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# The effect of nonsteroidal anti-inflammatory drugs on the equine intestine

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## Summary

**Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used in the management of pain and endotoxaemia associated with colic in the horse. While NSAIDs effectively treat the symptoms of colic, there is evidence to suggest that their administration is associated with adverse gastrointestinal effects including right dorsal colitis and inhibition of mucosal barrier healing. Several studies have examined the pathophysiology of NSAID associated effects on the large and small intestine in an effort to avoid these complications and identify effective alternative medications. Differences in the response of the large and small intestines to injury and NSAID treatment have been identified. Flunixin meglumine has been shown in the small intestine to inhibit barrier function recovery and increase permeability to lipopolysaccharide (LPS). A range of NSAIDs has been examined in the small intestine and experimental evidence suggests that those NSAIDs with cyclooxygenase independent anti-inflammatory effects or a COX-2 selective mode of action may offer significant advantages over traditional NSAIDs.**

## Introduction

The gastrointestinal tract of the horse is anatomically and physiologically complex. Due to certain anatomical features, the horse is susceptible to a variety of gastrointestinal lesions including volvulus and entrapment of the small and large intestines. The equine gastrointestinal tract requires endogenous prostanoids not only for the maintenance of mucosal blood flow and integrity, but for control of hind gut fermentation and volatile fatty acid production (Argenzio *et al*. 1974a). In the event of gastrointestinal injury, prostaglandins have been shown to be necessary for the restoration of mucosal barrier function (Blikslager *et al*. 1999). As nonsteroidal anti-inflammatory drugs (NSAIDs) are routinely administered to horses suffering from gastrointestinal disease, the effect of NSAIDs on gastrointestinal injury and recovery has been investigated. Using models of mucosal injury including ischaemia (Tomlinson and Blikslager 2004a; Little *et al*. 2007), bile acids (Campbell *et al*. 2002) and reactive oxygen metabolites (Inoue *et al*. 2007), studies have examined the effect of NSAIDs in both the small and large intestines. These studies have highlighted several differences in the response of the large and small intestines to injury and recovery, and to NSAID treatment. Knowledge of these differences is important in understanding the potential adverse effects of NSAIDs in the horse.

## The role of prostaglandins in the equine intestine

### *The cyclooxygenase system*

The roles of 2 cyclooxygenase enzyme isoforms have been described in the normal and healing intestine. Cyclooxygenase-1 (COX-1) is constitutively expressed in most tissues, and is believed to be responsible for PG production during normal physiological processes, while COX-2 is expressed at only low levels in normal tissue, but is upregulated in response to injury. In the equine gastrointestinal tract, constitutive expression of both COX-1 and COX-2 has been identified in the jejunum and the pelvic flexure of the large colon (Tomlinson and Blikslager 2005; Morton *et al*. 2009; Marshall *et al*. 2011). Ischaemic injury and 4 h of *in vitro* recovery was shown to have no significant effect on COX-1 expression in equine jejunum (Marshall *et al*. 2011) but an 18 h *in vivo* recovery results in an increase in COX-1 expression, suggesting a delayed increase in COX-1 protein expression (Tomlinson and Blikslager 2005). The expression of COX-2 increases more rapidly and is significantly greater after ischaemic injury and 4 h recovery (Marshall *et al*. 2011). Similar to the jejunum, ischaemic injury of the left dorsal colon results in a significant increase in COX-2 expression (Morton *et al*. 2009). NSAIDs are frequently used to treat colic in the horse, both to provide analgesia and to ameliorate signs of endotoxaemia (Shuster *et al*. 1997). NSAIDs inhibit the COX enzyme, which is critical in the conversion of arachidonic acid to prostaglandin (PG) $H_2$. Prostaglandin $H_2$ is then converted to variety of prostanoids by a range of specific synthases (Garavito and DeWitt 1999). Studies involving the porcine ileum have shown that prostaglandins $I_2$ and $E_2$ are required to restore mucosal barrier function following ischaemic injury (Blikslager *et al*. 1997).

### *Repair of the mucosal barrier*

Following mucosal injury, recovery of mucosal barrier function occurs as the result of 3 major processes (Blikslager *et al*. 2007).

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The villi of the small intestinal epithelium are capable of contracting in response to injury. This villus contraction is a major part of the mucosal response to injury and reduces the surface area of exposed basement membrane to be covered by epithelium. The first phase of contraction by the villus myofibroblasts is mediated by the enteric nervous system and is followed by a second phase of contraction, which requires endogenous prostaglandin synthesis (Moore *et al*. 1989; Erickson *et al*. 1990). The second process in the repair of the mucosal epithelium and recovery of barrier function is epithelial restitution (Blikslager *et al*. 2007). Following injury, the epithelial cells surrounding the denuded basement membrane produce plasma membrane extensions and begin to migrate into the defect (Gookin *et al*. 2003). In contrast to villus contraction and assembly of the tight junctions, epithelial restitution is not dependent on the production of endogenous prostaglandins (Gookin *et al*. 2003). The third major process in the restoration of mucosal integrity is the closure of the paracellular spaces (Blikslager *et al*. 2007). In order for the paracellular spaces to close and repair barrier integrity, the tight junctions located apically between the epithelial cells must be assembled. This process involves the recruitment of tight junction proteins to the apical paracellular space and is dependent on the action of endogenous prostaglandins (Blikslager *et al*. 2007). While the mechanisms by which prostaglandins stimulate the assembly of tight junctions are currently unclear, experimental studies suggest that they are mediated by their effects on ion channels and transporters (Moeser *et al*. 2008; Nighot *et al*. 2009). The effect of prostaglandins on recovery of mucosal barrier function is related to the initial stimulation of chloride secretion, mainly via ClC-2 chloride channels (Nighot *et al*. 2009), and inhibition of NHE2 electroneutral sodium exchangers (Moeser *et al*. 2008). Whether this effect is directly related to this secretory effect of prostaglandins causing collapse of the paracellular space, or tight junction signalling by ClC-2 and NHE2 resulting in organisation of the tight junctions is currently unknown. Therefore, prostaglandins play a crucial role in the process of barrier function recovery following injury to the gastrointestinal tract.

## Effect of NSAIDs on recovery of barrier function

### *Small intestine*

Studies of the effects of NSAIDs on the small intestine of the horse have focused on ischaemic injury of the jejunum. This injury is encountered clinically in cases of strangulating lesions of the jejunum such as small intestinal volvulus or strangulation by a pedunculated lipoma (Gerard *et al*. 1999). It has been demonstrated that flunixin meglumine retards recovery of the mucosal barrier in ischaemic equine jejunum as determined by the *ex vivo* measurement of transepithelial electrical resistance (TER) and the mucosal-to-serosal flux of mannitol (Tomlinson and Blikslager 2005). A clinically relevant measurement of mucosal barrier function is the mucosal-to-serosal paracellular flux of LPS (Koenig *et al*. 2009). Importantly, treatment with flunixin meglumine results in increased mucosal permeability to LPS following ischaemic injury and an 18 h recovery period (Tomlinson and Blikslager 2004a; Cook *et al*. 2009a). Flunixin meglumine inhibits the increase in thromboxane $B_2$ ($TXB_2$) and prostaglandin $E_2$ ($PGE_2$) observed during recovery from ischaemic injury both *in vitro* and *ex vivo* (Tomlinson and Blikslager 2005; Cook *et al*. 2009a). When ischaemic-injured equine jejunum is treated with a combination of flunixin meglumine and the prostaglandin $E_1$ ($PGE_1$) analogue misoprostol, the inhibitory effect on the recovery of barrier function is prevented (Tomlinson and Blikslager 2005). Therefore, the inhibition of barrier function in ischaemic equine jejunum by flunixin meglumine is probably due to the inhibition of cyclooxygenase in the intestine.

Following ischaemic injury, chemoattractants are released resulting in neutrophil migration and infiltration of injured tissues. This neutrophil infiltration and degranulation causes further tissue damage through physical opening of paracellular spaces and the release of proteases, e.g. elastase (Blikslager *et al*. 2007). It has been shown in a porcine experimental model that mucosal neutrophil infiltration following ischaemic injury is detrimental to mucosal barrier function (Gayle *et al*. 2002). Treatment with flunixin meglumine results in an increase in mucosal neutrophil infiltration in ischaemic equine jejunum over an 18 h recovery period (Cook *et al*. 2009b). The reason for this increase in neutrophil infiltration and its significance in the inhibition of mucosal barrier function recovery in the horse is unclear.

Following the identification of the detrimental effect of flunixin meglumine on mucosal barrier function recovery, several studies have examined alternative medications. Etodolac is an NSAID that was studied in an *ex vivo* model of equine ischaemic intestinal injury; however, there was no significant difference between the effect of etodolac and flunixin meglumine on the recovery of barrier function (Tomlinson *et al*. 2004). A later study revealed that etodolac has a COX selectivity ratio of 4.32 and is therefore not a COX-2 selective inhibitor in the horse (Davis *et al*. 2007). This may explain the inhibition of barrier function recovery by etodolac. Another NSAID that has been shown to preferentially inhibit COX-2 in the dog is meloxicam, which has a COX selectivity of 10 in a whole blood model (Brideau *et al*. 2001). A similar experiment using an equine whole blood model revealed a significantly lower COX selectivity ratio of 3.8 (Beretta *et al*. 2005). When meloxicam was examined in a model of ischaemic injury and recovery, it was found that it did not inhibit the recovery of mucosal barrier function as measured by TER and mucosal-to-serosal passage of inulin (Little *et al*. 2007). This study did not find a significant effect of either flunixin meglumine or meloxicam on the passage of LPS through ischaemic-injured tissue (Little *et al*. 2007). Both meloxicam and flunixin meglumine were shown to increase the mucosal neutrophil infiltration (Little *et al*. 2007). In addition to the inhibition of COX enzyme activity, it is possible that meloxicam has a COX-independent anti-inflammatory mechanism of action (Little *et al*. 2007). This is demonstrated by reduced upregulation of COX-2 protein expression in response to ischaemic injury following treatment with meloxicam (Little *et al*. 2007). However, as meloxicam was shown to have an equal or greater effect than flunixin meglumine on post operative pain in this experimental study, and it did not inhibit the recovery of mucosal barrier function, it appears to be a suitable alternative to flunixin meglumine for the treatment of colic (Little *et al*. 2007). As the efficacy of meloxicam as an analgesic is based on an experimental study, further investigation of the clinical efficacy of meloxicam is warranted.

In an effort to reduce adverse gastrointestinal effects in human medicine, COX-2 selective drugs have been developed. These drugs are often referred to as coxibs, and are designed to be potent inhibitors of COX-2 while allowing normal production of prostaglandins by COX-1 at clinically relevant concentrations (Cronstein 2002). Deracoxib is a coxib that is available for use in

veterinary medicine and has been shown to selectively inhibit COX-2 in the dog (Gierse *et al*. 2002). A study of the *in vitro* recovery of ischaemic-injured equine jejunum showed that deracoxib was similar to flunixin meglumine and inhibited the recovery of mucosal barrier function as measured by TER (Tomlinson and Blikslager 2005). However, unlike flunixin meglumine, deracoxib treatment did not result in a significant increase in the mucosal-to-serosal passage of mannitol (Tomlinson and Blikslager 2005). Furthermore, deracoxib inhibited the increase in $PGE_2$ and 6-keto-$PGF_{1\alpha}$, a stable metabolite of $PGI_2$, without inhibiting an increase in $TXB_2$ suggesting a COX-1 sparing effect (Tomlinson and Blikslager 2005). As this was an *in vitro* study the effect of deracoxib on neutrophil infiltration was not determined. Therefore, deracoxib may offer an alternative to flunixin meglumine in cases of small intestinal ischaemic injury. However, the clinical use of deracoxib is currently severely limited by the lack of a suitable intravenous formulation licensed for use in the horse.

Although meloxicam and deracoxib appear to offer advantages over flunixin meglumine for the treatment of small intestinal ischaemic injury (Tomlinson and Blikslager 2005; Little *et al*. 2007), neither is currently available for equine use in the USA and only meloxicam is available for the treatment of colic in Europe. However, a coxib has been recently approved for the treatment of musculoskeletal pain in the horse. This NSAID, firocoxib, has been shown to be a highly selective inhibitor of COX-2 in the horse with a COX selectivity ratio of 265 (McCann *et al*. 2002). In a clinical study, firocoxib was not associated with an increased risk of adverse gastrointestinal effects in the dog (Steagall *et al*. 2007). In an *ex vivo* study of ischaemic injury and recovery, treatment with firocoxib did not inhibit mucosal barrier function as measured by TER (Cook *et al*. 2009a). In this study, flunixin meglumine, but not firocoxib, was again shown to increase the permeability of the mucosa to LPS (Cook *et al*. 2009a). In horses treated with flunixin meglumine or firoxoxib there was no significant increase in the plasma $PGE_2$ during the recovery period, suggesting that both NSAIDs inhibit the action of COX-2 (Cook *et al*. 2009a). Only flunixin meglumine inhibited the increase in plasma $TXB_2$, suggesting that firocoxib did not significantly inhibit the action of COX-1 (Cook *et al*. 2009a). The effect of firocoxib on mucosal neutrophil infiltration is currently unknown.

The role played by mucosal neutrophils in the inhibition of mucosal barrier function recovery in the horse is unclear. Several studies have shown that there is an increase in mucosal neutrophil infiltration of injured jejunum in horses treated with flunixin meglumine, which is associated with reduced barrier function (Tomlinson *et al*. 2004; Little *et al*. 2007; Cook *et al*. 2009a). In contrast, treatment with meloxicam resulted in increased neutrophil infiltration without inhibition of barrier function recovery (Little *et al*. 2007). However, a recent study examining the effect of lidocaine on ischaemic injury and recovery of the equine jejunum suggested a role of mucosal neutrophil infiltration in barrier function (Cook *et al*. 2009c). In this study, while treatment with flunixin meglumine inhibited the recovery of mucosal barrier function, a combination of flunixin meglumine and lidocaine did not (Cook *et al*. 2009c). Interestingly, treatment with lidocaine significantly inhibited the mucosal infiltration of neutrophils associated with flunixin meglumine treatment (Cook *et al*. 2009c). However, an *in vitro* study found that lidocaine does not inhibit the migration and adhesion of equine neutrophils (Cook *et al*. 2009b). It appears that lidocaine prevents the inhibition of barrier function recovery associated with flunixin meglumine by an anti-inflammatory mechanism that is currently unknown and is indicated for clinical use in equine patients with small intestinal injury.

*Large intestine*

The large intestine of the horse has developed to perform the functions necessary for hindgut fermentation including mixing, secretion and absorption (Argenzio *et al*. 1974b). Following mixing in the caecum, ingesta enters the ventral colon every 3–4 min. The right and left ventral regions of the large colon are succulated and contain a large volume of mixed ingesta for fermentation. While the ventral colon does secrete a significant volume of fluid, in the normal situation there is a net absorption of water and sodium chloride (Argenzio *et al*. 1974b). However, when the right ventral colon is examined *in vitro*, production of endogenous prostanoids causes inhibition of sodium absorption and stimulation of chloride secretion (Clarke and Argenzio 1990). The *in vitro* treatment of normal right ventral colon with flunixin meglumine inhibits this increased chloride secretion (Freeman *et al*. 1997). Exposure of right ventral colon to reactive oxygen intermediates resulted in an increase in chloride secretion, which was abolished by treatment with flunixin meglumine (Inoue *et al*. 2007). However, as this model did not result in tissue injury or changes in mucosal barrier function the effect of flunixin meglumine on the recovery of the right ventral colon is unknown (Inoue *et al*. 2007). Since the recovery of barrier function has been associated with the stimulation of chloride secretion (Blikslager *et al*. 1999) and flunixin meglumine inhibits secretion by the right ventral colon (Freeman *et al*. 1997), it may inhibit recovery from ischaemic injury. However, ischaemic injury of the right ventral colon has not yet been investigated.

Following the large, sacculated ventral colon, the large colon of the horse loses its sacculations and narrows at the pelvic flexure. Although studies of the right ventral colon suggest that flunixin meglumine would inhibit recovery of the colon from ischaemic injury (Freeman *et al*. 1997), a study of the pelvic flexure found that treatment with flunixin meglumine did not adversely affect the recovery of mucosal barrier function (Matyjaszek *et al*. 2009). However, several differences between the response of the pelvic flexure and jejunum to ischaemic injury were discovered. In contrast to the results of previous studies in the jejunum (Tomlinson *et al*. 2004; Little *et al*. 2007; Cook *et al*. 2009a), the transepithelial electrical resistance (TER) of colonic mucosa following injury and recovery was significantly lower than uninjured mucosa (Matyjaszek *et al*. 2009). This is an interesting finding as it suggests a significant difference in the physiological response of the jejunal and colonic epithelia to injury. In jejunal tissue, an 18 h recovery results in a transepithelial electrical resistance significantly greater than that of uninjured tissue and the process by which this occurs is significantly inhibited by flunixin meglumine (Tomlinson *et al*. 2004; Little *et al*. 2007; Cook *et al*. 2009a). In contrast, the transepithelial electrical resistance of colonic tissue remains significantly lower than control tissue after 18 h of recovery and no significant effect of flunixin meglumine has been shown, possibly as the recovery process inhibited by flunixin meglumine is not occurring in the colon (Matyjaszek *et al*. 2009). Also, treatment with flunixin meglumine did not increase mucosal permeability to mannitol (Matyjaszek *et al*. 2009). While injury and recovery resulted in neutrophil infiltration of the *lamina*

*propria*, treatment with flunixin meglumine did not increase accumulation (Matyjaszek *et al*. 2009). The effect of ischaemic injury or flunixin meglumine on the transmucosal passage of LPS is unknown. Therefore, would it appear that there are significant differences in the responses of the equine jejunum and pelvic flexure to ischaemic injury and flunixin meglumine. These results suggest that the large colon will remain more permeable to intestinal contents, including LPS, for a period >18 h following ischaemic injury and that treatment of pain and endotoxaemia with flunixin meglumine will not influence the colonic recovery process. Therefore, the clinical strategy for the management of large colon injury should be aimed at preventing and treating the consequences of increased epithelial permeability including endotoxaemia.

The right dorsal colon is the shortest and widest section of the equine ascending colon. Like the ventral colon, it is sacculated and is a major site of fluid secretion and absorption (Argenzio *et al*. 1974b). In contrast to the ventral colon, a net secretion of fluid occurs in the dorsal colon (Argenzio *et al*. 1974b). Also, unlike the ventral colon, the right dorsal colon is well attached to the abdominal roof, caecal base and root of the mesentery. It is therefore not susceptible to volvulus and the resultant ischaemic injury. However, the administration of phenylbutazone, a nonselective COX inhibitor, has been shown to cause right dorsal colitis, which may result in death (McConnico *et al*. 2008). Experimental studies have shown that prolonged (21 days) administration of phenylbutazone decreases colonic volatile fatty acid production and mucosal blood flow (McConnico *et al*. 2008). While no study has examined the effect of prolonged phenylbutazone treatment on mucosal barrier function, the *in vitro* treatment of oxidant-injured right dorsal colon with phenylbutazone did not affect the recovery of barrier function (Rotting *et al*. 2004).

While studies of the effect of NSAIDs on the recovery of the colon from injury are limited, they do suggest that there may be significant differences in the response of the jejunal and colonic mucosal barrier to injury. As the colon is a physiologically complex organ, with varying functions throughout its length, further studies are required to determine the effect of injury location on the response to NSAID treatment. However, this may be difficult to assess *in vitro* as significant differences in the absorptive function have been identified when compared to the *in vivo* situation.

## Effect of NSAIDs on intestinal motility

The adverse effect of NSAIDs on the equine intestine may not be limited to the recovery of barrier function following injury. Studies of both the small and large intestine have attempted to investigate whether the administration of NSAIDs has an inhibitory effect on intestinal motility (van Hoogmoed *et al*. 1999, 2000, 2002; Menozzi *et al*. 2009). A series of *in vitro* experiments investigating the effect of a range of NSAIDs on the equine large intestine (dorsal colon, ventral colon and pelvic flexure) found that nonselective COX inhibitors including flunixin meglumine, phenylbutazone, ketoprofen and indomethacin had an inhibitory effect on contractile activity (van Hoogmoed *et al*. 1999, 2000, 2002). Etodolac, an NSAID believed to have a COX-1 sparing mechanism of action, was found to inhibit the contractile activity of the ventral colon at the highest concentration tested (10 µmol/l) only (van Hoogmoed *et al*. 2002). Treatment of equine large intestine with $PGE_2$ or $PGF_{2\alpha}$ has been shown to increase contractive activity providing further evidence that the inhibitory effect of NSAIDs is related to COX inhibition (van Hoogmoed *et al*. 1999). In contrast, a recent study of the effect of NSAIDs on ileal motility found no inhibitory effect of indomethacin but a decrease in contractile activity following treatment with COX-2 selective drugs including celecoxib (Menozzi *et al*. 2009). The authors of this study suggested the differences between the results of this study and previous studies may be explained by the different roles of prostaglandins in the small and large intestine and possible COX-independent effects (Menozzi *et al*. 2009).

## Conclusions

The equine gastrointestinal tract may be affected by a variety of conditions resulting in injury to the mucosal barrier. Restoration of barrier function is essential to prevent the passage of luminal contents, including LPS, into the systemic circulation resulting in endotoxaemia (Tomlinson and Blikslager 2004b). The routine use of NSAIDs in the treatment of pain and endotoxaemia in the horse has led to investigation of their effects on barrier function recovery. In the jejunum, the nonselective COX inhibitor flunixin meglumine has been shown to inhibit the recovery of mucosal barrier function, and increase neutrophil infiltration and permeability to LPS (Tomlinson *et al*. 2004). The COX-2 selective NSAIDs deracoxib (Tomlinson and Blikslager 2005) and firocoxib (Cook *et al*. 2009a) do not inhibit the recovery of barrier function in the jejunum. The inhibition of chloride secretion, an essential element in recovery of barrier function after ischaemic injury, has been demonstrated in the right ventral colon by treatment with flunixin meglumine (Freeman *et al*. 1997). Investigation of the effects of ischaemic injury in the large colon has focused on the pelvic flexure (Matyjaszek *et al*. 2009). In contrast to the jejunum, flunixin meglumine did not inhibit recovery of mucosal barrier function or increase neutrophil infiltration (Matyjaszek *et al*. 2009). Therefore, the regions of the equine gastrointestinal tract appear to vary in their response to ischaemic injury and to treatment with NSAIDs. Treatment strategies in cases of small intestinal injury should ideally include a choice of NSAID that does not inhibit the normal recovery process. In cases of large intestinal injury, the prolonged reduction in epithelial barrier function without influence by NSAID treatment suggests that prevention and treatment of endotoxaemia is a critical goal of treatment.

## Conflicts of interest

No conflicts of interest have been declared.

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