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Ischemia in equine intestine – mechanisms of injury and methods of treatment

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Summary

The importance of reperfusion injury in equine intestine is controversial and unclear. Large colon volvulus and small intestinal strangulation are the most devastating causes of intestinal ischemia in horses and result in severe mucosal damage, barrier dysfunction, endotoxic shock and death. Although essential for tissue recovery, reperfusion after these types of ischemia could exacerbate intestinal injury. Responses to ischemia and reperfusion (I/R) involve a series of synchronized biochemical, cellular and structural changes characterized by generation of reactive oxygen metabolites, activation of immune cells, and epithelial cell degeneration and death. The intensity of neutrophil activation and influx is central to the process of reperfusion injury and the associated inflammatory events. The classic paradigm of reperfusion injury is best displayed after the segmental hypoperfusion or low-flow ischemia model, in which arterial inflow is adjusted to 20% of normal baseline flow, and then allowed to return to normal or greater values during reperfusion. In such cases, injury during the ischemic phase is mild, compared with the reperfusion injury that follows. Although there is some evidence of reperfusion injury in equine colon and jejunum after low-flow ischemia, attempts to ameliorate this by pharmacologic means have met with only moderate success. A multimodal approach, using solutions designed to improve survival in transplanted organs, seems to hold the greatest promise, although these have not been embraced in the clinical setting. Expense and difficulties in delivery are the two most serious limitations. Recent studies to assess the effect of colonic I/R on equine pelvic flexure involved a complete ischemia model that was similar to the clinical lesion, and for periods of 1 to 2 hours of ischemia followed by 30 minutes, 4 hours, or 18 hours of reperfusion. Ischemia caused degeneration and detachment of epithelial cells, early apoptosis, and opening of tight junctions (TJs), all of which decreased transepithelial resistance (TER) and increased mannitol flux when the tissues were studied in vitro. Autophagy was a prominent feature in epithelial cells after 1 hour of ischemia. Reperfusion was characterized by apoptosis, epithelial regeneration, and closure of TJs with improved TER and reduced mannitol leakage. Neutrophils infiltrated colonic mucosa during reperfusion, and macrophages, mast cells and eosinophils were activated during I/R. Equine colonic mucosa subjected to ischemia can repair rapidly and regain barrier integrity during reperfusion, despite intense mucosal inflammation. Recently, attention has focused on inflammation induced by intestinal handling and on remote organ responses to intestinal injury, both of which could be highly relevent to postoperative management of horses that undergo colic surgery. The use of lidocaine in a continuous rate infusion is a popular approach to ameliorate the intestinal inflammatory response after colic surgery, although there is no evidence that it has an antiinflammatory effect in horses. Recent studies on use of nonsteroidal antiinflammatory drugs (NSAIDs) in horses have shown that selective cyclooxygenase inhibitors, such as firocoxib, might be more supportive of repair in small intestinal mucosa after ischemic injury than less selective NSAIDs, such as flunixin meglumine (FM). Similar studies in equine colon demonstrated that FM did not affect healing and barrier recovery in this organ after ischemia, so this NSAID might affect healing in different ways in these two parts of the equine intestinal tract. However, in the jejunum, the clinical relevance of the effect of FM is unknown and its postoperative benefits might well negate any minor effect on transepithlial permeability. Despite many years of investigation, the clinical relevance of I/R in equine colic patients is unknown. Experimental design, types and durations of I/R, differences between intestinal segments and even species differences could explain the failure to repeat the findings in laboratory animal studies. Also, endpoints to measure the response to pharmacologic prevention, problems with timing and dosages of drugs, and the relative importance of different pathways and mediators in response to ischemia could explain our failures.

Keywords: colic / horse / equine / ischemia / intestine / mechanism / injury / treatment / gastroenterology

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Intestinale Ischämie beim Pferd – Pathomechanismen und Behandklungsmethoden

Die Bedeutung von Reperfusions-Schäden am Intestinum des Pferdes ist unklar und wird kontrovers diskutiert. Durch eine Dünndarmstrangulation oder einen Volvulus des Dickdarms wird beim Pferd die am deutlichsten ausgeprägte intestinale Ischämie hervorgerufen und diese kann schwere mukosale Schäden, Dysfunktion der Darmschranke, endotoxischen Schock sowie den Tod des Pferdes verursachen. Obwohl die Reperfusion nach einer Ischämie für die Heilung des Gewebes essentiell ist, kann diese die vorliegenden Schäden noch verschlimmern. Reaktionen nach Ischämie und Reperfusion(I/R) umfassen eine Reihe von synchronisierten biochemischen, zellulären und strukturellen Veränderungen, charakterisiert durch die Synthese von reaktiven Sauerstoff-Metaboliten, Aktivierung von Immunzellen sowie Degeneration und Zelltod von Epithelzellen. Die Ausprägung der Aktivierung der neutrophilen Granulozyten und deren Infiltration sind im Prozess der Reperfusions-Schädigung und den damit verbundenen Entzündungsvorgängen von zentraler Bedeutung. Der klassische Verlauf einer Reperfusions-Schädigung kann am besten durch eine segmentale Hypoperfusion oder an einem Ischämie-Modell mit niedriger Durchflussrate dargestellt werden. Bei dieser wird der arterielle Zufluss auf 20% des normalen Blutflusses reduziert und danach während der Reperfusion eine normale oder gesteigerte Blutzufuhr gewährt. In diesen Fällen sind die Reperfusionsschäden schwerwiegender als diejenigen aufgrund der Ischämie. Obwohl es einige Anzeichen gibt, dass Reperfusionsschäden am Kolon und Jejunum nach einer Ischämie mit niedriger Blutflussrate auftreten, konnten diese durch pharmakologische Mittel nur mit mäßigem Erfolg gemildert werden. Ein multimodaler Ansatz, bei welchem Lösungen aus der Transplantationsmedizin verwendet wurde, erschien am vielversprechendsten, allerdings wurde dieses Verfahren noch nicht im klinischen Bereich eingesetzt. Die Kosten und Verfügbarkeit waren dafür die zwei wichtigsten limitierenden Faktoren. Neuere Studien zu Auswirkungen der I/R an der Beckenflexur simulierten eine komplette Ischämie und zwar über 1-2h gefolgt von 30min., 4h oder 18h der Reperfusion. Die Ischämie verursachte Degeneration und Ablösung der Epithelzellen, frühe Apoptose und Eröffnung der Tight Junctions und in vitro beurteilt einen reduzierten transepithelialen Widerstand (TER) und erhöhen Mannitolfluss. Die Autophagozytose war die auffälligste Veränderung der Epithelzellen nach 1h Ischämie. Die Reperfusion war durch Apoptose, epitheliale Regeneration sowie Schluss der Tight Junctions charakterisiert, welcher mit einem verbesserten TER und einem reduzierten Mannitolfluss einherging. Die Neutrophilen infiltrierten die Kolonschleimhaut

während der Reperfusion und Makrophagen, Mastzellen und Eosinophile wurden während der I/R aktiviert. Die Kolonschleimhaut des Pferdes kann während der Reperfusion Ischämieschäden schnell reparieren und die Integrität der Darmschranke wiederherstellen, trotz einer intensiven Entzündung der Schleimhaut. Neuerdings fiel der Fokus auf Entzündungsgeschehen, die durch Manipulation des Darmes hervorgerufen werden und auf Reaktionen entfernt liegender Organe auf intestinale Schäden. Beides kann für das postoperative Management von Pferden nach einer Kolikoperation von besonderer Bedeutung sein. Obwohl eine antiinflammatorische Wirkung von Lidocain beim Pferd nicht bekannt ist, wird dieses Medikament bei Kolikpatienten verbreitet infundiert, um die intestinale Entzündungsreaktion nach einer Operation zu verringern. Neuere Studien über die Verwendung nicht-steroidaler Antiphlogistika (NSAIDs) zeigten, dass selektive Cyclooxygenase-Hemmer, wie Firocoxib die Reparation von Dünndarm-Mukosa nach einer Ischämie-Läsion eventuell besser unterstützt als nicht-selektive NSAIDs wie zum Beispiel Flunixin Meglumin (FM). Ähnliche Untersuchungen demonstrierten, dass FM die Heilung und Wiederherstellung der Darmschranke nach einer Ischämie am Kolon des Pferdes nicht beeinflusst. So scheint FM die Heilung in Dünn- und Dickdarm auf unterschiedlichen Wegen zu beeinflussen. Wie auch immer, im Jejunum ist die klinische Relevanz der Wirkung von FM weiterhin unklar und der postoperative Nutzen könnte im Vergleich zu der geringgradigen Auswirkung auf die transepitheliale Permeabilität überwiegen. Trotz mehrerer Jahre Forschung ist die klinische Relevanz von I/R beim equinen Kolikpatienten weiterhin nicht klar. Aufbau der Studien, Typ und Dauer der I/R, Unterschiede bei der Wahl des untersuchten Darmsegments und sogar Unterschiede aufgrund der Artenzugehörigkeit könnten erklären, warum die Ergebnisse bei Studien an Labortieren nicht reproduzierbar waren. Die schlechte Wirksamkeit der Medikamente könnte durch Messzeitpunkt der Wirkung der medikamentösen Prävention, durch Probleme bei Dosierung und Wahl des adäquaten Zeitpunktes der Medikamentengabe sowie durch die relative Wichtigkeit der unterschiedlichen Reaktionswege und Mediatoren auf eine Ischämie hin erklärt werden.

Schlüsselwörter: Kolik / Pferd / Ischämie / intestinal / Mechanismus / Schädigung / Behandlung / Gastroenterologie

Introduction

A national survey performed in the USA in 1998 by the The USDA's National Animal Health Monitoring System (NAHMS) found that the national incidence of colic was 4.2 events/100 horses per year, that 1.4% of colic events resulted in surgical intervention, and the case fatality rate for all colic events was 11.0% (NAHMS 1998). Colic was the leading cause of death in horses after old age (NAHMS 1998). Survival of horses undergoing surgery for strangulating obstruction has improved in recent years, most likely the result of many factors, including improved surgery and anesthesia and early referral. However, equine surgeons have a strong interest in pharmacologic methods of modifying pathologic changes in intestine during the postischemic period and thereby prevent potentially fatal postsurgical complications, such as postoperative ileus (POI) and adhesions.

One aspect of postischemic damage to equine small intestine that has garnered much interest and generated some controversy is reperfusion injury. This is the paradoxical exacerbation of tissue damage that occurs when ischemic tissue is reoxygenated, and has been documented in several organs, including the gastrointestinal tract. If reperfusion injury does exist in equine intestine, the most exciting prospect of this finding would be the development of pharmacological methods to control the events directly responsible for the tissue damage it causes. The great attraction that reperfusion injury holds for equine surgeons also rests in the possibility that this mechanism could explain many complications of gastrointestinal tract surgery in horses, such as postoperative ileus (POI), postoperative endotoxemia, and adhesions. Although the tissue consequences of reperfusion injury to the colon and small intestine could be similar, different clinical complications could be expected for each. For example, the predominant changes in the colon would be those associated with endotoxemia and sepsis, whereas POI and adhesions could be the predominant consequences after small intestinal ischemia.

Intestinal Ischemia in Horses – Clinical Disease

Small intestinal strangulation comprises 85% of all small intestinal obstructive diseases in horses, and 68% of these require resection of the strangulated bowel (*Freeman* et al. 2000). In 32% of horses with strangulating lesions of the small intestine, resection might not be deemed necessary or possible, and then ischemic intestine is left in situ in the hope that it can recover fully (*Freeman* et al. 2000). The concern with such cases relates to the ability of affected intestine to heal and regain function without causing severe postoperative complications and death. Large colon volvulus can be diagnosed in up to 26% of horses that require emergency abdominal surgery (*Fisher* and *Meagher* 1985, *Mair* and *Smith* 2005), and is a rapidly progressive, life-threatening form of colic with fatality rates between 30 to 80% (*Snyder* et al. 1989, *Mair* and *Smith* 2005, *Ellis* et al. 2008). Although documented in horses of any age, breed and gender, broodmares are more likely to develop large colon volvulus 1 to 3 months after foaling (*Snyder* et al. 1989, *Embertson* et al. 1996).

Death in horses with large colon volvulus is mostly attributed to hypovolemic and toxic shock as a consequence of the abdominal compartment syndrome and bacterial toxins that rapidly enter the blood across the ischemic-damaged mucosa (*Snyder* et al. 1989). In horses with large colon volvulus, some ischemic intestine can be inaccessible for resection so that ischemic tissue is left in place and must even support anastomotic healing (*Driscoll* et al. 2008, *Ellis* et al. 2008). If the colon is considered viable or resection is not justified because of expense or a perceived perception of low risk for recurrence, then the entire organ is left in place to recover from the ischemic insult. Therefore, a horse that recovers from surgery for large colon volvulus is at some variable risk of reperfusion injury, whether the colon is resected or not.

Pathophysiology of Intestinal I/R

Reperfusion injury is the exacerbation of tissue damage that occurs when ischemic tissue is reoxygenated, and ischemia/reperfusion (I/R) injury has been well documented in mammalian gastrointestinal tract.

Method for Inducing Ischemia

An important prerequisite to understanding the importance of reperfusion injury in any tissue is to examine the nature and duration of the ischemic damage that precedes it. The classic

paradigm of reperfusion injury is best displayed after the segmental hypoperfusion or low-flow ischemia model, in which arterial inflow is adjusted to 20% of normal baseline flow and then allowed to return to normal or greater values during reperfusion (*Parks* et al. 1982, *Moore* et al. 1994, *Henninger* et al. 1992, *Van Hoogmoed* et al. 2000, 2004, *Dabareiner* et al. 1993, 2005). In such an approach, injury incurred during the ischemic phase is mild, compared with the reperfusion injury that follows (*Moore* et al. 1994). The clinical equivalent to the low-flow model could be small intestine subjected to distention and decompression (*Dabareiner* et al. 2001, *Faleiros* et al. 2008) or to endotoxin-induced hypoperfusion (*King* and *Gerring* et al. 1991). Because these segments are left in situ after removal of the strangulated tissue, progressive damage to them could delay return of gastrointestinal function and barrier integrity.

Strangulating obstruction of the intestine has typical features of a hemorrhagic strangulation, that can be attributed to occlusion of thin-walled veins (*Freeman* et al. 1988). Over time, arterial inflow will cease in response to altered Starling's forces in the intestinal wall (*Mortillaro* et al. 1976) and eventually from arterial obstruction between the swollen intestine and the site of strangulation (hernia, band, etc.). Some degree of continuous arterial inflow into the tissue could persist during strangulation, thereby creating low-flow ischemic conditions over a short period of time. However,the venous obstruction model of intestinal strangulation does not seem to display the characteristics of reperfusion injury in horses (*Laws* and *Freeman* 1995) and rats (*Park* et al. 1990). In some cases, small intestinal strangulation causes a combined venous and arterial occlusion, with abrupt loss of oxygen supply and rapid degeneration of the mucosa and its epithelium (*Meschter* et al. 1986). Therefore strangulating lesions can have a mixture of effects on tissue perfusion, not all of which are conducive to causing reperfusion injury. Possibly models with a rapid onset of near-maximal injury during strangulating allow little opportunity to display subsequent progressive damage as tissues are reperfused (*Parks* et al. 1982). Alternatively, the nature of the tissue damage produced by strangulation does not prime the necessary biochemical and pathophysiological processes for reperfusion injury.

In clinical cases of small intestinal strangulation, remaining viable margins display inflammatory changes marked by neutrophil infiltration in the seromuscular layer and mucosa/submucosa (*Gerard* et al. 1999), consistent with changes that characterize reperfusion injury (*Rowe* and *White* 2002). Neutrophilic inflammation in equine jejunal myenteric layers 18 hours after intraoperative manipulation to decompress small intestine coincides with the time point at which POI typically develops (*Little* et al. 2005). This finding suggests that inflammatory pathways rather than solely neurogenic pathways are responsible for POI in the horse (*Little* et al. 2005), and the postoperative consequences of such inflammation could be identical to those produced by reperfusion injury.

Ischemic Injury

Intestinal epithelial cells are highly energy dependent, so that reduced blood supply and decreased oxygenation cause rapid cellular injury and death (*Moore* et al. 1995, *Carden* and *Granger* 2000). Ischemia disrupts aerobic cellular metabolism, which decreases intracellular ATP and pH (*Moore* et al. 1995, *Rowe* and *White* 2002, *McMichael* and *Moore* 2004), inactivates transmembrane ion pumps, causes an intracellular accumulation of Ca^{2+}, Na^{+}, and lactate, and disrupts intracellular organelles, cytoskeletal elements and tight junctions (*Moore* et al. 1995, *Carden* and *Granger* 2000, *Li* et al. 2009, *Ivanov* et al. 2010). The biochemical steps responsible for I/R are initiated by oxidation of hypoxanthine to xanthine (X) by xanthine oxidase (XO), both of which are generated during the ischemic period.

Reperfusion Injury

Reactive oxygen species (ROS), or metabolites (ROM), and reactive nitrogen species (RNS), such as superoxide anions, hydroxyl radicals, hypochlorous acid (HOCl), and nitric oxide-derived peroxynitrite are generated rapidly in the presence of oxygen (Fig. 1) delivered during reperfusion (*Carden* and *Granger* 2000, *Collard* and *Gelman* 2001, *McMichael* and *Moore* 2004). Although the superoxide radical is not regarded as highly cytotoxic, it can generate secondary ROMs that are more toxic. Hydrogen peroxide produced by reduction of oxygen or from dismutation of the superoxide radical is very lipophilic and can readily cross cell membranes. Both superoxide radical and hydrogen peroxide react with intracellular transition metals, such as iron or copper, through the Haber-Weiss or the superoxide-driven Fenton reaction, to produce the highly reactive hydroxyl radical. The superoxide radical can also react with nitric oxide or HOCl to yield the hydroxyl radical. The hydroxyl radical is very short-lived but is extremely reactive with virtually all-known biomolecules in the site in which it forms, and probably is largely responsible for the effects of hydrogen peroxide on DNA. It causes structural damage and generation of phospholipid-derived mediators in cell membranes through lipid peroxidation.

Oxidants promote chemotaxis, activate leukocytes, and initiate cytokine gene expression through interactions with extracellular fluid, cell membrane lipids, and polyunsaturated fatty acids (*Granger* and *Parks* 1983, *Granger* and *Korthuis* 1995, *Dröge* 2002). They can also stimulate expression of cellular adhesion molecules, a crucial step in I/R that allows leukocytes to enter the injured tissue rapidly. Peroxynitrite, an active member of RNS can damage tissue by lipid peroxidation, oxidation of protein sulphydryl groups, and nitration of aromatic amino acids (*Radi* et al. 1991, *Beckman* and *Koppenol* 1996). Peroxynitrite formation has been demonstrated in activated leukocytes (*Ischiropoulos* et al. 1992, *Gagnon* et al. 1998, *Takemoto* et al. 2007) and endothelial cells (*Kooy* and *Royall* 1994), and it may be an important mediator of cytokine-induced epithelial hyperpermeability (*Chavez* et al. 1999).

Endogenous Antioxidants

Intracellular enzymatic antioxidants are the first line of cellular defense against ROMs and examples are superoxide dismutase (SOD), catalase, and glutathione peroxidase (*Grisham* et al. 1990). These are at considerably lower levels in

the human colon than in the human liver, which puts the colon at risk of reperfusion injury (*Grisham* et al. 1990). Ascorbate, uric acid, methionine, glucose, bilirubin, α-tocopherol, β-carotene, albumin, and ceruloplasmin are nonenzymatic antioxidants of extracellular fluid (*Grisham* et al. 1990). A free radical scavenger will donate an electron to a free radical, thereby becoming a radical species of lower reactivity than the harmful radical it scavenges (*Grisham* et al. 1990). In a tissue that undergoes reperfusion injury, presumably these protective mechanisms are overwhelmed.

injury that occurs upon reperfusion is thought to result from neutrophilic radicals and proteolytic enzymes (*Blikslager* et al. 1997, *Gayle* et al. 2000, *Souza* et al. 2004). Neutrophils residing in the interstitium might be as important as recruited neutrophils in generation of reperfusion injury, based on a study in cat small intestine (*Kubes* et al. 1992). Circulating neutrophils are recruited to sites of ischemic injury, roll along the endothelium, and become adhered to endothelium in postcapillary venules before they migrate across it to the inflamed tissue (*Witko-Sarsat* et al. 2000, *Luster* et al. 2005, *Chin*

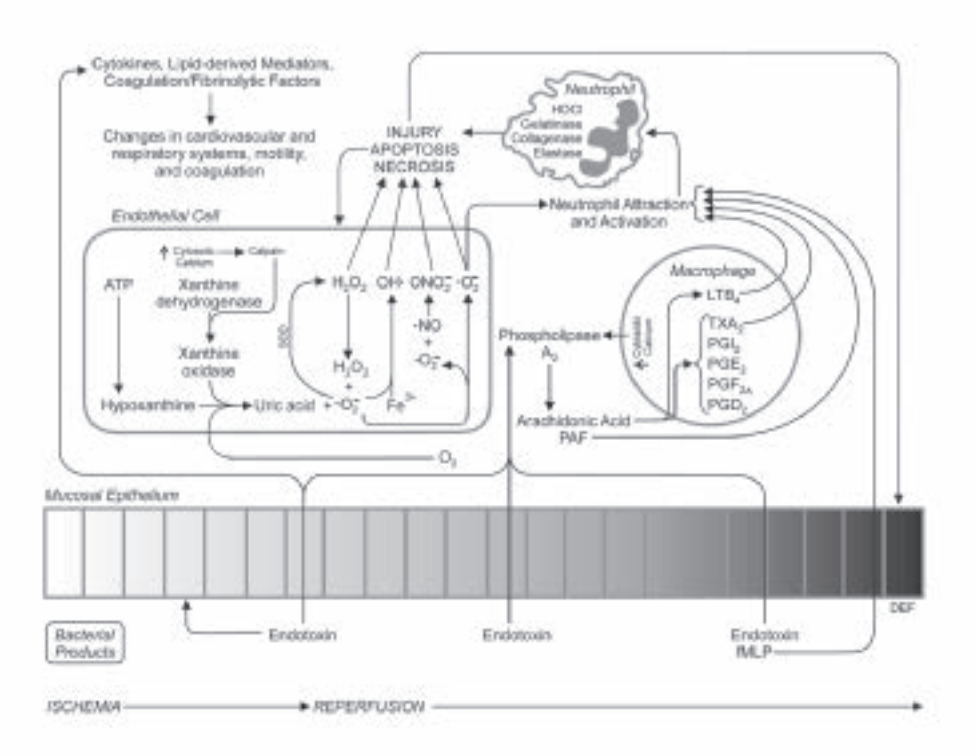

Fig. 1 Cascade of pathophysiological processes that characterize reperfusion injury in mammalian intestine, as explained in the text. In the epithelium, the cell injury in the mucosa gets progressively worse from left to right as indicated by darker shading.

Cyclooxygenase

Prostaglandins (PGs), synthesized by COX enzymes in many cell types in the intestinal lamina propria (*Krause* and *DuBois* 2000, *Morton* et al. 2009, *Hilton* et al. 2011) play a key role in regulating inflammatory reactions (*Krause* and *DuBois* 2000). Phospholipase A2, an enzyme in the cell membrane, is activated during ischemia by increased cytosolic calcium, and causes biologically important cell membrane phospholipids, such as platelet-activating factor (PAF) and arachidonic acid, to be released. Arachidonic acid metabolites are leukotrienes produced by the lipoxygenase pathway and prostaglandins produced through cyclooxygenase. Prostacyclin (PGI_2) is a vasodilator and it also inhibits platelet aggregation, whereas thromboxane A_2 is a potent vasoconstrictor, promotes platelet aggregation, and enhances neutrophil adherence and chemotaxis. Both leukotriene (LTB_4) and thromboxane A_2 are considered as mediators of neutrophil recruitment and microvascular dysfunction (*Krause* and *DuBois* 2000, *Morton* et al. 2009, *Hilton* et al. 2011).

Neutrophils

Intense leukocyte recruitment and activation is a crucial and early step in the I/R cascade (Fig. 2), and much of the tissue

and *Parkos* 2007). Firm adhesion is facilitated by neutrophil integrins and endothelial ICAM-1 (*Chamoun* et al. 2000, *Witko-Sarsat* et al. 2000). Migration of neutrophils into the mucosal lamina propria during reperfusion assists in recognizing and ingesting cell detritus and destroy invading bacteria (*Chamoun* et al. 2000, *Witko-Sarsat* et al. 2000, *Nathan* 2006, *Dale* et al. 2008).

Neutrophils contribute to the development of endothelial injury (*Granger* et al. 1986, *Hernandez* et al. 1987, *Kurose* et al. 1994, *Cooper* et al. 2004) and mucosal damage during intestinal I/R through an arsenal of pro-inflammatory factors, enzymes, antibacterial proteins and other toxic molecules (*Granger* 1988, *Grisham* and *Granger* 1988, *Grisham* et al. 1990, *Schoenberg* et al. 1991, *Kubes* et al. 1992, *Friedman* et al. 1998, *Gayle* et al. 2002, *Chin* and *Parkos* 2007). They secrete nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and myeloperoxidase (MPO), two enzymes that could contribute to formation of ROS during reperfusion (*Bhaskar* et al. 1995, *Granger* and *Korthuis* 1995). Neutrophils produce little hydroxyl radical but are an important source of HOCl that is produced from MPO and hydrogen peroxide and then oxidation of chloride (*Granger* 1988, *Grisham* and *Granger* 1988, *Grisham* et al. 1990). Neutrophils can secrete the proteases, elastase, collagenase, and gelatinase, into the extracellular fluid, where they can degrade the basement membrane and interstitial matrix of the endothelial cell.

Most of the damage mediated by neutrophils is called collateral or bystander damage, because it involves incidental contact of toxic molecules with surrounding cells (*Grisham* and *Granger* 1988, *Witko-Sarsat* et al. 2000) or physical damage while migrating to the site of injury (*Milks* et al. 1986, *Moore* et al. 1995, *Gayle* et al. 2000, *Nathan* 2006, *Chin* and *Parkos* 2007). Neutrophils increase intestinal permeability as they traverse intercellular spaces in restituted epithelium and this effect can be prevented by blocking neutrophil adhesion or scavenging superoxide (*Gayle* et al. 2002).

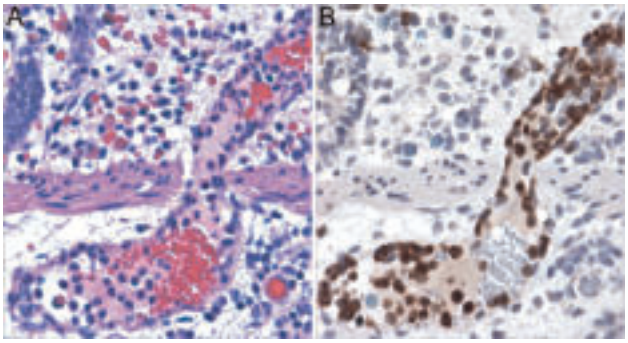

Fig. 2 Infiltration of neutrophils into the equine colonic mucosa after ischemia and reperfusion: A – H&E. B – Immunohistochemistry with anti-calprotectin antibody to show neutrophils as brown-stained cells (x400).

One of the antibacterial proteins produced by neutrophils is calprotectin (Fig. 2), an endogenous pro-inflammatory molecule of the innate immune system (*Johne* et al. 1997, *Yui* et al. 2003, *Foell* et al. 2007). Calprotectin is found to a lesser extent in monocytes and macrophages (*Johne* et al. 1997). Although the exact biological role of calprotectin is unknown, available evidence suggests it can modulate inflammatory reactions (*Johne* et al. 1997). If present in large amounts for a long period, calprotectin can cause local tissue destruction (*Yui* et al. 2003). Because of its strong association with neutrophil movement and location (Fig. 2), calprotectin can be used as a marker of acute and chronic inflammatory conditions such as rheumatoid arthritis, inflammatory bowel disease and intestinal I/R (*Foell* et al. 2004, *Striz* and *Trebichavsky* 2004).

Role of Other Immune Cells

In addition to neutrophils, other resident immune cells are well established in intestinal mucosa, such as macrophages, eosinophils, mast cells, and lymphocytes, could also contribute to the inflammatory response during I/R (*Galli* et al. 2008, *Schenk* and *Mueller* 2008, *Shea-Donohue* et al. 2010). Resident macrophages and attracted monocytes, strategically positioned in the subepithelial region, immediately recognize microorganisms and damage-associated signals, and tightly regulate an innate immune response (*Kono* and *Rock* 2008, *Laskin* et al. 2011, *Smith* et al. 2011). Eosinophils are pro-inflammatory leukocytes that reside in large quantities in the gastrointestinal lamina propria and can generate destructive radicals, lipid mediators, proteases and toxic granule proteins (*Walsh* 1997, *Hogan* et al. 2008) and produce the respiratory burst that generates ROS and peroxynitrite (*Van Dalen* et al. 2006, *Takemoto* et al. 2007). Eosinophilic granulocytes are frequently seen in close proximity to mucosal mast cells (*Meschter* et al. 1986, *Armetti* et al. 1999, *Munitz* and *Levi-Schaffer* 2004), and degranulation of mast cells is thought to be triggered by eosinophilic toxic proteins (*Piliponsky* et al. 1999). Mast cells are potent effector and immunomodulatory cells that can promote and increase inflammation, leukocyte recruitment, tissue remodeling, and tissue injury (*Kanwar* and *Kubes* 1994, *Penissi* et al. 2003, *Galli* et al. 2008, *Santen* et al. 2008, *Shea-Donohue* et al. 2010). They are thought to be important mediators of I/R-induced mucosal and microvascular dysfunction in the mouse intestine (*Kanwar* et al. 1998) and can be detrimental to intestinal barrier integrity (*Szabo* et al. 1999, *Penissi* et al. 2003, *Marshall* 2004).

Capillary Changes during Intestinal I/R

Decreased perfusion in submucosal postcapillary venules, referred to as "no-reflow" and "reflow-paradox" (*Beuk* et al. 2000), is a major factor in intestinal I/R injury (*Lefer* and *Lefer* 1993, *Seal* and *Gewertz* 2005, *Vollmar* and *Menger* 2011). The "no-flow" phenomenon is caused by intravascular hemoconcentration and platelet aggregation, thrombosis, leukocyte plugging, endothelial cell swelling, vasomotor dysfunction, fluid and protein movement into the interstitium, and capillary occlusion from edema-associated intestinal pressure (*Moore* et al. 1995, *Menger* et al. 1997, *Dabareiner* et al. 2005, *Seal* and *Gewertz* 2005, *Vollmar* and *Menger* 2011). In addition, reduced red blood cell velocity, red blood cell sludging (*Vollmar* and *Menger* 2011), and platelet adhesion to the capillary endothelium can also disrupt intestinal microvascular perfusion (*Massberg* et al. 1998, *Cooper* et al. 2003, *Vollmar* and *Menger* 2011).

Morphology, Ultrastructure, Cell Death, and Mucosal Repair After Intestinal I/R

The first histological evidence of epithelial damage begins within 20 minutes of total small intestinal ischemia, and is characterized by lifting of small clusters of epithelial cells, their detachment from the basement membrane, and subsequently death by apoptosis and necrosis (*Snyder* et al. 1988, *Meschter* et al. 1991, *Moore* et al. 1995, *Kong* et al. 1998). Diverse inflammatory stimuli can increase leakiness of the epithelial barrier integrity by disassembly of apical junctions (*Ivanov* et al. 2010).

Cell death by necrosis or apoptosis is triggered by mediators released during I/R (*Cummings* et al. 1997, *Ramachandran* et al. 2000, *Festjens* et al. 2006, *Zong* and *Thompson* 2006, *Rock* and *Kono* 2008). Both types of cell death can occur simultaneously in tissues exposed to the same stimulus, but the intensity of the stimulus determines which predominates (*Leist* et al. 1997). Unlike necrosis, apoptotic cell death is a controlled, programmed, energy-dependent event that regulates physiological cell turnover, without stimulation of the immune system (*Hall* et al. 1994, *Kono* and *Rock* 2008). It is associated with normal cell turnover in the gastrointestinal tract (*Hall* et al. 1994). The defining characteristic of apoptosis is shrinkage, chromatin condensation and margination, membrane blebbing, and segmentation and division into apoptotic bodies that can be phagocytized within several

hours (*Taylor* et al. 2008, *Kroemer* et al. 2009). Apoptotic cell loss might be a mechanism to control tissue damage, maintain a defensive barrier, regulate inflammation and hasten epithelial repair (*Ramachandran* et al. 2000, *Maderna* and *Godson* 2003, *Serhan* et al. 2008, *Maniati* et al. 2008). It typically affects single cells and does not induce an inflammatory response. Pronounced apoptosis can contribute to leaks in the epithelial barrier (*Shah* et al. 1997, *Abreu* et al. 2000, *Gitter* et al. 2000, *Schulzke* et al. 2006).

Resolution of Inflammation

Inflammation is essential for wound repair, and neutrophils have a key role in promoting tissue repair after I/R, (*Nathan* 2006, *Blikslager* et al. 2007, *Mantovani* et al. 2011). They can generate pro-reparative signals (PGE2, IL-1β) and anti-inflammatory mediators (lipoxins or adenosine), they inhibit their own diapedesis and recruitment, activate their own death by apoptosis, reduce generation of pro-inflammatory mediators and toxic radicals, control infection (*Serhan* and *Savill* 2005, *Martin* and *Wallace* 2006, *Nathan* 2006, *Blikslager* et al. 2007, *Dale* et al. 2008, *Serhan* et al. 2008). Neutrophils have been shown to augment recovery of ischemic injured porcine intestine by an IL-1β- and COX-2-dependent mechanism (*Shifflett* et al. 2004). Apoptosis of neutrophils and neutrophil clearance by inflammatory macrophages is a crucial step in reducing inflammation (*Haslett* et al. 1994, *Savill* et al. 2002, *Maderna* and *Godson* 2003, *Serhan* et al. 2008, *Martin* and *Wallace* 2006, *Eming* et al. 2007).

Epithelial Repair

In general, the healing process and restoration of the epithelial integrity and function can be classified in three phases (*Playford* and *Ghosh* 2005). The initial rapid response involves migration of surviving cells from the wound edge to cover the denuded area, and closure of leaky epithelial intercellular spaces and TJ (*Florian* et al. 2002). This process, termed restitution, is locally regulated by mediators arising from nerves, effector immune cells, fibroblasts, endothelial cells and subepithelial extracellular matrix, and begins within minutes after injury (*Florian* et al. 2002, *Playford* and *Ghosh* 2005, *Blikslager* et al. 2007). During restitution, cells bordering the injured mucosal zone flatten, aquire a squamoid appearance, and extend membrane pseudopodia before they migrate across denuded basement membrane (*McNeil* and *Ito* 1989, *Florian* et al. 2002). After cell-to-cell contacts are reestablished, junctional complexes between epithelial cells reform (*Blikslager* et al. 2007). Neutrophils are a rich source of PGE_2, which can facilitate mucosal repair in the intestine (*Blikslager* et al. 1997, *Blikslager* et al. 1999, *Martin* and *Wallace* 2006) through chloride secretion via ClC-2 chloride channels (*Moeser* et al. 2004).

In the small intestine, contraction of damaged villi through activation of lamina propria smooth muscle cells and myofibroblasts reduces the surface area of denuded basement membrane to facilitate resealing by migrating epithelial cells (*Florian* et al. 2002, *Blikslager* et al. 2007). Substantial damage of the jejunal epithelium after 30 minutes of ischemia in humans was completely resealed and fully recovered within 60 minutes of reperfusion (*Derikx* et al. 2008). In ischemic-injured porcine ileum, the repair process seemed to follow the preceding timeline initially, but full recovery of mucosal barrier function at 6 hours was followed by a significant increase in permeability at 18 hours, attributed to neutrophils traversing restituted epithelium (*Gayle* et al. 2002). Proliferation of crypt stem cells and their differentiation into mature epithelial cells follow 1 to 2 days after the damage has occurred. The third stage of tissue repair involves remodeling of the mucosa to its normal morphology, structure and function (*Mammen* and *Matthews* 2003, *Playford* and *Ghosh* 2005, *Blikslager* et al. 2007).

Colon versus Small Intestine

Reperfusion injury may be of less importance in the colon than in the small intestine, possibly because the colonic mucosa has relatively little xanthine oxidase (*Krenitsky* et al. 1974). The colon is a rich source of aldehyde oxidase, another enzyme that can generate free radicals (*Krenitsky* et al. 1974) and subepithelial inflammatory cells can produce reactive oxygen metabolites through an NADPH oxidase system (*McCord* 1987). In rabbits, polymorphonuclear leukocytes endogenous to the colon or attracted there by inflammation can interfere with healing of colonic anastomoses through oxygen radical production (*Shandall* et al. 1986).

Intestinal ischemia induced for 3 hours in dogs caused more changes in electrophysiological properties and permeability of the colon compared to the small intestine, but histopathological mucosal damage was similar in both (*Takeyoshi* et al. 1996). However, small intestinal epithelial cells at the tips of the villi are considered to be particularly sensitive to ischemia because they are located at the end of the central arteriole, where arterial oxygen concentration is normally low (*Takeyoshi* et al. 1996, *Kong* et al. 1998, *Vollmar* and *Menger* 2011). Reversible occlusion of the superior mesenteric artery for 30 minutes in rats reduced mucosal perfusion in the colon more than in the small intestine, but mucosal damage was significantly less in the colon (*Leung* et al. 1992). Infiltration of neutrophils was evident in both segments after 1h reperfusion, although mucosal injury was only exacerbated in the small intestine after I/R. *Hinnebusch* et al. (2002) assumed that the sensitivity of small intestinal epithelial cells to ischemia could also be associated with their differentiation state. Probably because villus cells are normally programmed for apoptosis, they might be primed for an apoptotic response to stressful stimuli (*Hinnebusch* et al. 2002).

Intestinal I/R in Horses

Strangulation obstruction in jejunum of anesthetized ponies for periods of 2 and 3 hours caused necrosis and loss of cells on villi on light and scanning electron microscopy (*Freeman* et al. 1988). Twelve hours after reperfusion started 25% segments were unchanged, 25% had deteriorated, and 50% had stunted villus remnants partly or completely lined with regenerating epithelium (*Freeman* et al. 1988). The last group provided the first evidence of restitution in equine jejunum after complete ischemia of sufficient severity to destroy villus lining (*Freeman* et al. 1988). In the colon, tissue injury usually begins in the

superficial part of the mucosa within 20 minutes of total ischemia, and within 60 minutes if partial ischemia is severe enough to cause damage (*Moore* et al. 1995). Lifting of epithelial cells due to fluid sequestration in the subepithelial space and loosening from the basement membrane are characteristic features at the beginning of mucosal injury in the colon (*Snyder* et al. 1988, *Moore* et al. 1995, Kong et al. 1998, Haglund and Bergqvist 1999). After 4 hours of low-flow ischemia, the colonic epithelium is completely denuded (*Snyder* et al. 1988, *Moore* et al. 1995). Complete necrosis of the mucosal epithelium to the base of the crypts occurs by 4 to 6 hours (*Snyder* et al. 1988, *Moore* et al. 1995).

Reperfusion

Equine small and large intestines behave differently in response to reperfusion (*Kooreman* et al. 1998, *Dabareiner* et al. 2001, *Rowe* and *White* 2002). There is some evidence that reperfusion injury can occur in the equine small intestine (*Dabareiner* et al. 1995/2001, *Van Hoogmoed* et al. 2001), with low-flow ischemic models providing the most compelling evidence of postischemic damage (*Van Hoogmoed* et al. 2001, *Rowe* and *White* 2002). The enzyme, xanthine oxidase (XO), is present in the equine small intestine, and is significantly increased after ischemic strangulation of the jejunum, which appears to follow the classic pathophysiology of I/R injury characterized by activation of the xanthine-oxidoreductase system during ischemia (*Prichard* et al. 1991, *Moore* et al. 1995, *Granger* 1988).

As in other species, the classic model of I/R injury dependendent on XO is not likely to contribute to postischemic damage in the large colon (*Kooreman* et al. 1998), because the enzyme is not present or in very low concentrations in the equine colonic mucosa (*Wilkins* et al. 1994, *Moore* et al. 1995a, *Blikslager* et al. 1997a, *Kooreman* et al. 1998). Thus, alternative oxidants, mitochondrial dysfunction, failure of mucosal postcapillary microcirculation, collateral tissue damage by infiltrating neutrophils, uncontrolled activation of an innate immune response, or simply a time-related continuation of injury initiated during ischemia, might be responsible for colonic mucosal damage after reperfusion (*Moore* et al. 1994, *Wilkins* et al. 1994, *Granger* and *Korthuis* 1995, *Blikslager* et al. 1997, *McAnulty* et al. 1997, *Rowe* and *White* 2002).

Reperfusion injury was demonstrated in the large colon after 3 hours of low-flow ischemia followed by 3 hours of reperfusion compared with changes seen after a comparable period of ischemia that was continued for the same period chosen for reperfusion (*Moore* et al. 1994). The estimated percentage depth of mucosal loss and cellular debris index were significantly greater during reperfusion, compared with a continued ischemia in another group of horses. There were trends toward greater percentage of surface mucosal disruption and mucosal edema during the early phase of reperfusion (3 to 4 hours) and greater mucosal hemorrhage, measured percentage depth of mucosal loss, and mucosal interstitium-to-crypt ratio during the late phase (4 to 6 hours) of reperfusion. The mucosal injury was attended by an influx of neutrophils (*Moore* et al. 1994). However, in this model, reperfusion did not affect production of prostaglandins (PGs) and cytokines (*Moore* et al. 1995) and response to antioxidants was not suggestive of reperfusion injury (*Moore* et al. 1995). Also, early evidence of repair by restitution was evident in this reperfusion model (*Moore* et al. 1994). Although this study focused on the neutrophil as the major inflammatory cell, eosinophils and other cells seemed to be involved to an undetermined extent (*Moore* et al. 1994).

In another study, mucosal lesions progressively worsened after twisting the colon for 2 or 3 hours followed by a 2-hour-reperfusion time period (*Meschter* et al. 1991, *Darien* et al. 1993, 1995), although these studies did not compare reperfusion with a comparable period of continued ischemia. Several studies on equine large colonic I/R failed to detect reperfusion injury (*Snyder* et al. 1988, *Reeves* et al. 1990, *Wilkins* et al. 1994, *Dabareiner* et al. 2001, *Matyjaszek* et al. 2009, *Morton* et al. 2009, *Grosche* et al. 2011).

Prevention of Equine Intestinal I/R

Drugs that have been used experimentally to inhibit reperfusion injury include allopurinol, mannitol, superoxide dismutase (SOD), manganese, folic acid, and 21-aminosteroids (*Freeman* 1992, *Vatistas* et al. 1993, *Moore* et al. 1995d, Vatistas et al. 1996). Pretreatment with free radical scavengers can reduce neutrophil infiltration into the mucosal interstitium during reperfusion (*Grisham* et al. 1990, *Otamari* and *Tagesson* 1989), presumably by interfering with production of chemoattractants by xanthine oxidase-derived oxidants (*Granger* et al. 1989).

In an in vitro model of anoxia/reoxygenation injury in equine jejunum, superoxide dismutase prevented epithelial damage, cell swelling, disturbances in cellular water and ion homeostasis, diminished amino acid absorption, and diminished protein synthesis induced by reoxygenation alone (*Johnston* et al. 1991). These results suggest that superoxide radicals can be generated in equine jejunal mucosa and can cause damage to that tissue in the absence of blood flow and neutrophil influx (*Johnston* et al. 1991). However, in an equine model of venous strangulation obstruction in jejunum and colon, human superoxide dismutase did not protect against reperfusion injury (*Freeman* 1992), although the model itself does not seem suitable for these purposes (*Laws* and *Freeman* 1995).

Dimethylsulfoxide (DMSO [a hydroxyl radical scavenger]) at 20mg/kg, IV, q12-24 hr, in 5L of 0.9% NaCl or lactated Ringer's solution has been used clinically to reduce injury from ischemia and reperfusion (*Rowe* and *White* 2002). DMSO as a pretreatment scavenges the hydroxyl radical, improves microvascular integrity and reduces neutrophil adherence associated with I/R in the intestine of cats and rats (*Parks* et al. 1984, *Sekizuka* et al. 1989, *Zimmerman* et al. 1990). At 1g/kg before ischemia or after release of a vascular occlusion, no benefit was evident in the equine jejunal mucosa (*Arden* et al. 1990, *Horne* et al. 1994). The severity of the equine large colon mucosal lesions were not changed when the same dose was given in the same manner before ischemia or 30 minutes before reperfusion (*Reeves* et al. 1990, *Moore* et al. 1995). *Moore* et al. (1995) actually found a tendency toward a greater mucosal injury in horses that received DMSO compared with those given a control solution. When DMSO reacts with the hydroxyl radical, methyl radicals and methylperoxy radicals

can be generated, which are less potent than the hydroxyl radical but can still react with membranes (*Raleigh* and *Kremers* 1981). High concentrations of DMSO can cause lipid peroxidation (*Raleigh* and *Kremers* 1981).

Dabareiner et al. (2005) used a lower dose of DMSO, 20 mg/kg IV before reperfusion, and demonstrated that it was partially effective in attenuating the I/R-induced permeability changes and edema formation in the equine jejunum. This lower dose also prevents adhesion formation in foal intestine 10 days after total ischemia for 60 minutes (*Rowe* and *White* 2002). The clinical benefits of DMSO in horses after colic surgery is unknown.

The 21-aminosteroids have shown some promise in equine intestine in vivo (*Vatistas* et al. 1993, *Vatistas* et al. 1996). In an I/R model in horses, the 21-aminosteroids did not affect inflammatory cell migration and activity and did not preserve mucosal surface area in the jejunum (*Vatistas* et al. 1996), but did in the large colon (*Vatistas* et al. 1993). In equine colonic mucosa subjected to low-flow ischemia for 3 hours, allopurinol (25mg/kg), manganese chloride (10mg/kg), and the 21-aminosteroid U-74389G (10mg/kg) did not protect against reperfusion injury (*Moore* et al. 1995).

The biochemical cascade of I/R generates RNS (Fig. 1), such as peroxynitrite, nitrate, NO, nitrogen dioxide or nitryl chloride (*Wallace* and *Ma* 2001). NO is an endogenous vasodilator and an inhibitory neurotransmitter to circular smooth muscle in equine jejunum and could contribute to POI after reperfusion injury (*Rakestraw* et al. 1996). NO can also mediate a component of the inhibitory transmission to circular muscle and taenia of the equine large colon (*Van Hoogmoed* et al. 2000). In this study, the nitric oxide synthase inhibitor NG-nitro-L-arginine methyl ester (L-NAME) had potential benefit in maintaining muscular activity, suggesting a potential role in treating POI in horses (*Van Hoogmoed* et al. 2000).

In the equine jejunum subjected to ischemia and reperfusion, Carolina Rinse solution (0.1 to 0.5 ml/kg/min) infused through the mesenteric artery to the affected segment maintained vascular permeability close to normal during the reperfusion period (*Dabareiner* et al. 2005). This solution, used originally to improve survival of organ transplants, contains allopurinol and glutathione as antioxidants, adenosine for vasodilation, a calcium channel blocker, and hydroxyethyl starch to provide oncotic support in a solution with pH of 6.5. Because these solutions involve administration via mesenteric arteries and they are expensive, their use in colic surgery would be limited in most cases. However, these solutions can ameliorate injury when instilled intraluminally, which could provide a clinical application for treating I/R injury (*Young* et al. 2002). Intraluminal administration of a customized rinse solution in the jejunum improved the histologic findings and mucosal translocation of albumin in horses with mild ischemic injury, compared with lactated Ringer's solution (*Van Hoogmoed* et al. 2002, 2004), similar to results with an IV delivery of the same rinse (*Van Hoogmoed* et al. 2001).

In the equine colon subjected to ischemia (torsion) and reperfusion, heparin at 80 IU/kg, IV, 30 minutes before reperfusion, increased blood flow, increased peripheral vascular resistance, and attenuated systemic hypotension during reperfusion (*Provost* et al. 1991). Heparin did not alter the mucosal damage, although it was considered beneficial and the enhanced blood flow did not induce a reperfusion injury (*Provost* et al. 1991). In a similar model, systemic administration of a specific PAF antagonist (WEB 2086) to ponies before induction of large-colon torsion did not change the severity of mucosal injury and inflammation in the colon compared with a saline-treated group (*Wilson* et al. 1994). This is consistent with findings in a low-flow model of ischemia in equine colon, in which no protective effects of PAF antagonist L-691,880 were observed on injury induced by I/R (*Moore* et al. 1998).

In Vitro Studies in Equine Intestine

In equine colonic mucosa in vitro, the reactive oxygen metabolites, superoxide anion, hydrogen peroxide, low concentrations of HOCl, or the hydroxyl radical, caused scattered apoptosis in surface epithelial cells and stimulated secretion, but did not cause the epithelial damage anticipated if these agents were responsible for reperfusion injury in this tissue (*Inoue* et al. 2007). However, the in vivo injury could be mediated by vascular damage and by an influx of neutrophils. The secretory response was probably PG-mediated based on the ability of flunixin meglumine (FM) to block it (*Inoue* et al. 2007). The colonic epithelium was severely damaged in vitro by concentrations of HOCl that could be achieved by immediate exposure to an inflammatory burst from invading neutrophils (*Inoue* et al. 1998). Of possible relevance to prevention of reperfusion injury in vivo was the finding that this injury could be ameliorated by coincubation with ascorbic acid (*Inoue* et al. 1998). A brief period of anoxia followed by reoxygenation of jejunal mucosa in Ussing chambers reduced the accumulation of radiolabeled L-alanine and caused cell swelling, as indicated by an increase in tissue water and tissue sodium content (*Johnston* et al. 1991).

Recent Research on I/R in Equine Colon

In our laboratory at the University of Florida, we studied I/R in the equine pelvic flexure with horses anesthetized and then euthanatized after all tissue samples are collected (*Graham* et al. 2011). Colonic mucosa was harvested after various periods of ischemia and reperfusion and examined by histological methods and for in vitro permeability changes in Ussing chambers (*Matjyaszek* et al. 2009, *Graham* et al. 2011). One group of horses was allowed to recover from anesthesia when blood flow was restored to ischemic colon, and and these horses were reanesthetized at 18 hours after reperfusion started. Then colonic mucosa was examined by histological methods and for in vitro permeability changes in Ussing chambers (*Matjyaszek* et al. 2009). This group of horses was closely monitored after the first surgery and postoperative pain was controlled with flunixin meglumine (FM) or butorphanol (*Matjyaszek* et al. 2009). They were euthanatized at the end of the second anesthetic period (*Matjyaszek* et al. 2009).

Morphological Changes

Structural changes in the colonic mucosa after I/R in these studies (Fig. 3) were similar to those recorded in other models of

combined arterial and venous occlusion and mural compression (*Reeves* et al. 1990). At 1 hour ischemia, minor histological alterations of the colonic mucosa were evident, with further progression after 2 hour ischemia (*Morton* et al. 2009, *Graham* et al. 2011, *Grosche* et al. 2011, 2012). Moderate hemorrhage into the lamina propria progressed over time during ischemia accompanied by profound interstitial and epithelial cell edema and increased interstitium-crypt ratio. With 2 hours of ischemia, further damage to the surface epithelium occurs as demonstrated by loss of 45% of the surface epithelium (*Morton* et al. 2009). Compared with control findings, the interstitium-crypt ratio and the mucosal hemorrhage score increased gradually and significantly as the period of ischemia increased (*Grosche* et al. 2012). There was no further significant change in either variable after reperfusion.

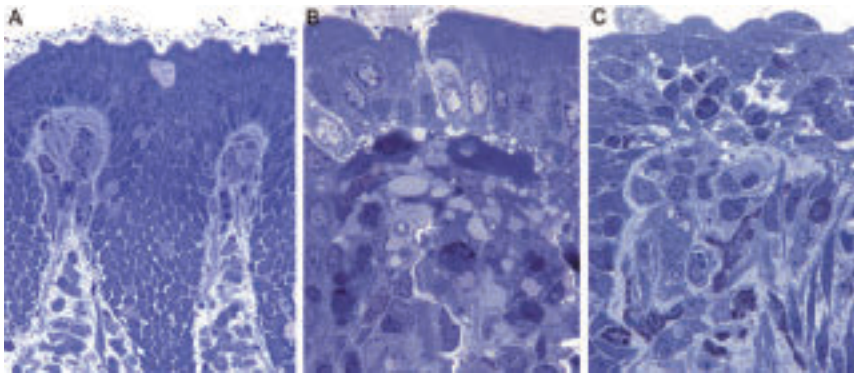

Fig. 3 Structural changes of the equine colonic epithelium and subepithelium after ischemia and reperfusion: A – control, B - 1 hour of ischemia, C – 1 hour of ischemia followed by 4 hours of reperfusion (toluidine blue, x400). Ischemia was characterized by cell edema, microvilli disintegration, apoptosis and single cell necrosis, subepithelial fluid accumulation and detachment of epithelial cells from the vacuolated basement membrane (B). No further exacerbation of mucosal damage was evident after reperfusion. Epithelial defects were covered by a continuos layer of shortened epithelial cells that appeared tightly adhered to each other resulting in formation of enlarged paracellular spaces and subepithelial clefts. Numerous lymphocytes, neutrophils and eosinophils infiltrated the subepithelial clefts and migrated through the paracellular space towards the intestinal lumen. Large macrophages containing phagocytic vacuoles were located in the subepithelium (C).

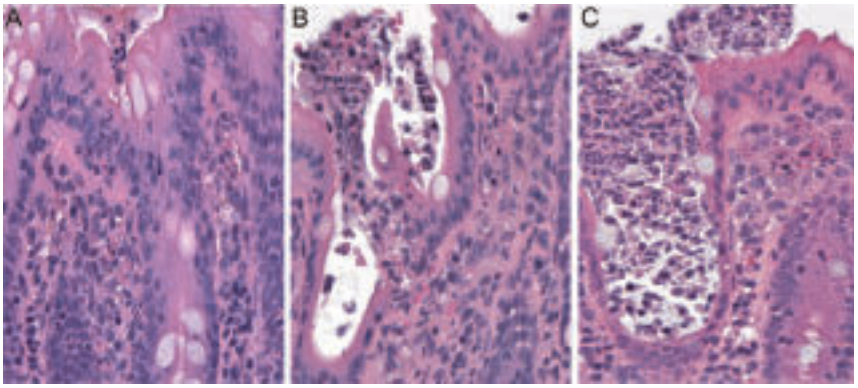

Fig. 4 Accumulation of neutrophils within the lamina propria of the equine colonic mucosa, and migration of neutrophils into the intestinal lumen after reperfusion: A – 1 hour of ischemia followed by 1 hour of reperfusion, B - 1 hour of ischemia followed by 2 hours of reperfusion, C - 1 hour of ischemia followed by 4 hours of reperfusion (H&E, x400).

An active inflammatory process was apparently initiated during ischemia (Fig. 4). Numerous lymphocytes, phagocytic active neutrophils and eosinophils infiltrated the subepithelial clefts and migrated through the paracellular space towards the intestinal lumen during ischemia. The lamina propria contained large vacuoles and enlarged macrophages containing phagocytic vacuoles and granules. Subepithelial mast cells, lymphocytes and eosinophils were swollen and necrotic. Numerous neutrophils were attached to subepithelial venules, and they migrated into the lamina propria.

Apoptosis

Apoptosis is a major cause of cell death after intestinal I/R in equine small and large intestines (*Rowe* et al. 2003, *Grosche* et al. 2012). Semithin sections embedded in epon and stained with toluidine blue (TB) clearly demonstrated nuclear chromatin condensation and margination, characteristics of apoptotic cells that we previously identified with the TUNEL method after I/R in equine colon (*Grosche* et al. 2012). Chromatin condensation and margination, and formation of apoptotic bodies, were increased by ischemia, and persisted during reperfusion in the present study.

Functional Changes

One hour of ischemia induced experimentally in the colon of horses caused mild injury that should have allowed some expression of reperfusion injury over the next 4 hours (*Graham* et al. 2011). When transepithelial resistance (TER) and mannitol permeability of the mucosa was measured in Ussing chambers, the reperfused tissues performed significantly better than ischemic tissues and as well as the control tissues (Figs. 5 and 6). This is not consistent with the expected results if reperfusion actually exacerbated ischemic damage, but is evidence of restored barrier function instead. In fact, histomorphometric examination revealed restitution in reperfused tissues with almost complete mucosal recovery after 4 hours in vivo. This recovery was against a background of rapid and intense neutrophil influx throughout reperfusion (*Grosche* et al. 2008, *Grosche* et al. 2011a/b). Ischemia for 1 hour in the equine colon caused minor histological alterations but led to severe epithelial barrier failure characterized by decrease of TER and increased mannitol leakage (*Graham* et al. 2011; Figs. 5 and 6).

Calprotectin can be used as a marker of acute and chronic inflammatory conditions (*Eckert* et al. 2004, *Foell* et al. 2004, *Striz* and *Trebichavsky* 2004, *Little* et al. 2005, *Faleiros* et al. 2009). In horses, calprotectin expression correlated signifi-

cantly with neutrophil infiltration in colonic (Fig. 2), and calprotectin expression increased during colonic I/R in horses (*Grosche* et al. 2008, 2011, *Matyjaszek* et al. 2009). Studies on large colon I/R injury in horses showed restitution and functional recovery of ischemic damaged colonic mucosa after 4 hours of reperfusion characterized by normalization of TER and transmucosal mannitol flux (*Graham* et al. 2011).

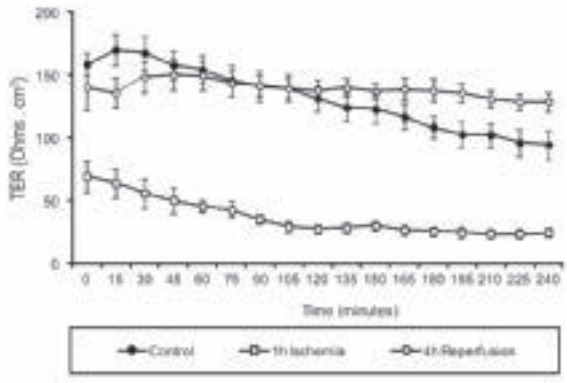

Fig. 5 Mean ± SEM values for transepithelial resistance (TER) of colonic mucosa in Ussing chambers from 6 anesthetized horses after 1h ischemia, or after 1h ischemia and 4h reperfusion, or from adjacent control segments. Time 0 = time of tissue collection at end of ischemia or end of reperfusion under anesthesia. Immediately after collection, tissues were mounted in Ussing chambers and incubated with Krebs-Ringer Bicarbonate solution (KRB) for 240 minutes. Control tissues and 4-hour reperfusion tissues are not different from each other, but both are significiantly different from the ischemic group at all time points ($P<0.05$).

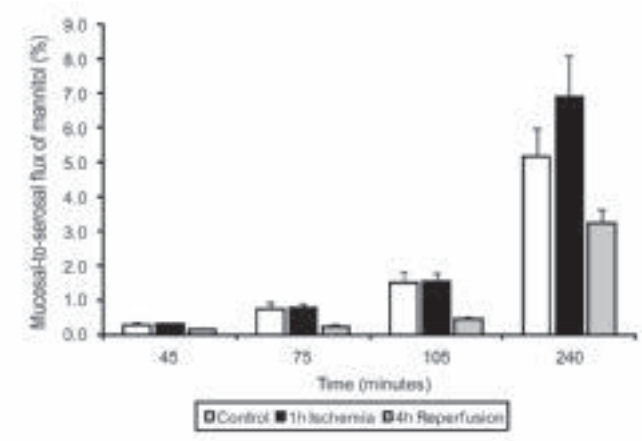

Fig. 6 Mean ± SEM values for mannitol flux across colonic mucosa in Ussing chambers from 6 anesthetized horses after 1h ischemia, or after 1h ischemia and 4h reperfusion, or from adjacent control segments. The reperfused tissues were significantly less permeable to mannitol than the ischemic tissues ($P<0.05$).

Apoptotic cells in the colonic epithelium were evident in semithin sections stained with TB, on TEM, and with the classic TUNEL method (*Grosche* et al. 2011, 2012). Apoptosis (measured by the number of apoptotic cells/mm2 of mucosa) and apoptotic index (number of apoptotic cells per 1,000 epithelial cells) increased significantly from the control findings during ischemia, although the values did not change during reperfusion (*Grosche* et al. 2012). These findings support the hypothesis that apoptosis has an important role in cell death in the equine colonic mucosa and that necrosis contributed less than one third to epithelial cell death in the present study (*Grosche* et al. 2011b). Although studies have revealed an activation of apoptosis during intestinal ischemia with further exacerbation during reperfusion (*Shah* et al. 1997, *Ikeda* et al. 1998, *Rowe* et al. 2003), the role of programmed cell death on tissue damage and dysfunction remains unknown (*Toth* et al. 2007).

Ultrastructural Changes

Improvement in transepithelial permeability in reperfused tissues compared with ischemic tissues could not be explained by light microscopsy (*Graham* et al. 2011), which led to a detailed study of ultrastructural changes in the same tissues (*Grosche* et al. 2011). In semithin sections stained with toluidinie blue (TB), minor histological alterations of the colonic epithelium were evident after 1 hour of ischemia as cell edema, microvilli disintegration, apoptosis, subepithelial fluid accumulation and detachment of epithelial cells from the vacuolated basement membrane (Fig. 3). Edema, necrotic debris, and swollen or necrotic immune cells (lymphocytes, eosinophils, mast cells) were evident in the subepithelial space, and cytoplasmic granules in subepithelial mast cells and eosinophils were reduced.

On transmission electron micropsy (TEM), epithelial cells had less electron-dense cytoplasm than in control tissues, the cytoplasm appeared vacuolated, and mitochondria and organelles were reduced and swollen (Fig. 7). The mitochondrial matrix was lucent, and the christae were dilated and disrupted, most of the nuclei appeared rounded and enlarged, and the condensation and margination of nuclear chromatin indicated early apoptotic features. Most of the epithelial cells were shorter and dilated compared with controls, microvilli were reduced in size and number, and the apical part of terminal tight junctions was partly disrupted or dilated. Separation of epithelial cells by intercellular fluid accumulation and expanded TJ (Fig. 8) could explain epithelial barrier failure after ischemia, which recovered after reperfusion (*Graham* et al. 2011). Large numbers of autophagosomes were evident in the cytoplasm after 1 hour of ischemia. Small groups of epithelial cells detached from the distorted basement membrane to form subepithelial clefts, but these detached epithelial cells remained connected to each other by their apical cellular junctions. Only single epithelial cells in these groups appeared necrotic.

Epithelial injury was not exacerbated by reperfusion for 1 and 4 hours. Within 1 hour of reperfusion, epithelial repair could be detected as a covering of small epithelial defects by interconnections between detached epithelial cells or between membrane extensions from intact neighboring cells. The paracellular space was dilated and infiltrated with neutrophils, lymphocytes and eosinophils (Fig. 7). Numerous macrophages with large phagocytic vacuoles, neutrophils and mast cells were located in the subepithelial lamina propria after 4 hours of reperfusion.

A prominent feature during reperfusion was an enlargement of the paracellular and subepithelial space over time that further separated detached epithelial cells from the basement mem-

brane (Fig. 7). However, these cells became shorter and appeared to adhere to each other at the luminal surface by membrane extensions and intact apical cell junctions, thus covering the large underlying clefts (*Grosche* et al. 2011). These findings were evident within 1 hour of reperfusion and could combine with closure of tight junctions to explain recovered epithelial barrier function after 4 hours of ischemia (*Graham* et al. 2011).

Large membrane-bound vacuoles containing necrotic debris, bacteria, and apoptotic bodies were observed in the cytoplasm of epithelial cells, possible evidence that these cells became phagocytic during reperfusion. Numerous neutrophils migrated into the intestinal lumen in close proximity to the epithelial surface, and contained vacuoles with phagocytized bacteria and necrotic debris (Fig. 4). Some apoptotic cells and apoptotic bodies were evident within the epithelium. Despite the intense infiltration and apparent activation of inflammatory cells in close proximity to the epithelium, epithelial cells were able to recover barrier integrity during reperfusion.

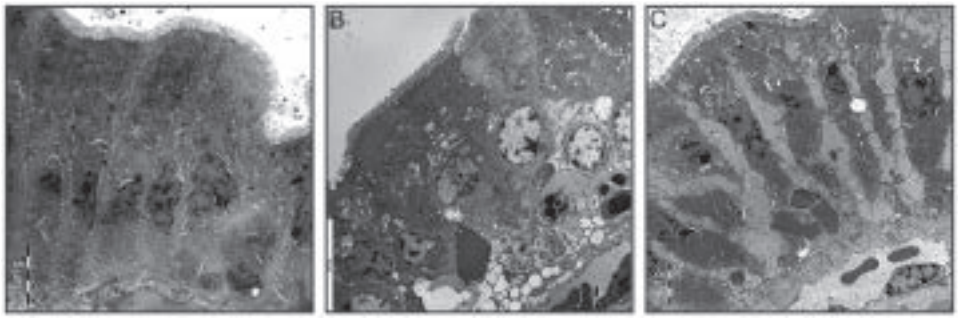

Fig. 7 Ultrastructural changes of the equine colonic epithelium after ischemia and reperfusion: A – control, B – 1 hour of ischemia, C – 1 hour of ischemia followed by 4 hours of reperfusion (TEM). After ischemia, detached epithelial cells appeared swollen and vacuolated, and their nuclei were characterized by chromatin condensation and margination. Most of the epithelial cells were shorter and dilated, microvilli were reduced in size and number, and the apical part of terminal tight junctions was partly disrupted or dilated (B). Enlargement of the paracellular and subepithelial space separated detached epithelial cells from the basement membrane during reperfusion (C).

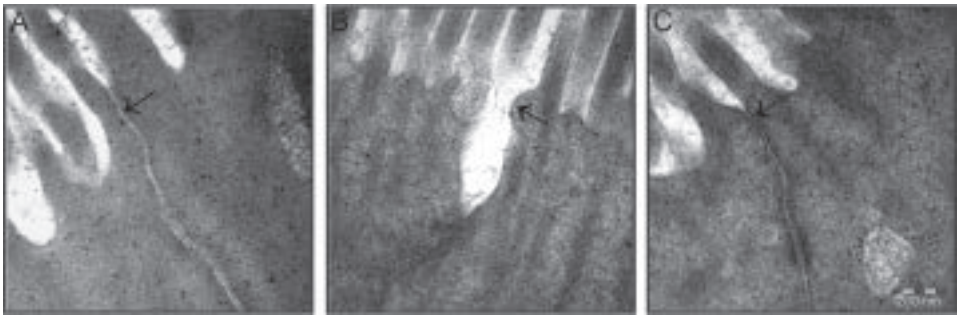

Fig. 8 Apical junction complex of the equine colonic epithelium after ischemia and reperfusion on TEM: A – control, B – 1 hour of ischemia with open tight junction, C – 1 hour of ischemia followed by 4 hours of reperfusion with resealed tight junction (arrows identify tight junctions).

Autophagy in colonocytes after ischemia in this study could represent a homeostatic process that removes damaged or surplus organelles, supplies nutrients and energy, eliminates intracellular pathogens and toxic proteins, and delivers endogenous antigens for presentation (*Levine* and *Deretic* 2007). Amino acids or fatty acids recovered through autophagy may be used for ATP production (*Sadoshima* 2008). Although autophagy in epithelial cells could cause single cell death after ischemia, it might also favor epithelial cell survival and epithelial repair. Because epithelial cells can phagocytize adjacent cells, apoptotic cells and bacteria (*Monks* et al. 2005, *Neal* et al. 2006), they could control the inflammatory response and minimize injury after I/R in horses. Although a larger epithelial defect requires restitution by migration of surviving cells in the periphery of the injury, the earliest mechanism of recovery in our model appeared predominantly to involve reattachment between remaining cells in the zone of epithelial damage (*Grosche* et al. 2011). The final fate of damaged epithelial cells cannot be established from this study.

Prostaglandins

Prostaglandins play a key role in regulating inflammatory reactions, and we demonstrated that COX-2 was rapidly induced at sites of ischemia-induced inflammation in the colonic mucosa of horses (*Matyjaszek* et al. 2009, *Morton* et al. 2009, *Grosche* et al. 2011). Although COX enzymes regulate the production of potent pro-inflammatory PGs (Crofford 2001), evidence is growing that COX-2 expression may contribute to resolution of gastrointestinal inflammation, and might be crucial in regulating mucosal healing (*Blikslager* et al. 1999, *Wallace* and *Devchand* 2005). COX-2 expression in the epithelium and by lamina propria immune cells was significantly upregulated after ischemia and after 1 hour of reperfusion in the present study, a time point where epithelial repair began (*Grosche* et al. 2011). *Shifflett* et al. (2004) found enhanced recovery of barrier function in ischemic-injured porcine ileum that was mediated by upregulation of COX-2 and activation of neutrophils. This reparative effect of PGE2 to ischemia-injured intestinal mucosa is initiated through chloride secretion in restituting epithelium (*Moeser* et al. 2004). Generation of COX-2 continued, and was associated with mucosal restitution after 18 hours of reperfusion (*Morton* et al. 2009). Thus, increased COX-2 expression after ischemia in ischemic-injured equine colonic mucosa could be a response of the colonocytes to prevent further damage and initiate early recovery during reoxygenation (*Savill* et al. 2002, *Karrasch* et al. 2006).

Neutrophils

After ischemic injury for 2 hours, accumulation and transepithelial migration of neutrophils persisted in mucosa for at

least 18 hours of reperfusion (*Grosche* et al. 2008, *Matyjaszek* et al. 2009). After 18 hours of reperfusion, TER was reduced in ischemic tissues compared with nonischemic controls, but mannitol fluxes were unchanged (*Matjyaszek* et al. 2009). This is in contrast to 4 hours reperfusion after 1 hour of ischemia, in which both TER and mannitol fluxes were similar to these measurements for nonischemic controls but significantly better than for ischemia only (*Graham* et al. 2011). Although we did not have an 18-hour reperfusion group after 1 hour ischemia to allow direct comparison between 1 hour and 2 hours of ischemia, we consider the 2-hours ischemia to be considerably more severe than the shorter interval.

Direct counting of neutrophils within large tissue areas, such as full-thickness mucosa, is difficult and cumbersome using standard histological techniques (H&E), because separation from other cells with shared staining characteristics that accumulate in the inflammatory reaction is highly subjective (*Moore* et al. 1994). Methods for measuring neutrophil infiltration, such as counting in a small portion of the tissue, a scoring system, tissue MPO activity, and leukocyte scintigraphy, do not help to localize the cells as they migrate through the tissue (*Moore* et al. 1994, *Gayle* et al. 2002). The neutrophil cytosolic protein, calprotectin can be detected in tissues by immunohiostochemistry (Fig. 2) and is a valuable marker of neutrophil activity in gastrointestinal tract diseases (*Johne* et al. 1997, *Poullis* et al. 2003, *Striz* and *Trebichavsky* 2004).

After 4 hours of reperfusion, neutrophils disappeared from submucosal venules because of transmigration and accumulation within the adjacent tissue (*Grosche* et al. 2011). After 18 hours of reperfusion, calprotectin-stained cells declined significantly in the venules. Pattern of changes in calprotectin-positive cells suggested that cells left the venules during reperfusion, migrating towards the epithelium at 1 hour of reperfusion, to reach the intestinal lumen at 2 hours of reperfusion. We did not examine reperfusion time periods between 4 hours and 18 hours, so that mucosal neutrophil traffic during that period is unknown. De novo synthesis of P-selectin and expression of E-selectin could explain the second peak of neutrophil accumulation after 18 hours reperfusion in the present study (*Grosche* et al. 2008). *Little* et al. (2005) also found an increased neutrophilic inflammation within all intestinal layers of equine jejunum at 18 hours after ischemia.

Our results (*Grosche* et al. 2008, 2011, *Graham* et al. 2011) would suggest that there was no relationship between the severity of mucosal injury and neutrophil influx, although the neutrophil activity profile in equine colon in our model was compatible with that proposed to cause reperfusion injury (*Moore* et al. 1995). *Dagleish* et al. (1999) have shown that activated horse and human neutrophils concurrently release ROS, elastase and antiproteinase. However, in human beings, the antiproteinase released by the neutrophil may be locally destroyed by ROS that are also produced by the neutrophil. This allows human neutrophil elastase to destroy lung tissue, which characterizes the effect of neutrophils in human chronic obstructive pulmonary disease (COPD). However, the equine neutrophil releases several isoforms of antiproteinase and only one of these is susceptible to oxidants (*Patterson* and *Bell* 1989). Therefore, the equine neutrophil may have greater ability to limit the activity of its own elastase compared with the human neutrophil. Horses with heaves can have intense neutrophil infiltration in their airways but without extensive destruction of lung parenchyma, which develops in human COPD (*Jefcoat* et al. 2003). In equine jejunum subjected to arterial low-flow ischemia and reperfusion in an extracorporeal circuit, leukocyte depletion by a filter interposed in the circuit generally did not attenuate the effects of low-flow ischemia and reperfusion on equine small intestine (*Van Hoogmoed* et al. 2001).

Eosinophils

In equine colon, the role of neutrophils during I/R cannot be distinguished from the role of resident eosinophils (*Meschter* et al. 1986, *Moore* et al. 1994, *McConnico* et al. 1999, *Rötting* et al. 2003). Intestinal accumulation of eosinophils has been described in horses with experimentally-induced and naturally occuring colitis, I/R injury and parasitism (*Moore* et al. 1994, *McConnico* et al. 1999, *Edwards* et al. 2000, *Archer* et al. 2006, *Rötting* et al. 2008). Whereas eosinophils accumulate within the human gastrointestinal tract in disease (*Rothenberg* et al. 2001), eosinophilic granulocytes are resident in the gastrointestinal lamina propria in healthy horses (*Rötting* et al. 2008). Most of the eosinophils in equine colon are found near the muscularis mucosae and are rarely located close to the surface of the mucosa (*Rötting* et al. 2008, *Grosche* at al. 2011, 2012).

During ischemia and in some extent during reperfusion the absolute number of mucosal eosinophils in equine colon decreased while migrating towards the epithelium because of loss into the lumen or through apoptosis (*Grosche* et al. 2011, 2012). Movement towards the lumen is consistent with findings in vitro in that an oxidant (HOCl) can induce eosinophils to migrate towards the damaged surface epithelium (*Rötting* et al. 2003). The observation that the number of eosinophils in the intestinal mucosa was significantly greater in horses that survived naturally occurring strangulation obstruction led to the assumption that eosinophils may play a role in suppressing inflammation (*Meschter* et al. 1986).

Other Cells of the Inate Immune System

Subepithelial mast cell numbers were unchanged during I/R, but they displayed features of degranulation after 1 hour of ischemia, evident by the decreased number of intracellular granules and their sub-membranous localization (*Grosche* et al. 2011). Such changes could be interpreted as degranulation and release of pro-inflammatory molecules into the interstitium. Although the number of mucosal macrophages did not change during I/R in the present study (*Grosche* et al. 2001), their increased phagocytic activity during I/R suggested a possible role during recovery from ischemic injury in horses (*Grosche* et al. 2011).

Nitrotyrosine

Formation of nitrotyrosine from nitration of the aromatic amino acid tyrosine could be a potential marker of RNS generation (*Halliwell* 1997, *Eiserich* et al. 1998), including peroxynitrite, nitrate, NO, nitrogen dioxide or nitryl chloride (*Wallace* and

Ma 2001). Peroxynitrite is a short-lived oxidant species and potent inducer of cell death and tissue damage (*Beckman* et al. 1996, *Ischiropoulos* et al. 1992, *Grisham* et al. 1999, *Mirza* et al. 1999, *Kubes* and *McCafferty* 2000, *Takemoto* et al. 2007, *Szabo* et al. 2007, *Kono* and *Rock* 2008). It is generated by activated leukocytes (*Gagnon* et al. 1998, *Takemoto* et al. 2007) and endothelial cells (*Kooy* and *Royall* 1994) during I/R (*Kono* and *Rock* 2008). Ischemia increased nitrotyrosine production by eosinophils significantly from the control value in equine colon, but reperfusion had little if any effect on its production (*Grosche* et al. 2012). These findings suggest that resident eosinophils in equine colonic tissues are subjected to oxidative stress in the early stage of I/R, when they could contribute to tissue damage. The same response was noted for mucosal and submucosal leukocytes, compared with the control values, but there was a significant peak in production after 18 hours of reperfusion in colon that followed 2 hours of ischemia (*Grosche* et al. 2012). This could represent a defense mechanism against transepithelial passage of bacteria or bacterial products during this stage of reperfusion.

Nitrotyrosine staining in mucosal leukocytes of horses with naturally acquired small intestinal strangulation obstructions was increased, compared with findings in horses without gastrointestinal tract diseases (*Mirza* et al. 1999). A possible cytotoxic role of NO in small intestinal strangulation obstructions was proposed (*Mirza* et al. 1999). Administration of peroxynitrite into the colon of rats produced widespread injury and inflammation similar to that recorded in inflammatory bowel disease (*Rachmilewitz* et al. 1993), which provides evidence that it could initiate intestinal inflammation and tissue damage (*Singer* et al. 1996, *Shah* et al. 2004). Although peroxynitrite has limited extracellular stability and diffusion range, it could be cytotoxic towards invading pathogens and act as a potent microbicidal compound (*Muijsers* et al. 1997).

Remote Lesions

In a rat model of 1 hour complete ischemia and 4 hours of reperfusion, neutrophils and their products mediated most of the associated injury in lung and liver during reperfusion, but did not contribute to intestinal injury, despite a marked increase in intestinal neutrophils (*Simpson* et al. 1993). In horses that were subjected to distension of the small colon by a latex balloon surgically implanted in the lumen and inflated to a pressure of 40 mm Hg for 4 hours, similar observations were made (*Faleiros* et al. 2008). The mucosa was not affected by luminal distension, but neutrophil accumulation and edema were observed in the distension group, as well as hemorrhage, fibrin deposition, and increased MPO activity in the seromuscular layer. Similarly, there was greater accumulation of neutrophils in the lung samples from the distension group than in those from a sham-operated group, based on light micropsy and MPO assay (*Faleiros* et al. 2008).

In a study on small intestinal I/R alone, without any manipulation of the colon, an eosinophilic and neutrophilic inflammatory response was observed in the colon after small intestinal reperfusion (*Hopster-Iversen* et al. 2011). These findings provide new evidence of a systemic inflammatory response, followed by remote lesions, in horses with intestinal obstruction but without direct vascular obstruction. Contrary to these results, 1 hour of induced large colon torsion in ponies followed by 3 hours of reperfusion caused extravascular neutrophil accumulation in all sections of colon and cecum, but not in liver and lung (*Wilson* et al. 1994).

Potential Treatments and Modifiers

A novel solution (OPS) used in kidney transplant patients was able to preserve viability in an isolated segment of equine colon in the absence of oxygen and blood (*Polyak* et al. 2008). The solution represents a multimodal approach to treating reperfusion injury but is expensive and its components did not improve epithelial repair in equine colon in vitro (*Graham* et al. 2011). The selected components are inexpensive and each has the potential to improve tissue recovery from an ischemic injury. L-arginine is an indirect precursor of NO (*Polyak* et al. 2008) and therefore has the potential to hasten tissue healing and modulate the inflammatory response. L-glutamine can support several metabolic pathways during intestinal stress (*Kojima* et al. 1998).

Acetylcysteine can protect mucosa through its antioxidant properties and by providing sulfhydryl groups required for replenishment of glutathione, which is an important intracellular antioxidant (*Cotgreave* 1997). In mucosa from equine right dorsal colon, acetylcysteine prevented mucosal damage and reduced eosinophil migration in tissues subjected to brief chemical damage in vitro, when the tissue was exposed to the damaging agent (HOCl) and acetylcysteine simultaneously (*Rötting* et al. 2003). In another study on HOCl-injured mucosa from the equine right dorsal colon in vitro, phenylbutazone and indomethacin did not seem to interfere with restitution of oxidant-injured mucosa of equine colon, butyrate and PGE_2 had no effects, and glutamine facilitated mucosal restitution (*Rötting* et al. 2004).

Nonsteroidal Anttinflammatory Drugs

Cyclooxygenase-1(COX-1), which is constitutively expressed in most tissues, is largely responsible for prostaglandin (PG) production and associated house-keeping functions under normal physiological conditions. COX-2 is expressed at low levels in normal tissue, but is upregulated in response to injury and is the enzyme responsible for many of the harmful responses associated with ischemic intestinal injury in the horse. In the equine gastrointestinal tract, both COX-1 and COX-2 are constitutively expressed in the jejunum and pelvic flexure (*Tomlinson* et al. 2004, *Morton* et al. 2009, *Marshall* et al. 2011). COX-2 expression may contribute to resolution of gastrointestinal tract inflammation, and it might be crucial in regulating mucosal healing (*Blikslager* et al. 1999, *Krause* and *DuBois* 2000, *Wallace* and *Devchand* 2005, *Fukuta* et al. 2006). In most studies, COX-2 expression was identified in lamina propria immune cells, such as neutrophils, macrophages, lymphocytes and mast cells, and generation of COX-2-derived PGD_2 was associated with inhibition of granulocyte infiltration in a rat colitis model (*Reuter* et al. 1996, *Wallace* 2006).

Flunixin meglumine (FM) is the nonsteroidal antiinflammatory drug (NSAID) most commonly used to inhibit the PG-media-

ted responses associated with pain and endotoxemia in horses with colic. While FM can effectively treat the clinical signs of colic, there is evidence to suggest that its administration has adverse gastrointestinal effects, such as right dorsal colitis and impaired recovery of the mucosal barrier (*Marshall* and *Blikslager* 2011). In equine right dorsal colon in vitro, phenylbutazone caused apoptosis and blocked anion secretion, but did not affect mucosal permeability (*Richter* et al. 2002).

Recent studies have examined the in vitro measurements of recovery in intestine that was subjected to ischemic injury in vivo, comparing the effects of different NSAIDs on recovery of mucosal barrier function (*Marshall* and *Blikslager* 2011). Whether the NSAIDs were given in vivo or added to the bathing solution in vitro, the histological measures of recovery were largely unaffected by these drugs. However, FM did appear to impair functional recovery of mucosal barrier properties and allowed low molecular weight markers of permeability to traverse the ischemic-injured mucosa, including lipospolysaccharide (LPS; *Cook* et al. 2009). NSAIDs with a more COX-2 selective profile (e.g. firocoxib) may support repair of mucosal barrier defects compared with the more traditional NSAIDs, such as FM (*Marshall* and *Blikslager* 2011). There is a difference in the response of the large and small intestines to ischemic injury and NSAID treatment. In the equine pelvic flexure, FM does not inhibit barrier function recovery and increase permeability to low-molecular weight compounds after ischemia (*Matyjaszek* et al. 2009).

The major limitation of the preceding studies on the equine small intestine is that they lack obvious clinical relevance for the postoperative management of horses that had surgery for a small intestinal lesion. Endotoxemia should not develop in these horses, whether they had a resection and anastomosis or ischemic intestine was left in place after the strangulation was corrected. If endotoxemia does develop in these horses, it signifies a major complication (obstruction, leakage). In fact, there should be very little LPS in the small intestine after surgery, because the responsible organisms are at low numbers compared with the colon. Also, any LPS that would gain entry across the mucosa would enter the portal vein and be removed in the liver (*Mimura* et al. 1995). Any LPS that escapes hepatic elimination would be unlikely to cause clinical signs in a horse treated with FM (Fig. 9). Also, the critical measurements were made in vitro and might not accurately reflect in vivo events. Nonetheless, these findings do underscore the need for seeking more selective NSAIDs for treating horses with small intestinal strangulation, while recognizing that FM should be used as needed until that time arrives.

Lidocaine

Initial interest in lidocaine focused on its use as an agent to restore motility in horses after colic surgery and thereby prevent POI (*Cook* and *Blikslager* 2008). A survey of Diplomates of the American College of Veterinary Surgeons revealed that lidocaine continuous rate infusion (CRI) was the most commonly used prokinetic agent in horses after colic surgery (*Van Hoogmoed* et al. 2004). Intraoperative lidocaine is also used to reduce the minimum alveolar concentration of inhalation anesthetics in horses and may have been responsible for a reduction in POI prevalence in a study on surgical colic patients (*Cohen* et al. 2004). In a multicenter study, a constant rate infusion of lidocaine for 24 hours in horses with nasogastric reflux attributable to POI or enteritis significantly reduced duration of reflux, compared with horses treated with saline solution CRI during the same period (*Malone* et al. 2006). Also, lidocaine CRI significantly reduced the hourly volume of reflux and decreased duration of hospitalization (*Malone* et al. 2006). In horses that had small intestinal surgery only in a single hospital, lidocaine CRI reduced the prevalence of POI and improved short-term survival (*Torfs* et al. 2009).

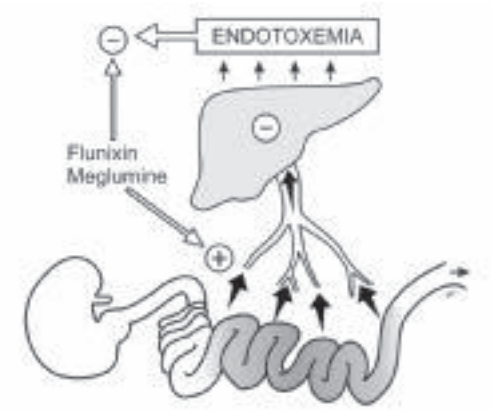

Fig. 9 Flunixin meglumine (FM) can delay recovery of barrier integrity in ischemic-injured jejunum so that lipopolysaccharide (LPS) can cross the mucosa and enter the portal venous system, to cause postoperative endotoxemia. However, FM can block the effects of LPS, which is likely to be diminished also by the liver as it receives LPS through the portal vein. Black arrows indicate LPS flow from the injured jejunum (shaded) and minus signs represent inhibition of LPS effects by liver and FM. The plus sign indicates promotion of LPS leakage under the influence of FM.

Although the widespread use of lidocaine after colic surgery was initially based on the presumption that it was a prokinetic agent (*Van Hoogmoed* et al. 2004), this does not appear to be the case (*Milligan* et al. 2007, *Rusiecki* et al. 2008). Therefore, any clinical benefit of lidocaine is attributable to other mechanisms of action. Intravenous lidocaine can shorten the duration of POI in the human colon after abdominal surgery, possibly by: 1) reducing circulating catecholamines by inhibition of the sympathoadrenal response; 2) suppressing activity in the primary afferent neurons involved in reflex inhibition of gut motility; 3) stimulating smooth muscles directly; and 4) decreasing inflammation in the bowel wall through inhibition of prostaglandin synthesis, inhibition of granulocyte migration and the release of lysosomal enzymes and free radicals (*Rimback* et al. 1990).

The perceived clinical benefit of lidocaine CRI in horses after gastrointestinal tract surgery is currently attributed to novel anti-inflammatory effects on neutrophils, which require a concentration that is less than the concentration necessary to block sodium channels (*Hollmann* et al. 2001). Macrophages within the muscularis layers of the intestine are activated by surgical manipulation of the intestine, which causes them to release proinflammatory cytokines and prostaglandins (*Bauer* et al. 2002). An influx of monocytes and neutrophils into the muscularis layers follows, and further release of

inflammatory products reduce muscle contractility and ultimately cause POI (*Bauer* et al. 2002). In horses, neutrophilic inflammation has been identified in the jejunum 18 hours after experimentally induced ischemia and after jejunal manipulation (*Little* et al. 2005). The temporal relationship between onset of clinical signs of POI and peak small intestinal inflammation in horses suggest a cause and effect relationship (*Little* et al. 2005).

In vitro assays have revealed that lidocaine inhibits neutrophil adhesion, phagocytosis, and the production of free radicals (*Azuma* et al. 2000). In addition, lidocaine can reduce the expression of endothelial adhesion molecules in epithelial cells, endothelial cells and neutrophils and could thereby inhibit neutrophil adhesion and migration (*Lan* et al. 2004). In rats with experimentally induced obstructive ileus, IV administration of lidocaine prevents fluid secretion into the lumen of the small intestine and reduces edema formation in the intestinal wall (*Nellgard* et al. 1996). Lidocaine may also prevent ischemic-induced intracellular sodium overload caused by sodium influx through the epithelial sodium channel that is blocked by lidocaine (*Cook* and *Blikslager* 2008). These findings could be highly relevant to management of POI in horses, based on ability of intestinal inflammation to inhibit smooth muscle activity (*Bauer* et al. 2002).

Of considerable importance to equine surgeons is the recent finding that lidocaine can improve smooth muscle contractility in vitro in equine jejunum after it was subjected to I/R injury (*Guschlbauer* et al. 2010). In another study, systemic administration of lidocaine 15 minutes before reperfusion significantly reduced the mean grade for mucosal damage in ischemic-damaged equine jejunum, compared with responses to treatment with saline solution (*Marshall* et al. 2006). Treatment with lidocaine also ameliorated the negative effects of FM on recovery of the mucosal barrier on equine jejunal mucosa (*Cook* et al. 2008). In horses with ischemia-injured jejunum, lidocaine administered IV during reperfusion reduced plasma prostaglandin E2 metabolite concentration and mucosal COX-2 expression (*Cook* et al. 2009). Systemically administered lidocaine also ameliorated the neutrophilic infiltration in ischemic-injured equine jejunum induced by FM, but did not affect neutrophil infiltration in the absence of FM (*Cook* et al. 2009).

Currently, there is little information on the effectiveness of lidocaine in the management of I/R in the large colon (*Cook* and *Blikslager* 2008). Also, there is little evidence that lidocaine might actually be antiinflammatory in horses. In an in vitro study on equine neutrophils, lidocaine did not inhibit neutrophil migration or adhesion at therapeutic concentrations, and it actually increased migration and adhesion at higher concentrations (*Cook* et al. 2009). Despite its ability to reduce inflammation and reduce lung function in asthmatic patients, lidocaine appeared to increase total cell numbers and percentage of neutrophils in bronchoalveolar lavage fluid from horses with heaves (*Wilson* et al. 2010) Lidocaine does not inhibit inflammatory events in either the laminae or skin in the horse in the black walnut model of laminitis, further evidence that its antiinflammatory effect might not be applicable to all diseases characterized by inflammation (*Williams* et al. 2010). Also lidocaine can cause muscle fasciculations, ataxia, masking of pain from laminitis, (*Hardy* and *Rakestraw* 2006) delayed intestinal transit (*Rusiecki* et al. 2008), and possibly incisional infection (*Stephen* et al. 2004).

Conclusions

Despite the intense influx of neutrophils during reperfusion in our I/R model in equine colon, and the presence of neutrophils within the intercellular space and TJ after 4 hours of reperfusion (*Grosche* et al. 2011), transepithelial barrier function was not impaired at this time, as determined by TER and transmucosal mannitol flux (*Graham* et al. 2011; Figs. 5 and 6). Therefore, the debate continues on whether intestinal I/R injury after reperfusion is clinically relevant in the horse, or simply a result of progression of tissue changes that have been activated under hypoxic conditions (*Snyder* et al. 1988, *Blikslager* et al. 1997, *Rowe* and *White* 2002).

Other pathomechanisms that can exacerbate intestinal damage after reperfusion might be involved in small intestinal I/R injury as well (*Laws* and *Freeman* 1995, *Vatistas* et al. 1996, *Vatistas* et al. 1998, *Dabareiner* et al. 2001). Experimental design, types and durations of I/R, differences between intestinal segments and even species differences could explain the failure to repeat the findings in laboratory animal studies. Also, endpoints to measure responses to pharmacologic manipulation, problems with timing and dosages of drugs, and the relative importance of different pathways and mediators in the response to ischemia could explain our failures.

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