surrogate for naturally occurring disease, they may not exactly mimic spontaneous disease. Therefore, the clinical significance of IR injury in equine medicine is controversial. Although numerous medications have been investigated for mitigating the effects of IR injury, these therapeutics need to be examined in controlled clinical trials. Thus, it is important for equine practitioners to understand the basis of IR injury as it applies to equine colic and to ascertain the clinical significance of IR injury and the efficacy of proposed therapeutics in light of the current literature.

Evidence of Ischemia-Reperfusion in Equine Intestinal Disease

The major supposition regarding IR injury is that further damage to previously ischemic intestine results primarily from oxidative injury by (1) ROS produced by xanthine oxidase (XO) during reintroduction of oxygen and (2) hypochlorous acid (HOCl) from neutrophils that have accumulated in the intestinal interstitium. Various studies have examined morphologic lesions associated with intestinal injury. For more information, please see the companion article, “Mechanisms of Oxidative Injury in Equine Disease.”
after ischemia alone and compared these findings with tissues that have been subjected to ischemia with subsequent reperfusion. An early study examining small intestinal lesions in cats exposed to 3 hours of intestinal ischemia versus 3 hours of ischemia followed by 1 hour of reperfusion reported increased mucosal injury in tissues subjected to IR. The authors concluded that the vast majority of mucosal injury associated with ischemia occurred during reperfusion of ischemic intestine and not during the ischemic period. Multiple equine studies have also demonstrated a similar progression of intestinal injury during IR.

In equine models involving 120 minutes of small intestinal ischemia alone, lesions identified included mild neutrophilic infiltrate, nuclear alterations, cytoplasmic vacuolization of surface mucosal epithelial cells, mild hemorrhage, and edema. Comparatively, lesions associated with 120 minutes of ischemia followed by 120 minutes of reperfusion demonstrated a much more pronounced neutrophilic inflammatory response along with increased loss of epithelial cells, significantly worse mucosal lesion grade, and decreased surface area and volume. Increased microvascular permeability has also been documented with IR models of equine small intestine; increased permeability may subsequently contribute to mucosal edema, neutrophil infiltration, and swollen endothelial cells. Thus, during IR, intestinal alterations include increased microvascular permeability, mucosal edema, neutrophil infiltration, hemorrhage, and necrosis. Similar histomorphologic changes have been demonstrated in the large intestine of horses subjected to experimental IR.

Conversely, other equine studies have documented that reperfusion of ischemic jejunum or colon resulted in mucosal lesions similar to those associated with ischemia alone. The different models used to examine IR injury, specifically the type of ischemia induced, must be considered. Some studies suggest that IR injury occurs with low-flow (e.g., 20% baseline blood flow) ischemia that is followed by reperfusion. In contrast, further cellular damage does not occur during reperfusion of ischemic tissues subjected to total vascular occlusion. Therefore, it is possible that ischemia induced by complete vascular obstruction may induce different pathophysiologic processes in intestine compared with low-flow (partial) ischemia. Moreover, it has been suggested that most intestinal injury occurs after reperfusion if the ischemic period is partial and of moderate duration (1 to 3 hours); comparatively, if the ischemia is characterized by complete intestinal vascular occlusion and/or is prolonged (i.e., >3 hours), tissue damage sustained during the ischemic period predominates and further injury during reperfusion may be inconsequential.

As studies have implicated ROS, reactive nitrogen species, or both as significant contributors to IR injury in other species, investigations have attempted to identify whether similar oxidative mechanisms of IR injury occur in horses. Histomorphologic deterioration of equine jejunal and colonic mucosa has been observed during reperfusion of intestinal segments following a 2-hour ischemic period. The mucosal deterioration correlated with oxidative processes in the jejunum, including increased concentrations of malondialdehyde and conjugated dienes, both indicators of lipid peroxidation during reperfusion. Increased concentrations of malondialdehyde have also been measured in an ischemic model of the equine colon.

Further evidence of the role of oxidative injury to the intestines has been suggested by in vitro studies that have demonstrated damaging effects of HOCl, a highly toxic oxygen metabolite released by neutrophils, on the colon of horses. Oxidative injury during IR injury has been documented in ischemic small and large intestines of horses based on increased numbers of neutrophils and myeloperoxidase activity during reperfusion. Myeloperoxidase—an enzyme within azurophilic granules in neutrophils and other leukocytes—promotes formation of HOCl and serves as a marker of neutrophil emigration and oxidative activity. In addition, peroxynitrite, produced by the reaction between nitric oxide and superoxide radicals, is a highly reactive radical associated with IR injury. Increased presence of peroxynitrite has been indirectly demonstrated in clinical cases of equine small intestinal strangulating obstruction. In vitro studies have also suggested that superoxide radicals might be involved in the pathogenesis of IR injury in equine jejunum.

Additionally, the percentage of XO, an integral enzyme involved in production of superoxide radicals during reperfusion, increased from 27% at baseline to 37% after 1 hour of ischemia in an equine small intestine model, providing further evidence that ROS may be involved in equine IR injury. In contrast, other studies failed to demonstrate increased activity of XO in equine jejunum or large colon during IR models. Furthermore, inhibition of XO had no beneficial effect in one equine model of intestinal IR. One study also suggested that the mucosal interstitium of the equine small intestine lacks a sufficient number of neutrophils, which are implicated as a primary source of ROS, to markedly worsen injury during reperfusion. In summary, some information implicates oxidative injury in intestinal IR injury, but the true significance of these findings has not been determined.

Potential Therapeutics for Ischemia-Reperfusion Injury

Although the clinical significance of IR injury is inconclusive at this time, many medications, used alone or in combination, have been investigated in various species in efforts to attenuate oxidative damage associated with intestinal IR injury. These medications are intended to reduce production of ROS by providing an agonist or antagonist to essential enzymes involved in IR injury, scavenging free radicals, binding iron, or decreasing the migration of neutrophils into the intestine—and therefore their activation—during reperfusion. The XO inhibitor allopurinol and its active metabolite oxypurinol have demonstrated decreased ROS production during endotoxemia or exercise in horses. Allopurinol is a hypoxanthine analogue that competitively inhibits the reaction between XO and hypoxanthine, thus depressing production of superoxide radicals (FIGURE 1). Thus far, experimental administration of allopurinol during equine IR models has not decreased the degree of intestinal injury. Another enzymatic antioxidant, superoxide dismutase (SOD), converts superoxide radicals to hydrogen peroxide (FIGURE 1, step 6). One study has measured decreased SOD activity during
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Experimental ischemia of equine colon, and an in vitro investigation suggested that SOD improved the histologic score and may have a protective role in equine IR injury. However, clinical studies are still necessary.

Although the clinical benefit of the industrial solvent dimethyl sulfoxide (DMSO) for scavenging hydroxyl radicals is controversial, some models of equine intestinal IR have demonstrated positive effects with intravenous administration of DMSO after experimental induction of ischemic injury. Beneficial effects include decreased serosal and submucosal edema formation and decreased intestinal adhesion formation; both studies used a DMSO dosage of 20 mg/kg. However, other studies, using a DMSO dosage of 1 g/kg, have not demonstrated improvement with the use of DMSO. Administration of DMSO (1 g/kg IV) demonstrated a trend toward greater depth of intestinal mucosal loss in one equine study compared with control samples. A potential reason for this finding is that DMSO can react with hydroxyl radicals, resulting in the generation of methyl radicals and methyloxo radicals that can react with membrane lipids. The dosage of DMSO must also be considered. A standard dosage of DMSO has not been established in horses: a range from 20 mg/kg to 1 g/kg has been used. The two equine studies in which 20 mg/kg was used yielded positive results; however, this low number of studies is not sufficient for formulating conclusions regarding the dose. In general, concrete evidence of beneficial effects of administering DMSO to treat IR injury is lacking.

Sequestration of iron may also protect animals from IR injury. Ferrous iron is necessary for the Fenton reaction, which is essential for forming hydroxyl radicals. Endogenous iron-binding proteins, such as transferrin and ferritin, sequester iron to decrease the formation of hydroxyl radicals. In addition, exogenous iron chelators such as deferoxamine have been used for similar purposes, but no equine-specific information on using this drug as monotherapy is available. The 21-aminosteroids are compounds that exert therapeutic properties of corticosteroids, such as attenuation of lipid peroxidation induced by postischemic oxygen-derived free radicals and inhibition of nicotinamide adenine dinucleotide phosphate oxidase generation of superoxide by neutrophils, without the...
deleterious effects associated with glucocorticoids or mineralocorticoids. The overall efficacy of 21-aminosteroid (U-74389G) has been disappointing, with no improvement observed in most equine IR models when this drug class is used. One study documented a trend toward improved intestinal histologic grade with the use of U-74389G, but statistical significance was not achieved. Other medications that are potentially beneficial for treating IR injury in horses include a platelet-activating factor (PAF) antagonist (t-691,880), high-molecular-weight (HMW) dextrans, manganese chloride (MgCl₂), and acetylcysteine. It has been suggested that release of ROS during reperfusion of ischemic intestine stimulates the synthesis of PAF via activation of phospholipase A₂. Subsequently, PAF attracts and activates neutrophils, which then release oxidants and degradative enzymes, resulting in tissue damage. Unfortunately, administration of PAF antagonist to horses did not prevent or decrease colonic mucosal injury associated with low-flow IR. The use of other agents that block neutrophil chemotaxis or endothelial adhesion has been suggested, but no information on the use of these agents in horses is available. Increased microvascular permeability secondary to intestinal IR injury has been documented in equine models, resulting in interstitial edema. Some studies in rats have suggested that HMW dextrans can reduce microvascular permeability and tissue injury associated with IR injury, but administration of HMW dextrans during an equine intestinal IR model failed to demonstrate significant improvement in intestinal histopathology. Manganese chloride, an inorganic manganese salt that has superoxide-scavenging properties, has been experimentally administered during an equine IR model of the colon, but beneficial effects were not demonstrated. Alternatively, acetylcysteine, which has antioxidant properties and can replenish reduced glutathione, has demonstrated a protective effect against HOCl in equine in vitro models, suggesting that it may have therapeutic value. However, in vivo equine studies involving experimentally induced or naturally acquired gastrointestinal tract ischemia are required.

NSAIDs are routinely administered to horses with ischemic intestinal disease, principally because these drugs counteract negative cardiocirculatory effects and abdominal pain associated with IR-initiated prostanoic production. However, recent studies investigating the use of nonselective cyclooxygenase (COX) inhibitors, such as flunixin, during ischemic intestinal injury have raised concern regarding routine administration of NSAIDs in clinical cases of ischemic intestinal injury in horses. Prostaglandins are critical for recovery of ischemic-injured intestinal tissues. COX-1 is constitutively expressed in most tissues, including intestine, and maintains various physiologic (“housekeeping”) functions in health, whereas inducible COX-2 is upregulated by numerous stimuli and is associated with inflammation. While nonselective COX inhibitors provide analgesic and antiinflammatory benefits, indiscriminate inhibition of both COX isoforms hinders production of beneficial housekeeping prostaglandins and may predispose the equine intestinal tract to injury or impair recovery of injured intestine. This theory has been substantiated by studies demonstrating that the nonselective COX inhibitor flunixin prevented recovery of equine jejunum after an ischemic episode. Specifically, equine jejunum exposed to flunixin increased intestinal permeability compared with untreated control tissues. Furthermore, in vitro studies documented increased lipopolysaccharide flux in ischemic-injured equine jejunum from horses treated with flunixin. Thus, despite the known beneficial effects of flunixin, experimental evidence in horses suggests possible deleterious effects of flunixin, such as delayed cellular restitution and enhancement of lipopolysaccharide flux in ischemic-injured equine intestine.

Based on these findings, further studies have evaluated preferential COX-2 inhibitors such as meloxicam, which may circumvent some deleterious effects associated with nonselective COX inhibitors. Meloxicam has postoperative analgesic effects comparable to those of flunixin without impeding the cellular recovery of ischemic-injured equine jejunum. Although further studies are warranted, meloxicam may be a useful alternative to flunixin for postoperative treatment of equine colic. Other selective COX-2 inhibitors (deracoxib, etodolac) did not demonstrate benefits similar to those of meloxicam. Ultimately, before modifications of postoperative colic therapy are applied (e.g., eliminate administration of flunixin after intestinal surgery), clinically demonstrated detrimental effects of flunixin on intestinal permeability must be documented and outweigh the positive effects of the drug, including its ability to ameliorate clinical signs of endotoxic shock and deleterious effects of endotoxin on intestinal motility.

Because many of the aforementioned medications have yielded equivocal results when used individually, combination therapy has also been investigated. Various iterations of rinse solutions that contain substances intended to improve circulation, provide energy, preserve endothelium, and scavenge free radicals have been used to perfuse human donor organs before transplantation. A few of these rinse solutions have been investigated in equine intestinal IR models in an attempt to improve intestinal viability subsequent to IR. Carolina rinse solution contains electrolytes, hydroxyethyl starch (oncotic support), allopurinol and glutathione (antioxidants), deferoxamine (an iron chelator), nicardipine (a calcium channel blocker), adenosine (an enhancer of microcirculation), and fructose and glucose (ATP substrates). The solution has been administered via local jejunal arterial perfusion, intraluminally, or topically to jejunum in a few equine IR models. These studies demonstrated that administering Carolina rinse solution had a protective effect on IR injury of the small intestine because it attenuates capillary permeability, decreases edema formation, and decreases serosal accumulation of neutrophils. However, no studies have been reported regarding the efficacy of the solution for improving survival in experimental or naturally acquired intestinal IR injury in horses.

In one study, a customized solution containing essential electrolytes, energy sources, and free radical scavengers demonstrated positive results in an in vivo model of IR injury of the equine jejunum. The study also documented significantly improved histologic evidence (greater intestinal villous area and length).
Box 1. Clinical Conditions Resulting in Strangulating Obstruction of the Equine Intestine

**Small Intestine**
- Volvulus
- Epiploic foramen entrapment
- Intussusception
- Inguinal hernia
- Umbilical hernia

**Large Intestine**
- Volvulus of ascending colon
- Intussusception (cecum or ascending colon)
- Mesenteric rent

and maintenance of mucosal permeability in horses after luminal administration of customized solution compared with control-group horses. A commercial organ preservation solution (Vasosol, Pike Laboratories, Eagle, PA) containing various antioxidants, cellular fuel sources, oncotic support, and vasodilators has also been investigated in an ex vivo study involving equine large colon. In this study, harvested segments of large colon perfused with a modified organ preservation solution maintained biochemical indices (pH, PaO₂, electrolytes, glucose, lactate) and vascular homeostasis, whereas deterioration in measured parameters was recorded in segments of colon perfused with autologous blood. The authors concluded that the modified organ perfusion solution could maintain the integrity of the large colon during a 12-hour period of isolated pulsatile perfusion, in the absence of blood and oxygen, and may have a future role in clinical cases of intestinal IR in horses.

Although numerous studies investigating potential therapeutics for IR injury have been conducted, the limitations of these studies must be realized. Specifically, many of the studies have been in vitro. Additionally, a standardized time for drug administration (e.g., before IR, during ischemia, before reperfusion) has not been established, which may reperfusion studies. Furthermore, no norelaxant survival studies involving experimental or naturally acquired equine gastrointestinal ischemia or IR have been reported. Finally, clinical studies on spontaneously occurring disease are necessary to evaluate therapeutic efficacy because of possible disparities between experimental and naturally occurring colic.

**Clinical Relevance of Ischemia-Reperfusion Injury**

Box 1 lists potential causes of strangulating obstruction of the small and large intestines. In many of these conditions, nonviable (ischemic) intestine can be resected via laparotomy, thus circumventing IR injury completely. However, in some instances, the viability of intestinal segments cannot be accurately determined clinically and some surgeons may choose to leave these intestinal segments in place rather than perform a resection. Furthermore, some causes of colic do not allow complete removal or resection of ischemic intestine, which therefore must be left in situ. In these cases, IR injury may be clinically relevant and induce further damage. Therefore, equine practitioners should be cognizant that IR may cause injury in these situations and should consider the use of the aforementioned medications to potentially reduce further damage from IR injury. Until further studies evaluate these medications, definitive recommendations cannot be made.

**Conclusion**

This article presents basic information about IR injury and potential therapeutics for clinical cases of intestinal strangulation in horses. Abundant, detailed information on IR injury is available. While evidence suggests that IR injury is a relevant pathophysiologic mechanism in equine colic, other studies do not support the notion of IR injury in horses. This controversy may be reflected in the lengthy list of medications that have been administered experimentally to attenuate the effects of IR. A comprehensive list of additional agents that have been experimentally investigated in rats for attenuating intestinal IR injury in people and horses is available. Large prospective trials involving some of the aforementioned medications alone or in combination may help elucidate IR injury in horses. In addition, investigation of alterations of intestinal absorptive function, bacterial translocation, and injury to distant organs subsequent to intestinal IR injury should be considered in horses.

**References**


27. Granger DN, Parks DA. Role of oxygen radicals in the pathogenesis of intestinal ischemia. Physiologist 1983;26:159-164.


1. In treating IR injury, the mechanism of action of allopurinol is inhibition of
   a. the reaction between XO and hypoxanthine, thus decreasing superoxide production.
   b. myeloperoxidase, thus decreasing HOCl production.
   c. catalase, thus decreasing hydrogen peroxide production.
   d. glutathione, thus decreasing hydroxyl radical production.

2. IR injury to ischemic intestine is thought to be primarily due to oxidative injury by
   a. ROS produced by XO during reintroduction of oxygen.
   b. HOCl production by macrophages that have accumulated in the intestinal interstitium.
   c. ROS produced by overproduction of hydrogen peroxide.
   d. ROS produced by interaction between vascular endothelium and iron.

3. According to the theory of IR, most injury to intestinal mucosa is thought to occur
   a. during reperfusion of ischemic intestine and not during the ischemic period.
   b. before reperfusion is established.
   c. during excessive production of mitochondrial ROS.
   d. after 3 hours of ischemia.

4. Which of the following has not been observed after 120 minutes of reperfusion following an ischemic episode in the equine intestine?
   a. less pronounced neutrophilic infiltration
   b. increased loss of epithelial cells
   c. increased microvascular permeability
   d. mucosal edema

5. Peroxynitrite is a highly reactive radical produced by the reaction between
   a. superoxide radicals and hydrogen peroxide.
   b. nitric oxide and HOCl.
   c. hydrogen peroxide and hydroxyl radicals.
   d. superoxide radicals and nitric oxide.

6. Medications for decreasing IR injury may work by all of the following mechanisms except
   a. increased production of protective ROS and subsequent reduction in epithelial damage.
   b. reduced production of ROS by providing agonists or antagonists to essential enzymes.
   c. scavenging of free radicals and binding of iron.
   d. decreased migration of neutrophils into the intestine during reperfusion.

7. NSAIDs are routinely administered to horses with ischemic intestinal disease; however, recent experimental evidence suggests that
   a. NSAID administration also reduces intestinal damage.
   b. nonselective COX inhibitors may inhibit intestinal healing.
   c. NSAIDs should never be administered to treat IR injury.
   d. NSAIDs do not provide an analgesic effect.

8. In theory, deferoxamine may help treat intestinal IR injury by
   a. inhibiting neutrophil migration into areas of IR.
   b. releasing endogenous antioxidants, such as ascorbic acid, into the blood.
   c. binding iron.
   d. preventing upregulation of inflammatory cytokines.

9. The inflammatory cell that most often contributes to oxidative damage to the intestine due to IR injury is the
   a. neutrophil.
   b. macrophage.
   c. eosinophil.
   d. lymphocyte.

10. Acetylcysteine may have therapeutic value because it can
    a. increase absorption of tocopherol.
    b. inhibit only COX-1.
    c. block neutrophil chemotaxis.
    d. replenish glutathione.