

The Pharmacokinetics of Firocoxib after Multiple Oral Doses to Neonatal Foals

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## ABSTRACT

The purpose of this study was to determine the safety and pharmacokinetic profile of firocoxib in healthy neonatal foals. Foals are more sensitive to the side effects of nonsteroidal anti-inflammatory drugs, (NSAIDs), particularly due to immature renal clearance mechanisms and ulcerogenic effects on gastric mucosa. Firocoxib, a novel second generation NSAID, is reported to have reduced side effects due to its COX-2 selectivity. The pharmacokinetic profile of firocoxib in neonates has not been established, making reliable dosing difficult. We hypothesized that firocoxib given per os at the labeled dose to neonatal foals would be absorbed and not be associated with clinically significant adverse events.

Seven healthy American Quarter Horse foals of mixed gender were administered 0.1mg/kg firocoxib orally q24h for nine consecutive days, commencing at 36h of age. Blood samples were collected for firocoxib analysis using high pressure liquid chromatography with fluorescence detection at 0 (dose #1 only), 0.25, 0.5, 1, 2, 4, 8, 16 and 24 hours after doses #1, 5 and 9. For all other doses (2, 3, 4, 6, 7 and 8) blood was collected immediately prior to the next dose (24 hour trough). Elimination samples (36, 48, 72, 96, 120 and 144 hours) were collected after dose #9. Safety was assessed via physical examinations, changes in body weight, gastroscopy, complete blood count, serum biochemistry and urinalysis.

Firocoxib was rapidly absorbed following oral administration with minimal accumulation after repeat dosing. After the initial dose, an average peak serum concentration ( $C_{\max}$ ) of  $89.50 \pm 53.36$  ng/mL (mean  $\pm$  SD) was achieved ( $T_{\max}$ ) in  $0.54 \pm 0.65$  hours. Steady state was obtained after approximately 4 doses and the average maximum concentration ( $C_{\text{avg}}$ ) in serum was  $39.1 \pm 8.4$  ng/mL. After the final dose, the mean terminal half-life ( $T_{1/2\lambda}$ ) was  $10.46 \pm 4.97$  hours. Firocoxib was not detected in plasma 72 hours after the final dose ( $<2$ ng/mL). Bioavailability could not be determined as currently, there is no accompanying intravenous dose of firocoxib for this age group to permit the calculation. No significant abnormalities were noted on blood work, urinalysis or gastroscopy. This study demonstrated that firocoxib is absorbed after oral administration in neonatal foals with no observable adverse effects after multiple doses.

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## Chapter 1

### Introduction

Nonsteroidal anti-inflammatory drugs, (NSAIDs), encompass some of the most widely used medications in human and veterinary medicine worldwide. NSAIDs are utilized for their anti-inflammatory, analgesic, anti-thrombotic and anti-pyretic effects. In veterinary medicine, NSAIDs are utilized for various inflammatory conditions including those producing musculoskeletal, ocular or visceral pain, fever, sepsis and endotoxemia.

NSAIDs exert these therapeutic effects by inhibiting cyclo-oxygenase (COX) in the arachidonic acid cascade. Disrupting this inflammatory cascade inhibits the hallmark effects of inflammation and the resultant loss of function, namely pain, heat, redness and swelling. However, alongside the beneficial, therapeutic effects of blocking COX come numerous adverse effects. Adverse effects occur primarily through inhibition of the COX-1 isoenzyme, which produces prostanoids that serve important housekeeping functions in the body. Over the last few decades, research has focused on finding NSAIDs that primarily block the COX-2 isoenzyme (coxibs) and allow COX-1 to maintain its homeostatic functions. However, the roles of COX-1 and COX-2 are not so well defined, and recent discovery of further forms of COX, including COX-3, complicates this further. The role of leukotrienes (LOX) in inflammation can also not be ignored.

Adverse effects impact the clinical application and dosing regimens of NSAIDs. Adverse effects may occur in the gastrointestinal, renal and cardiovascular systems in particular, and there is an innumerable amount of published data on NSAID toxicity in humans and animals. Recent research has focused on safer but therapeutically equivalent NSAIDs, particularly the promising class of coxibs. Coxibs are thought to be less toxic than traditional NSAIDs due to greater COX-2 selectivity and this assumption has been supported by numerous human and animal studies. Firocoxib is one of the few drugs in this class that has been approved for use in animals, particularly horses.

Due to differences in metabolism, efficacy between species and age of the patient, extensive pharmacokinetic and pharmacodynamics investigation is warranted before new NSAIDs can be utilized in medical practice. Many studies have elucidated the differences in drug disposition between adults and neonatal animals. When NSAIDs are used for neonates in particular, appropriate dosing needs to be established through pharmacokinetic studies. Due to differences in metabolism, side effects of NSAIDs may be more pronounced in neonates, particularly from COX-1 inhibition. Therefore, COX-2 selective NSAIDs, including firocoxib, provide a promising option, but little published data is available about this class of drugs in neonates. Currently, information on firocoxib focuses on adult equids, and primarily on its use for musculoskeletal issues. For drugs such as firocoxib to be used safely in neonates, the first step is to ascertain pharmacokinetic information on the drug in this age group.

This thesis will review current information on NSAIDs, including a brief overview of historical background of NSAIDs, the arachidonic acid cascade and the mechanism of action of NSAIDs on inflammation. A discussion of NSAID use in horses, and in particular the equine neonate, will follow and include information on available pharmacokinetic data and adverse effects. Finally, a review of the current information on the selective COX-2 NSAID, firocoxib, will be provided. This literature review will provide background and information as to why further research into the use of firocoxib in horses, particularly neonates, will be of benefit to veterinary therapeutics.

## Literature Review

Inflammation was described 2000 years ago by the ancient Greeks as pain, swelling, inflammation, redness and loss of function. The first record of treating inflammation in the form of rheumatic pain is in the Ebers papyrus, an Egyptian medical papyrus about 1500 BC, which refers to the use of decoctions or plant extracts, including willow bark or leaves. The Reverend Edward Stone of Chipping Norton, a country parson in Oxfordshire, reported the first ‘clinical trial’ of willow bark in 1763 in the *Philosophical Transactions of the Royal Society*, where he describes dried and pulverized willow bark dispersed in drink curing fever in 50 patients (1). Although the use of willow for pain, rheumatism and fever was described by early historical figures including Hippocrates, Celsus, Pliny the Elder and Dioscorides, salicin, the active principle in the common white willow (*Salix alba*) was not identified until 1829 by German scientists. Salicylic acid was initially compounded in Germany in 1874 (2) and the first commercial use of sodium salicylate in humans was in 1875 (3). Aspirin (acetylsalicylic acid) was first sold as a powder in 1899, after a chemist working for Bayer in Germany, Felix Hoffman, developed a more palatable form for his father with severe rheumatism (1).

In 1952, phenylbutazone was introduced onto the market to treat rheumatoid arthritis and gout in humans. It soon became apparent that the drug produced significant bone marrow toxicity in humans, including agranulocytosis and aplastic anemia, resulting in its withdrawal from the human drug market (4). During the 1960s, prostaglandins were discovered and a second NSAID, indomethacin, was developed. Since then, an NSAID revolution has occurred, with over 30 new NSAIDs being introduced to the human and veterinary pharmaceutical markets (3). Although diverse in chemical structure, all NSAIDs share the therapeutic properties of being anti-inflammatory, anti-thrombotic, analgesic and anti-pyretic, achieved through inhibition of COX in the arachidonic acid cascade.

In veterinary medicine, the use of NSAIDs in horses is first documented by Dun in 1895, in *Veterinary Medicine. Their Actions and Uses*, where he describes the use of

acetylsalicylic acid (aspirin). Aspirin was the sole NSAID used in veterinary medicine until the introduction of phenylbutazone, which although soon withdrawn from the human market (5), remains the most prescribed NSAID for horses today.

## Arachidonic Acid Cascade

### Introduction to the Arachidonic Acid Cascade

Inflammation occurs as a result of cellular injury, which initiates a series of cellular events via the arachidonic acid cascade. Arachidonic acid, the precursor to eicosanoids, is acquired from linoleic acid in the diet and esterified into cell membrane phospholipids. It is a 20-carbon polyunsaturated fatty acid (5, 8, 11, 14-eicosatetraenoic acid), which is not present free in cells, instead it is found esterified in membrane phospholipids (6). Stimulation by numerous factors, (including microbial products, and mechanical, chemical or physical stimuli), triggers the enzymatic action of phospholipase A<sub>2</sub> on cell membrane phospholipids, thereby initiating the arachidonic acid cascade (7). Activation of phospholipase A<sub>2</sub> occurs with biochemical signals including increased cytoplasmic calcium and activation of various kinases in response to external stimuli. The enzymes cyclooxygenase and lipoxygenase, metabolize arachidonic acid, which catalyzes the formation of cyclic endoperoxides into the various eicosanoids. The cyclooxygenases generate prostaglandins, while the lipoxygenases produce leukotrienes and lipoxins. These eicosanoids then bind to the G protein-coupled receptors on numerous types of cells, mediating the whole inflammatory process (6). Further details of this process and the specific role of the eicosanoids are described below. *Figure 1* displays the steps and physiologic effects of various eicosanoids in the arachidonic acid cascade. *Table 1* displays some of the main target tissues of the prostanoids and leukotrienes.

### Formation and Effects of Prostanoids

Prostaglandins (PGs) and thromboxanes, known collectively as prostanoids, are produced by most cell types including mast cells, macrophages and endothelial cells. They act as autocrine and paracrine lipid mediators, with signaling occurring at or near the site of synthesis. Numerous enzymes regulate arachidonic acid, so it remains esterified until mobilized by phospholipases (PLA<sub>2</sub>). Various stimuli of cells, including mechanical trauma, cytokines, growth factor, collagen, adenosine diphosphate (ADP) in platelets,

bradykinin or thrombin in endothelium, cause synthesis of prostaglandins by arachidonic acid released from cell membranes. At the endoplasmic reticulum and cell membrane, arachidonic acid is released by phospholipases, and presented to prostaglandin endoperoxidase H synthetase (PGHS), more often referred to as cyclooxygenase (COX), resulting in the formation of the various prostaglandins and thromboxanes. At least two isoforms, COX-1 and COX-2 exist, and discussion of these and another isoform, COX-3, will follow in another section. Prostanoids, are organized into series by basis of their structural features, which is letter coded (PGD, PGE, PGF, PGG and PGH) and then a subscript numeral for the number of double bonds in each compound. The prostanoids primarily associated with inflammation are PGE<sub>2</sub>, PGD<sub>2</sub>, PGF<sub>2α</sub>, PGI<sub>2</sub> (prostacyclin) and TXA<sub>2</sub> (thromboxane).

Each prostaglandin is derived by the action of a specific intermediate on the arachidonic acid pathway and has various local effects in inflammation. Thromboxane, for instance, is a potent platelet aggregator and causes vasoconstriction. Prostacyclin, on the other hand, is a potent inhibitor of platelet aggregation and causes vasodilation. PGD<sub>2</sub>, which is the main prostaglandin made by mast cells, and PGE<sub>2</sub>, are associated with edema formation via vasodilation and increased permeability of post-capillary venules. PGD<sub>2</sub> is a chemoattractant for neutrophils and PGF<sub>2α</sub> stimulates contraction of small arterioles, as well as uterine and bronchial smooth muscles (8).

Systemically, prostaglandin involvement in inflammation is related in part to PGE<sub>2</sub>, which causes hyperalgesia and is upregulated in cytokine-induced fever during infections. In the gastrointestinal tract, PGE<sub>2</sub> and PGI<sub>2</sub> are important in maintaining gastrointestinal mucosal integrity by reducing gastric acid secretion, increasing secretion of bicarbonate in the duodenum, increasing protective mucus production and vasodilating mucosal blood vessels (9). The kidney, and in particular, the renal medulla, is a major producer of prostaglandins including PGE<sub>2</sub>, PGI<sub>2</sub>, PGF<sub>1α</sub> and TXB<sub>2</sub>. Prostaglandins are locally active substances and so different segments of the nephron produce varying amounts and forms of prostaglandin. Species differences also exist, with human glomeruli, for instance, producing predominantly PGI<sub>2</sub>, while rat glomeruli have higher

levels of PGE<sub>2</sub> and PGF<sub>2α</sub>. The particular significance for prostaglandins' role in the kidney is their effects on renal blood flow and glomerular filtration. Similar to other organs, PGE<sub>2</sub> and PGI<sub>2</sub> are vasodilatory while TXA<sub>2</sub> is a potent vasoconstrictor. Additionally, renal prostaglandin synthesis is important in maintaining renal function in various disease states including chronic renal failure, volume depletion and congestive heart failure, while PGE<sub>2</sub> and PGI<sub>2</sub> will enhance renin release (10). Therefore when addressing the role of prostaglandins in inflammation, it is important to consider these substances have both beneficial and harmful effects, particularly in the gastrointestinal and renal systems.

#### Formation and Effects of Leukotrienes

Leukotrienes are primarily produced by lipoxygenase enzymes secreted by inflammatory cells including polymorphonuclear leukocytes, macrophages and mast cells (11). In general, leukotrienes are chemoattractants for leukocytes and have vascular effects. In neutrophils, 5-lipoxygenase converts arachidonic acid to the leukotriene precursor, 5-hydroxyeicosatetraenoic acid (5-HETE), which is also a chemotactic for neutrophils. Alongside the prostanoids, the various leukotrienes have specific functions in inflammation. LTB<sub>4</sub>, for example, is a potent chemotactic and activator of neutrophils. It causes aggregation and adhesion of cells to venular endothelium, release of lysosomal enzymes and generation of reactive oxygen species (ROS). ROS destroy microbes phagocytosed by leukocytes, but also produce endothelial cell damage and increase vascular permeability. They injure other cells including parenchymal and red blood cells, and are known to inactivate anti-proteases which leads to unopposed protease activity and increased extracellular matrix destruction, particularly of elastic tissues such as the lungs. The cysteinyl containing leukotrienes, LTC<sub>4</sub>, LTD<sub>4</sub> and LTE<sub>4</sub>, cause intense vasoconstriction, bronchospasm and increase vascular permeability of venules (12). Therefore, when evaluating individual effects of leukotrienes and prostanoids, it is clear that both major pathways of the arachidonic acid cascade, mediated through either cyclooxygenase or lipoxygenase, produce many mediators of inflammation in the body.

## The Production and Role of Lipoxins

In contrast, the lipoxins, which are also formed by arachidonic acid from the lipoxygenase pathway, are associated with inhibition of inflammation. Leukocytes, particularly neutrophils, produce intermediates in lipoxin synthesis, which are converted to lipoxins by platelet interaction with the leukocytes. Lipoxins principally act to inhibit cellular components of inflammation and leukocyte recruitment, partly through inhibiting neutrophil chemotaxis and adhesion to endothelium. There is an inverse relationship between production of lipoxins and leukotrienes, which suggests lipoxins are endogenous negative regulators of leukotrienes, with a role in resolving inflammation (13).

## Cyclooxygenase (COX) Isoforms

### Introduction to COX Isoforms

COX is the enzyme that converts arachidonic acid, via oxidation and reduction reactions, into prostaglandin G<sub>2</sub> (PGG<sub>2</sub>) and prostaglandin H<sub>2</sub> (PGH<sub>2</sub>), and is present for only minutes to seconds before being broken down into inactive compounds (14). COX was first discovered in 1976, and identified as the substance responsible for producing PGH<sub>2</sub> from arachidonic acid and the target of NSAIDs (15). It was not until 1991 that a second COX isoform (COX-2) was identified (16). The term COX-3 was first designated to a splice-variant of COX-2 in 2000 (17), then a splice-variant of COX-1 in 2002 (18, 19). The roles and expression of these isoenzymes continue to be investigated.

### COX-1

In basic terms, COX-1 is considered homeostatic since it is constitutively expressed and has enzyme activity in many organs, including the kidneys, stomach, intestine and platelets. Both COX-1 and COX-2 isoforms display constitutive and inducible activity, but COX-1 is primarily responsible for the physiologic functions of eicosanoids,



including gastrointestinal mucosal protection, renal blood flow and vascular homeostasis (11, 20-22).

However, not all the homeostatic roles of COX-1 are protective. For instance, ischemic reperfusion injury is exacerbated from vasodilation of gastric blood vessels and increasing gastric mucosal ulceration is associated with increasing expression of COX-1 in the lamina propria of mononuclear cells (22, 23). COX-1 is overexpressed in ovarian cancer and has been suggested as a therapeutic target (24) and it has been shown that both isoforms are increased in equine jejunal mucosa after two hours of ischemia (25).

## COX-2

It is well established that upregulation of COX-2 expression occurs with acute and chronic inflammation, after stimulation by proinflammatory cytokines and mitogens (21). It is also increased in ischemia and involved in the pathogenesis of certain cancers, including transitional cell carcinomas of the bladder in dogs (26). Although COX-2 mRNA has been identified in the stomach, intestine, spleen, cerebral cortex, lung, ovary, kidneys and in the liver in dogs, the COX-2 enzyme was not identified, which would be expected for locations where it did not have homeostatic functions, but was primarily present when upregulated with inflammation (27).

COX-2 is also constitutively expressed in various tissues where it has homeostatic functions, including roles in renal function, ulcer healing in the gastrointestinal tract, constitutive expression in the proximal colon, functions in the brain, bone repair and in female reproduction (26, 28, 29). Additionally, its inhibition will delay ulcer healing and worsen colitis. There is also evidence that it is detrimental to inhibit COX-2 during the resolution phase of inflammation, because synthesis of beneficial anti-inflammatory prostaglandins are affected (22). This is evidenced by certain prostaglandins peaking at different times in inflammation, for example in a mouse model, PGE<sub>2</sub> (which promotes inflammation, hyperalgesia and fever) was shown to peak at 4 hours and PGD<sub>2</sub> synthase expression at 48 hours after endotoxin administration (30). As opposed to PGE<sub>2</sub>, PGD<sub>2</sub>

has anti-inflammatory properties including blocking pro-inflammatory prostaglandin production and by inhibiting nuclear factor  $\kappa$ B (22).

### COX-3

The role of COX-3 has not been as well established. It appears COX-3 is made from the COX-1 gene but has differences in its mRNA. COX-3 mRNA is expressed in the canine and human cerebral cortex, and in the human heart. COX-3 is inhibited by various NSAIDs including diclofenac, ibuprofen and aspirin, to varying degrees. The significance of COX-3 to the inflammatory pathway and disease has not yet been determined and this remains an area for further scientific investigation (18, 22).

### NSAIDs

#### Mechanism of Action of NSAIDs

In 1971, Sir John Vane demonstrated that the NSAIDs, aspirin and indomethacin, inhibited enzymatic production of prostaglandins. He determined that this was due to inhibition of COX in the arachidonic acid cascade, and won a Nobel Prize in 1982 for his work (3). The molecular basis for COX inhibition by aspirin was first described in 1975 (31). These researchers demonstrated aspirin to acetylate a microsomal protein in sheep and bovine seminal vesicles and human platelets, which occurred with the same time course and concentration as COX. This was deemed to explain the anti-inflammatory and anti-thrombotic actions of aspirin. Further research (32) has shown that COX-1 and COX-2 are membrane bound proteins that exist as dimers, and have considerable structural similarities. Arachidonic acid accesses the active site by a hydrophobic channel. This is blocked irreversibly by interpolation of an acetyl residue on Serine 530 and Serine 516 for COX-1 and COX-2, respectively, by aspirin (acetylsalicylic acid). Other NSAIDs interact competitively with the active site. Minor differences exist between the isoforms. For example, the signal residue of COX-1 is seven residues longer than COX-2, while the N-terminus of COX-1 has eight residues and the C-terminus of

COX-2 has 18 residues (32). Variation in specificity of COX-1 and COX-2 inhibitors is due to minor differences in their amino acid composition, specifically that the smaller valine residues at positions 434 and 523 on COX-2 allows for formation of a side pocket, which is the active site for selective COX-2 drugs. COX-1 has larger isoleucine residues at the aforementioned positions, which block the entrance of this molecular gate and prevent binding by COX-2 selective drugs (22). Another contributor to COX-2 specificity is the type of residue in position 513, near the surface of the protein. For COX-1, this is an aromatic histidine residue and for COX-2, it is a charged arginine residue. The imidazole ring of the histidine residue is unable to interact with COX-2 inhibitors, but arginine interacts with COX-2 sulfonamide groups (32). Finally, COX-2 inhibitors have hydrogen bonding to residues in COX-2, which are not present in COX-1 (22).

#### Pharmacology of NSAIDs

*Table 2* lists various NSAIDs available for veterinary use by chemical class. NSAIDs are weakly organic compounds with pK<sub>A</sub>'s from 3 to 5 and are ionized at physiologic pH. They are highly protein bound (>98%), have low volumes of distribution, and due to increased blood flow and vascular permeability, display fast penetration into and prolonged elimination from inflamed tissues (7). They have good bioavailability after oral and subcutaneous administration, though absorption may be delayed after oral dosing in horses due to binding to ingesta. There is limited excretion of the parent drug in urine due to the high degree of plasma protein binding limiting ultrafiltration through glomerular capillaries. Excretion is generally by renal tubular secretion. Due to medium to high lipid solubility, they readily penetrate the blood brain barrier. As weak acids, NSAIDs may have poor penetration into cells due to the relatively acid pH of intracellular fluid. Most are metabolized by the liver via oxidation, reduction, hydrolysis and conjugation, into inactive compounds, though some metabolites are active, including phenylbutazone into oxyphenbutazone and aspirin into salicylate. There are marked differences in clearance and terminal plasma half-life between species (7, 33). Donkeys eliminate phenylbutazone more rapidly than horses and mules, while flunixin has a

significantly shorter mean residence time, smaller area under the curve and faster mean body clearance (34). In general, neonates display reduced clearance and longer half-lives (33).

Low protein concentrations in synovial fluid are responsible for the relatively low penetration of NSAIDs into synovial compartments (60% of mean plasma concentration). Due to increased protein in inflamed joints, and increased protein binding of NSAIDs to protein within the joint, drug penetration in presence of synovitis is increased. This was evidenced in an equine study where intra-articular concentration of ketoprofen were 6.5 times greater in inflamed joints than in normal horses (35). In dogs, meloxicam shows preferential accumulation in inflamed joints (36). However, as protein bound drugs are inactive, this phenomenon does not necessarily correlate with a greater efficacy of NSAID therapy in joint inflammation.

#### Toxicity of NSAIDs in Horses

As has been identified earlier in this review, the toxicity of NSAIDs is primarily related to the inhibition of the COX-1 isoenzyme and its ability to perform homeostatic functions in the body (7, 37-39). This is exemplified in mice which lack COX-1, as they display resistance to gastric ulceration and a generalized reduction in inflammation associated with the arachidonic acid cascade (40). Side effects reported in horses with NSAID usage include gastrointestinal lesions (ulcerations and erosions), renal toxicity, plasma protein binding effects, hepatotoxicity, coagulation effects, chondrodestruction and perivascular & intramuscular irritation with inappropriate administration. Some of these side effects and relevant published literature will be discussed below.

#### Gastrointestinal Toxicity

In the gastrointestinal tract, prostaglandins serve to maintain adequate blood flow, motility, secretion and to promote mucosal cytoprotection. Inhibition of these functions by blocking prostaglandin production will promote gastrointestinal injury (7).

Prostaglandin E<sub>2</sub>, for example, protects gastric mucosa from acid damage by maintaining adequate blood flow, inhibiting gastric acid secretion (which is stimulated by feeding, inhibin or histamine) and by inducing mucus and electrolyte secretion into the intestinal lumen (41). Additionally, NSAIDs can cause direct irritation to gastrointestinal mucosa, and studies using oral dosing have resulted in worse oral lesions than intravenous administration (42).

In a study evaluating commonly utilized NSAIDs in equine practice, the glandular portion of the stomach was shown to undergo the most deleterious effects from administration of phenylbutazone, flunixin and ketoprofen. In addition, phenylbutazone produced edema of the small intestines and erosions and ulceration in the large colon and the horses receiving phenylbutazone displayed a significant decrease in serum total protein and albumin concentration (43). In a model of ischemic-induced injury to equine jejunum, flunixin demonstrated inhibition of mucosal repair in vitro but not increased permeability to LPS of the ischemic tissues (44). However, the negative effects of a particular NSAID are not consistent throughout the gastrointestinal tract. In a model of colonic ischemia, flunixin significantly lowered pain scores and did not affect recovery or barrier integrity of ischemic injured colonic mucosa (45).

In summary, the gastrointestinal associated side effects of NSAIDs in horses include mucosal ulceration, (oral, esophageal, gastric, duodenal, cecal and right dorsal colonic), diarrhea and an associated protein-losing enteropathy, hypoproteinemia and associated ventral edema, difficulty with prehension and mastication from oral ulceration, anorexia, dullness and weight loss (5, 46). Additionally, intestinal mucosal damage can lead to breakdown of the protective barrier, resulting in translocation of bacteria into the circulation and endotoxemia (47).

### Renal Toxicity

Prostaglandins are also involved in normal renal function, impacting on renal circulation through vasodilation, renin secretion, and sodium and water excretion. There is

constitutive expression of both COX-1 and COX-2 isoforms in the kidney. Both traditional (phenylbutazone, flunixin) and COX-2 selective (firocoxib) NSAIDs have been reported to cause acute renal failure from renal papillary necrosis, particularly in association with dehydration or increased dosage, because adequate renal perfusion is not maintained by prostaglandin and medullary ischemia occurs (43, 48). For instance, during periods of hypovolemia or hypotension, PGI<sub>2</sub> and PGE<sub>2</sub> would normally cause afferent arteriolar dilation, which maintains renal blood flow and glomerular filtration rate. This effect is blocked by the administration of NSAIDs and dehydration, hypotension or pre-existing renal disease will increase the likelihood of acute renal failure developing (7).

### Cardiovascular Effects

In humans, long-term inhibition of COX-2 without simultaneous inhibition of COX-1 is linked to an increase in cardiovascular adverse events. This is believed to be due to affecting the balance between COX-2-derived vascular endothelial prostacyclin offsetting the thrombogenic properties of COX-1-derived thromboxane. However, in animals, cardiovascular disease is not often related to thromboembolic events. Adverse cardiovascular events have not been reported in clinical trials in dogs and horses where firocoxib was dosed up to 42 days (49).

The inhibition of thromboxane synthesis impacts platelets' ability to aggregate. Aspirin's inhibition of platelet aggregation is irreversible, until new platelets form without the influence of aspirin (50, 51). This can be of therapeutic benefit for cases of jugular vein thrombosis in horses, for example. In humans, aspirin reduces the risk for myocardial infarction or stroke, but has the unwanted side effect of increased risk of hemorrhage. Selective COX-2 inhibitors, including firocoxib, may not have the characteristic of inhibiting platelet aggregation.

Within cell membranes, NSAIDs affect processes including the oxidation of nicotinamide adenine dinucleotide phosphate in neutrophils and macrophage-based phospholipase C. The salicylates, ibuprofen, indomethacin and piroxicam are particularly inclined to inhibit

neutrophil function. NSAIDs have been shown to affect the formation of proteoglycans by chondrocytes, transmembrane ion transport and cell-to-cell binding. They can also unmask T-cell suppressor activity which may cause a decrease in rheumatoid factor (3).

### Hepatocellular Toxicity

There are a few reports of elevations in hepatic values with the use of firocoxib in dogs and horses (49), though there are no reports of clinically adverse effects. The use of another COX-2 selective NSAID, carprofen, has however been associated with more significant hepatotoxicity in dogs, with one case report of death in a dog following treatment with meloxicam and carprofen (52). Although reports of elevated hepatic values are relatively common, reported adverse effects are generally minimal and liver values improve with cessation of administration (53). No significant hepatocellular effects of NSAID administration are reported for horses.

### Neonates and NSAIDs

#### Differences in Drug Metabolism

There are significant pharmacokinetic differences between animal and human neonates and adults (54, 55). The structural and functional characteristics of neonates which influence drug disposition include deficiencies in drug metabolizing enzymes, glomerular filtration and renal tubular secretory mechanisms, plasma proteins that influence drug binding and a relative increase in volume of body fluid in neonates. These factors all play a role in susceptibility to toxic effects of certain drugs, and specifically NSAIDs. In a study which evaluated the pharmacokinetics of flunixin in healthy foals less than 24 hours old (56), the volume of distribution was much larger and plasma clearance was markedly reduced. The differences resulted in a longer plasma elimination half-life and the elimination rate constant was reduced in foals when compared to adult horses. The conclusion from this study was that foals may require larger doses at longer dosing intervals to achieve the same plasma concentrations as adult horses. Similar conclusions

were drawn from findings in a study evaluating ketoprofen in healthy foals less than 24 hours old (57), which also reported a larger volume of distribution, markedly reduced clearance, longer half-life and reduced elimination rate constant in foals. Recent research in foals less than six weeks of age using the COX-2 selective inhibitor, meloxicam, at 0.6 mg/kg PO after a single dose and q12h for 14 days, identified a similar time to maximum plasma concentration as adults. However, elimination half-life, and therefore drug clearance, was more rapid in foals than adults (58). Hence, characterization of the pharmacokinetic disposition of specific NSAIDs in neonatal foals is essential and should not rely on adult studies, as considerable changes in pharmacokinetics occur as animals mature.

#### Toxicity of NSAIDs in Neonates

There are few published reports of NSAID toxicosis in foals. In a 1988 (42) study reporting effects of chronic flunixin meglumine therapy in foals, flunixin was administered at 1.1 mg/kg PO divided into 2 doses (n = 3) or 1.1 mg/kg IM once daily (n = 7) for 30 days. There were also comparable control groups. Renal lesions were not observed in any of the foals, however all of the foals dosed per os with flunixin developed oral ulcers and on post-mortem examination, all foals receiving flunixin had developed erosions of the glandular portion of the stomach. Two types of erosions were noted. In the pyloric region, there were irregular areas of mucosa with a heavy polymorphonuclear cell infiltration on the eroded surface. The other type of erosions observed were linear, crease-like lesions up to several centimeters in length in the fundic portion of the glandular mucosa. The pathogenesis of gastric ulceration in NSAID toxicosis was thought to be due to inhibition of PGE<sub>2</sub> synthesis.

In another study evaluating flunixin administration to neonatal foals, foals were administered flunixin from two days of age for five days, at doses of 0.55, 1.1, 2.2 and 6.6 mg/kg intravenously (59). Some foals developed diarrhea, but the most relevant finding was that foals in the 6.6 mg/kg group had more prominent gastrointestinal lesions than the other groups, particularly in the cecum, including petechiations. Additionally,



loss of total protein occurred. Hematological and serum biochemical changes were not statistically significant. No renal lesions were identified in this study. The severity of glandular stomach mucosal ulcerations with administration of flunixin to foals were comparable to those seen with phenylbutazone dosed at 10 mg/kg PO for 12 to 42 days (60).

Recent work with meloxicam in foals less than six weeks of age identified no significant adverse events when dosed at 0.6 mg/kg PO q12h for 14 days. Monitoring for adverse events consisted of physical examinations, monitoring of body weight, complete blood count and serum biochemistry evaluation, urinalysis including urine enzyme concentrations, gastroscopy and abdominal ultrasonography. After a seven day washout period, ten foals were then dosed at three times the recommended dose (1.8 mg/kg PO), twice daily for seven days. No significant changes in physical examinations, complete blood count or serum chemistry were observed. Mild gastric ulceration (grade 1) was present in two foals at the commencement of the higher dose, and one of these foals developed a single grade 2 lesion at the end of the seven day study period. Fecal occult blood tests and abdominal ultrasound were within normal limits (58). Though very limited safety data for COX-2 selective NSAIDs in young foals is available, the results of a recent study are promising for better therapeutic alternatives in this age group.

Although no specific information is available for foals, the fact that dehydration increases the incidence of nephrotoxicosis from NSAID administration is well established (48, 61). Even in healthy foals, ensuring adequate hydration when administering NSAIDs is important in limiting the incidence of toxicosis.

Firocoxib

Current Literature

Firocoxib, is a highly COX-2 selective NSAID, which was developed specifically for the veterinary market by Merial Ltd® (Duluth, Georgia, USA) in 2004. The major

metabolites of firocoxib are descyclopropylmethylfirocoxib and its gluconuride conjugate. Whole blood tests determined that both metabolites have little or no pharmacologic activity (62). Currently, published literature is available for pharmacokinetic trials in adult dogs, cats and horses, with some studies investigating toxicity and clinical efficacy for various disease states also available in these species.

In canine whole blood in vitro assays, firocoxib displays a 350- to 430-fold selectivity for COX-2 over COX-1. When comparing other COX-2 selective NSAIDs, firocoxib shows greater COX-2 selectivity than deracoxib and carprofen. In dogs, the COX-1:COX-2 ratio for IC<sub>50</sub> values for firocoxib, deracoxib and carprofen are 384, 12 and 7 respectively, and for IC<sub>80</sub> values are 427, 12 and 6, respectively. Pharmacokinetic parameters in dogs display rapid and complete absorption after oral administration, with a peak plasma concentration one hour after oral administration. There was low systemic clearance and a plasma elimination half-life of 5.9 ± 1.1 hours. There was minimal first-pass removal from circulation by the liver, good distribution into body tissues and once or twice daily dosing was deemed appropriate (63).

When comparing inhibition of COX activity of various NSAIDs in horses, dogs and cats, firocoxib appears to be equipotent to deracoxib, meloxicam, indomethacin and ketoprofen, 30-fold more potent than carprofen and 90-fold more potent than phenylbutazone. Potency of each compound was determined by establishing the concentration at which 50% of COX activity was inhibited (IC<sub>50</sub>). Activities of COX-1 and COX-2 were determined by measuring TXB<sub>2</sub> and PGE<sub>2</sub> concentrations in whole blood with and without addition of each compound (64). Firocoxib is also a weak inhibitor of COX-1 compared to other NSAIDs. It was shown to be effective prophylactically and therapeutically in attenuating lameness in dogs with urate crystal-induced synovitis, which is a standard method of assessing efficacy in canines (63). In another preclinical trial utilizing the urate crystal-induced lameness, firocoxib demonstrated greater efficacy than carprofen in a dose-dependent manner (65).

Clinical trials in dogs have been fairly extensive and generally involve the use of firocoxib for osteoarthritis. In 2006, an extensive 1000 dog clinical trial across the United States, firocoxib was provided as the sole NSAID therapy for dogs suffering from osteoarthritis (49). Dogs were evaluated by owners and veterinarians at 10 and 40 days, with 88.2% dogs considered mildly to greatly improved by owners and 87.4% considered improved by veterinarians at 10 days. On day 40, veterinarians rated 92.8% of dogs improved and owners rated 90.8% of their animals as improved. Owners rated 86% of dogs as happier or more active, suggesting an improvement of quality of life with firocoxib treatment. Side effects reported were mild, affected a small percentage of animals and included vomiting, and elevations in serum BUN, creatinine and liver enzymes. These were generally without outward clinical signs.

Two other extensive clinical trials from 2006, (20, 66), compared the use of firocoxib to etodolac and carprofen, which are NSAIDs commonly utilized in small animal practice. In a positive-control, double-blinded, multicenter clinical trial comparing firocoxib and etodolac, 249 client-owned dogs with osteoarthritis were treated with either drug for 30 days, with examinations on days 0, 14 and 29. The drugs were comparable in efficacy, and firocoxib displayed significantly greater improvement from baseline than etodolac for lameness at a trot on days 14 and 29, and for lameness at a walk, pain on manipulation and range of motion on day 29. Additionally, fewer abnormal health events were recorded by owners of dogs treated with firocoxib than etodolac. In a double-blind, randomized, controlled, multicenter field study in 218 dogs with osteoarthritis comparing the efficacy of firocoxib and carprofen over 30 days, veterinarians reported that 92.5% of dogs treated with firocoxib and 92.4% of dogs treated with carprofen had improved. Dogs treated with firocoxib had a significantly greater reduction in lameness and had 36% fewer side effects than dogs treated with carprofen. Gastrointestinal problems were most commonly reported (20).

In a long-term, (52 week), prospective study involving 39 dogs with osteoarthritis treated with firocoxib, 96% of the 25 dogs that completed the study had improved (67). Three dogs dropped out due to treatment failure, four due to side effects related to treatment

(including one dog with a fatal duodenal perforating ulcer after inadvertent administration of a double dose), and the remainder were for reasons unrelated to firocoxib administration. In general, there was a low rate of side effects, with gastrointestinal signs including diarrhea (1%) and vomiting (2.5%) being reported. Serum creatinine, an indicator of renal function, rose above the reference range in two dogs and resulted in their exclusion from the study. Creatinine values returned to within the reference interval after cessation of firocoxib administration. Improvement over the first 15 days (82.5%) was slightly lower than previously reported at 93.4% (20). This was the first study of coxibs in veterinary species over an extended time period and results were encouraging with 64% of dogs improving for overall score from day 90 to 360 (67).

The effect of firocoxib in a model of canine gastric mucosal healing has been evaluated (68). Inhibition of COX-2 is associated with delayed mucosal healing in mice (69) and results of the canine study conferred with this finding. Goodman et al. (2009) determined that in vivo, firocoxib is highly COX-2 specific, has a greater decrease in PGE<sub>2</sub> production compared to tepoxalin and a placebo, does not alter mucosal prostaglandin concentrations (compared with a placebo) but slows pyloric mucosal healing and is associated with larger mucosal lesions when compared to tepoxalin and a placebo. The study concluded that further work is needed to investigate how mucosal healing is altered by compounds which suppress prostaglandin synthesis. Results from this study imply that consideration needs to be made for the use of firocoxib in canines and possibly other species with gastrointestinal mucosal defects.

Clinical trials in dogs report comparable or better efficacy for firocoxib to other commonly utilized NSAIDs in small animal practice. The studies report fewer adverse effects with firocoxib than other NSAIDs and these are predominantly gastrointestinal related. Effects on mucosal healing of selective COX-2 inhibitors including firocoxib require further investigation. No adverse interactions with other medications the patients were receiving were reported in any of the trials and overall, the use of firocoxib in canine osteoarthritis was considered favorable. Pharmacokinetic evaluation in companion

animals identified once daily dosing, good oral bioavailability and a relatively long elimination half-life.

### Pharmacokinetics in Adult Horses

Firocoxib was developed by Merial Ltd.<sup>®</sup> into an oral paste formulation (marketed as *Equioxx*<sup>®</sup>) specifically for horses. An intravenous injectable formulation is also available. Most of the safety information that is currently available has been determined by Merial Ltd.<sup>®</sup> (NADA 141-253). Other information available in horses is mainly in relation to pharmacokinetic parameters and clinical trials for firocoxib's use in osteoarthritis in adult horses.

Pharmacokinetic studies in adult horses show firocoxib to be a highly selective COX-2 inhibitor, with a COX-1/COX-2 IC<sub>50</sub> ratio of 263-643 in the horse, (62). The drug follows linear pharmacokinetics after multiple oral and intravenous dosing. Time to peak serum concentration after a single oral dose of  $7.8 \pm 4.80$  hours (mean  $\pm$  SD) (70). T<sub>max</sub> in adults after a single oral dose has also been reported at  $3.9 \pm 4.40$  hours, (62). The elimination half-life in adults is  $29.6 \pm 7.5$  hours, (62) and the average maximum serum concentration following a single oral dose of firocoxib is  $45.0 \pm 11.3$  ng/mL, though also reported as  $75.0 \pm 33.0$ , (62, 70). After multiple daily oral doses, the average maximum serum concentration is  $173 \pm 44.0$  ng/mL, (Letendre, 2008). In adult horses, average bioavailability is 79%. The drug displays a high volume of distribution at 1.5 L/kg, which is likely due to it being highly lipophilic. It is well distributed throughout the body and was detected in synovial fluid at approximately 30% of plasma concentration. When comparing oral and intravenous dosing, concentration-time profiles were similar, displaying parallel slopes with comparable half-lives that were three times longer than half-life of the drug in dogs. Additionally, firocoxib's half-life is five to ten times longer than reported for other NSAIDs, including flunixin and phenylbutazone, supporting once daily dosing of firocoxib (62). Total systemic clearance of firocoxib in adult horses ( $27.9 \pm 11.3$  mL/kg/h) is similar to other NSAIDs. As mean renal clearance is much lower at  $0.26 \pm 0.09$  mL/kg/h than total body clearance, it was concluded that hepatic clearance

via metabolism of the drug is the primary elimination mechanism for firocoxib in horses (70).

### Efficacy and Clinical Applications for Equine Practice

Much of the current equine data on firocoxib focuses on its effectiveness as an anti-inflammatory and analgesic agent for osteoarthritis. To determine an effective dose of firocoxib for chronic equine lameness (71), researchers used a force plate to evaluate doses of 0.05, 0.1 and 0.25 mg/kg q24h PO, in horses with chronic lameness presumed due to osteoarthritis, including navicular disease. Lameness improved greater than one grade with 0.25 mg/kg and 0.1 mg/kg q24h PO doses of firocoxib, from which it was concluded that 0.1 mg/kg q24h PO of firocoxib is effective at attenuating lameness in horses with chronic osteoarthritis. These results were similar to a field study where firocoxib at 0.1 mg/kg q24h PO was as efficacious as phenylbutazone in horses with chronic, naturally occurring osteoarthritis (72). In another prospective, randomized, controlled, double-blinded multicenter field trial for firocoxib, 96 client-owned chronically lame horses with osteoarthritis were evaluated after 14 days of oral firocoxib (73). Horses were administered firocoxib (n = 48) or vedaprofen (n = 48) and evaluated on days 1, 7 and 14. By day 14, 83% of the firocoxib horses had improved, versus 65% of vedaprofen-treated horses. Although statistically not significant, there was a four-fold lower incidence of side effects in the firocoxib treated group. In a 253 client-owned horse study conducted by Merial Ltd.<sup>®</sup>, veterinarian assessment judged 84.4% of horses improved based on lameness, pain on manipulation, range of motion and joint swelling (NADA 141-253). Studies evaluating firocoxib for treatment of osteoarthritis in adult horses show promising results for efficacy and a low incidence of side effects.

In a study evaluating the effect of firocoxib compared to flunixin meglumine on recovery of ischemic-injured jejunum and analgesia, it was concluded that unlike flunixin, firocoxib did not inhibit recovery of ischemic-injured mucosa in the jejunum, both drugs were effective analgesics and that firocoxib may be superior in horses recovering from ischemic intestinal injuries (74). These conclusions were made based on finding lower

transepithelial resistance and increased lipopolysaccharide permeability (which leads to endotoxin translocation) in the flunixin-treated horses versus firocoxib or saline controls. Thus, although as a selective COX-2 inhibitor, firocoxib may inhibit mucosal healing (68), it shows promise after ischemic injury in the gastrointestinal tract.

Horses often receive long-term NSAID therapy for ocular inflammation, particularly when glucocorticoids are contraindicated. NSAIDs are effective in ocular inflammation because they inhibit the effects of prostaglandin in the eye, which include miosis, increased vascular permeability of the blood-ocular barrier, conjunctival hyperemia, alterations in intraocular pressure and influx of fibrin, protein and cells (75). Firocoxib provides a useful anti-inflammatory alternative for horses at risk for adverse side effects associated with traditional NSAIDs, including flunixin. It has been determined that flunixin lessens the accumulation of inflammatory mediators in the equine eye.

Researchers investigated ocular penetration of firocoxib and flunixin administered orally over seven days, to determine whether firocoxib may be a viable alternative to the current drug of choice, flunixin, for ocular inflammation (76). They determined that on days 3 and 5, firocoxib was present in aqueous humor to a greater extent than flunixin in healthy equine eyes, with an aqueous to serum ratio of  $3.59 \pm 3.32\%$  (mean  $\pm$  SD) for flunixin and  $11.99 \pm 4.62\%$  (mean  $\pm$  SD) for firocoxib. Though clinical efficacy in cases of ocular inflammation has not been reported for firocoxib, this study showed that orally administered firocoxib penetrates the aqueous humor better than flunixin and may be an alternative for horses at risk of NSAID toxicity.

### Toxicity Data for Horses

Research into long term dosing and higher than recommended therapeutic doses of firocoxib, are limited to safety studies conducted by Merial Ltd.<sup>®</sup>. The label (NADA 141-253) states that *Equioxx*<sup>®</sup> can be administered up to 14 days to control pain and inflammation associated with osteoarthritis in horses at an oral dose of 0.1 mg/kg once daily. In their target animal safety studies, toxicity was seen at the recommended dose once administration exceeded 30 days. It also states that the safe use in horses less than



one year of age, horses used for breeding and in pregnant or lactating mares has not been evaluated. In a controlled field study using 127 horses from three to 37 years of age, orally administered firocoxib for 14 days was associated with diarrhea in two horses, loose stool in one horse and excitement in one horse. In a target animal safety study, eight healthy horses were administered firocoxib at 0, 1X, 3X and 5X the recommended labeled dose for 42 days. Delayed healing was noted in preexisting oral ulcers of the lips, tongue and gingiva in horses receiving the 1, 3 and 5X dosage. One horse in the 5X group developed a mildly elevated BUN and creatinine, prolonged buccal mucosal bleeding time (BMBT) and dilated renal pelvis. Another 5X horse developed prolonged BMBT, bilateral tubulointerstitial nephropathy and bilateral papillary necrosis. One horse in the 1X group developed papillary necrosis. All animals remained clinically healthy with normal hematology, clinical chemistry and urinalysis values.

Another target animal safety study dosed at 0X, 2.5X, 7.5X and 12.5X the recommended labeled dose to 6 horses per group for 92 days. An additional group was dosed at 12.5X the recommended labeled dose and was monitored until day 147 to 149. All treatment groups showed treatment-related adverse events including ulceration of the lips, gingiva and tongue, erosions of the skin on the mandible and head, and gross and microscopic lesions of tubulointerstitial nephropathy. Renal papillary necrosis was seen in the 2.5X and 12.5X groups, while some of the 12.5X horses displayed elevated hepatic enzymes (AST, GGT, SDH and ALT). Surprisingly, stomach ulceration of the margo plicatus and glandular region were more common in the 2.5X and 7.5X groups, but not found in the 12.5X group. The group of horses monitored until day 147 to 149 showed recovery from the skin and oral ulceration but not from tubulointerstitial nephropathy. In a study evaluating efficacy and safety of phenylbutazone dosed at 4.4 mg/kg PO q24h (n = 126) and firocoxib dosed at 0.1 mg/kg PO q24h (n = 127) administered orally to adult horses with naturally occurring osteoarthritis for 14 days, there were no adverse treatment-related events reported and all serum biochemical and hematological values remained within reference intervals (72). Safety studies and field trials identify firocoxib as a relatively safe NSAID when administered to healthy horses. As with other NSAIDs,



adverse effects are typically related to the gastrointestinal or renal systems, though renal abnormalities may be more likely than with other NSAIDs.

## Significance of Study

NSAIDs are used extensively for treatment of pain and inflammation in horses. The mechanism of action of NSAIDs is through inhibition of COX activity, thereby inhibiting precursors for a variety of pro-inflammatory prostanoids produced by the arachidonic acid cascade. In general, toxic effects of NSAIDs are related to inhibition of COX-1 and its homeostatic, protective functions in the gastrointestinal and renal systems in particular. The anti-inflammatory effects are primarily related to the inhibition of the largely inducible, COX-2 isoform, which is up regulated at sites of inflammation. The current NSAID focus is on developing drugs which limit their influence on COX-1 and target COX-2 for safer, but still effective, anti-inflammatory therapy. This concept is of particular importance to neonates, which are more susceptible to the toxic effects of NSAIDs due to differences in metabolism.

Firocoxib is the first NSAID of the coxib class to be registered for use in horses. Of the coxibs, it has the highest selectivity for COX-2, thereby providing greater COX-1 sparing than other NSAIDs currently available. Target animal safety studies show promise for a considerable safety margin in horses and field trials in adults have identified firocoxib as being as effective for therapy in osteoarthritis as established NSAIDs available on the veterinary market.

Due to its potential for limited toxicity, firocoxib shows promise as a therapeutic option for neonatal foals, however pharmacokinetic evaluation in this age group is lacking. As neonates and adults display differences in drug pharmacokinetics, adult data cannot be extrapolated to neonates and studies to evaluate the pharmacokinetic profile of firocoxib in equine neonates are warranted. Additionally, numerous practical benefits for using oral firocoxib exist for foals. Oral administration is easier and avoids the need for repeated painful injections. Additionally, firocoxib's relatively long half-life suggests once daily dosing is adequate in adults.

Firocoxib has potential as an effective anti-inflammatory therapy in neonates due to its highly selective inhibition of COX-2 and reduced risk of toxicosis associated with administration of nonselective NSAIDs.

Chapter 2

Manuscript

THE PHARMACOKINETICS OF FIROCOXIB AFTER MULTIPLE ORAL DOSES  
TO NEONATAL FOALS

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## Abstract

The purpose of the study reported here was to determine the pharmacokinetics and safety profile of firocoxib in neonatal foals. Seven healthy foals were administered 0.1mg/kg firocoxib orally q24h for nine consecutive days, commencing at 36h of age. Blood was collected for firocoxib analysis using high pressure liquid chromatography with fluorescence detection at 0 (dose #1 only), 0.25, 0.5, 1, 2, 4, 8, 16 and 24 hours after doses #1, 5 and 9. For all other doses (2, 3, 4, 6, 7 and 8) blood was collected immediately prior to the next dose (24 hour trough). Elimination samples (36, 48, 72, 96, 120 and 144 hours) were collected after dose #9. Safety was assessed via physical examinations, changes in body weight, gastroscopy, complete blood count, serum biochemistry and urinalysis.

Firocoxib was rapidly absorbed following oral administration with minimal accumulation after repeat dosing. Steady state was obtained after approximately 4 doses. After the final dose, the terminal half-life was approximately 11 hours. Firocoxib was not detected in plasma 72 hours after the final dose (<2ng/mL). No significant abnormalities were noted on blood work, urinalysis or gastroscopy.

This study demonstrated that firocoxib is absorbed after oral administration in neonatal foals with no observable adverse effects after multiple doses.

## Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) represent some of the most widely used medications in human and veterinary medicine worldwide. NSAIDs are utilized therapeutically in horses for their anti-inflammatory, analgesic, and anti-pyretic effects, particularly in cases of endotoxemia, gastrointestinal and musculoskeletal disease. They are also a mainstay of therapy for neonatal sepsis, particularly flunixin meglumine, and have antithrombotic effects.

Firocoxib (3-(cyclopropylmethoxy)-4-(4-(methylsulfonyl)phenyl)-5,5-dimethylfuranone) is the first of the coxib class of NSAIDs to be approved for use in horses (Equioxx<sup>®</sup>, Merial Ltd, Duluth, Georgia, USA; NADA 141-253). This novel, second generation NSAID, is reported to have reduced adverse effects due to its high selectivity for COX-2. It is approved for administration in horses to mediate pain and inflammation associated with degenerative joint disease. Several studies have described the pharmacokinetic profile of firocoxib in adult horses (62, 70). However, the pharmacokinetic profile of firocoxib in neonates has not been established.

The mechanism of action of NSAIDs is inhibition of cyclooxygenase (COX) activity, thereby inhibiting the precursors for a variety of pro-inflammatory prostanoids, particularly prostaglandins and thromboxanes (2). The discovery of multiple isoforms of COX in the 1990s, especially the constitutive COX-1 and inducible COX-2, has revolutionized the field of NSAID pharmaceutical development. It is apparent that COX-1 has primarily homeostatic physiologic functions including maintenance of renal blood flow, gastrointestinal mucosal integrity and platelet aggregation. For example, in the gastrointestinal tract, prostaglandin E<sub>2</sub> and prostaglandin I<sub>2</sub> (prostacyclin) maintain gastric integrity by reducing gastric acid secretion, increasing production of protective mucus, vasodilating mucosal blood vessels and increasing duodenal bicarbonate secretion (9). COX-1 expression is not typically a component of inflammatory conditions. In contrast, COX-2 is primarily an inducible enzyme, with up regulation occurring in acute and chronic inflammation and ischemia. Suppression of this COX isoform is considered

responsible for reducing inflammation. However, COX-2 also has some constitutive functions in the body, including in the intestines, central nervous system and juxtaglomerular apparatus of the kidney (22, 77).

The toxicity of nonselective NSAIDs in equids, which suppress production of both COX-1 and COX-2, has been well established (78). Drugs in this category include flunixin meglumine and the NSAID most commonly administered to horses, phenylbutazone (72). Adverse effects associated with NSAID administration, even at therapeutic doses, include gingival and gastric ulceration, hypoproteinemia and renal papillary necrosis (38, 48, 61). Evidence is increasing for the ability of selective COX-2 inhibitors to inhibit inflammation without disrupting normal organ function that is primarily maintained by COX-1 expression (77, 79).

Foals are more sensitive to the side effects of NSAIDs, particularly the ulcerogenic effects on gastric mucosa, primarily due to differences in pharmacokinetic clearance mechanisms (42, 60). The pharmacokinetic differences between neonates and adult horses of non-selective NSAIDs, including flunixin meglumine and phenylbutazone, have been established (80, 81). These variations in drug disposition are due to differences in relative volumes of body fluid, plasma protein levels, renal function and deficiencies in drug metabolizing enzymes, and typically result in the need for lower doses or extended dosing intervals, in order to avoid adverse effects.

Due to the increased risk for toxicity of NSAIDs in neonates, it appears that a coxib class of drug would be the safer anti-inflammatory choice in the ill equine neonate. This may be particularly relevant in conditions such as sepsis and endotoxemia, where reduced organ perfusion may enhance toxicity of traditional (nonselective) NSAIDs. To date however, little work has been done with coxibs, (specific COX-2 inhibitors), in neonates. To the authors' knowledge, there is to date only one published report on the pharmacokinetics of a coxib, (meloxicam), in neonatal foals (82).

The purpose of the present study was to determine the pharmacokinetic profile of firocoxib in healthy neonatal foals, and to monitor the foals for the development of adverse gastrointestinal and renal effects following multiple doses. We hypothesized that firocoxib given per os at the labeled dose to neonatal foals would be absorbed and not be associated with clinically significant adverse events.



## Materials and Methods

### Animals

Seven healthy neonatal American Quarter Horse foals of mixed gender, (six colts and one filly), were used in this study. All mares were observed pre- and post-parturition as part of a student-based foal watch program at Virginia Tech. After birth, foals were observed for normal developmental and behavioral patterns. Foals were determined to be healthy based on physical examination findings, complete blood count, serum biochemistry, urinalysis and plasma IgG concentrations evaluated at 24 hours of age.

During the study period, physical examinations were performed twice daily and body weight evaluated every two days. Foals were housed in stalls with their mares and provided with small paddock turnout twice daily. They were allowed to suckle normally. Throughout the study period, mares were provided with *ad libitum* access to mixed alfalfa/grass hay and water, as well as two kilograms of a commercial mare and foal feed, (16% protein, 6% fat and 12% fiber<sup>1</sup>), twice daily.

At 30 hours of age, foals were sedated with xylazine<sup>2</sup> (0.5mg/kg intravenously) and a 16 gauge, 5¼ inch over-the-wire polyurethane catheter<sup>3</sup> was placed using aseptic technique into the right jugular vein. Catheters were maintained *in situ* for the initial 10 days of the study. Immediately after catheter placement, gastroscopy was performed and the stomach evaluated for presence of ulcers.

The Virginia Polytechnic Institute and State University, Institutional Animal Care and Use Committee approved the use of animals for this study and all study protocols.

### Drug Administration

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<sup>1</sup> Legends Mare and Foal Textured®, Southern States Cooperative, Genworth, VA, USA

<sup>2</sup> AnaSed® Injectable, Lloyd Incorporated, Shenandoah, IA, USA

<sup>3</sup> MILA International®, Erlanger, KY, USA

Dosing of firocoxib<sup>4</sup> (0.1mg/kg q24 hour per os) as a 0.82% (wt/wt) paste was commenced when foals were 36 hours of age and continued for nine consecutive days at 24 hour intervals (i.e. last dose was administered when foals were 10 days of age). Foals were weighed every 48 hours and the firocoxib mass specific dose adjusted accordingly. Doses of firocoxib were individually prepared and administered by oral dosing in a prefilled syringe.

### Sample Collection

Whole blood samples (5mL) were collected via the indwelling jugular catheter in heparinized tubes<sup>5</sup> for firocoxib analysis at times 0 (day 1 of study only) and 0.25, 0.5, 1, 2, 4, 8, 16 and 24 hours after dosing for doses 1, 5 and 9. For all other doses (2, 3, 4, 6, 7 and 8), blood was collected immediately prior to the next dose (considered the 24 hour trough concentration) to ensure that steady state was achieved. Catheters were flushed with 5ml heparinized saline (2% heparin solution) and then 0.5ml heparin<sup>6</sup> (1000 USP units/mL) was infused into the catheter and extension line until the next sampling time to prevent blood clot formation. The heparin was removed from the line and discarded at the next sampling time. Following firocoxib dose #9, additional elimination samples were collected at 36, 48, 72, 96 and 120 hours post dosing via venipuncture. Following collection, samples were immediately centrifuged<sup>7</sup> and the plasma was then separated and stored at -80°C until analysis.

### Monitoring

Between three and five free catch urine samples were obtained on different days of the study from six of the seven foals. Urinalyses were performed, including specific gravity and urinary GGT-to-creatinine ratio. Gastroscopy was performed immediately after catheter placement (day 0) and on day 10 to evaluate for presence of gastric ulcers. Foals

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<sup>4</sup> Equioxx® (firocoxib), Merial, The Animal Health Division of Sanofi, Duluth, GA, USA

<sup>5</sup> BD Vacutainer, BD Franklin Lakes, NJ, USA

<sup>6</sup> Heparin, SAGENT Pharmaceuticals, Inc. Schaumburg, IL, USA

<sup>7</sup> International Clinical Centrifuge Model CL, International Equipment Co., 115 VAC, 50/60 HZ, 1.2 AMP, Needham, MA, USA.

were sedated with 0.5mg/kg xylazine intravenously and gastroscopy performed (nasogastric) with a one meter endoscope<sup>8</sup>. Blood was submitted for complete blood count and serum biochemistry on days one and 11. On days three and seven, blood was submitted for evaluation of blood urea nitrogen (BUN) and creatinine only.

## Drug Analysis

Firocoxib concentrations in plasma were determined using HPLC with fluorescence detection with modifications from a previously published method (83). Samples were analyzed at PKDM Department of Merck Ltd. (Bridgewater, NJ). The current analysis method differed from that previously described in that fluorescence detection rather than UV detection was utilized for increased accuracy and to allow for a lower limit of quantitation at 5ng/mL (versus 25ng/mL with UV detection). Additionally, a smaller sample size (0.2 vs 1-2mL plasma) was used. In brief, plasma samples were thawed at room temperature, vortexed and centrifuged. Acetonitrile (1mL) followed by 0.2mL plasma were added to the well of a 96-well precipitation plate and left to sit for approximately three minutes. A vacuum was applied and the eluate collected in 2 to 4mL collection plate before being evaporated to dryness. The residue was reconstituted in 0.2mL of 40% acetonitrile in water and a 0.05mL aliquot injected into a HPLC with fluorescence detection. Separation was accomplished on a Zorbax XBD precolumn (4.6 x 12.5 mm) and Zorbax RxC18 column (150 x 3 mm, 3.5 µm) using a mobile phase with 45% acetonitrile, 55% water and 0.25% trifluoroacetate anhydride. Two HPLCs were used for this analysis, one at a flow rate of 0.5 mL/min, and the other at 0.8 mL/min. The column was maintained at 40°C with an excitation wavelength of 250 nm and emission wavelength of 375 nm. The standard curve ranged from 2.5 to 250 ng/mL. Acceptability of bioanalytical sets was based on the standard curves and fortified control sample results. Quality controls were prepared by adding an aliquot of standard solution to 200 uL of plasma. Three quality controls at each of three levels were prepared with

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<sup>8</sup> Olympus® Exera GIF-160, Olympus America, Center Valley, PA, USA

each set. The linear regression parameters for each set were determined with statistical software<sup>9</sup>.

### Pharmacokinetic Analysis

Pharmacokinetic parameters for multi-dose firocoxib were calculated using the noncompartmental analysis model (log/linear trapezoidal) of WinNonlin® version 5.0.1 pharmacokinetic software<sup>10</sup>. Individual animal and average pharmacokinetic measures were obtained. The maximum ( $C_{\max}$ ) and minimum ( $C_{\min}$ ) plasma concentrations and times ( $T_{\max}$ ,  $T_{\min}$ ) to those values were determined directly from the plasma concentrations of firocoxib. The average maximum and minimum concentrations at steady state ( $C_{ss,\max}$  and  $C_{ss,\min}$ ), and the terminal half-life ( $t_{1/2}$ ) were determined. Area under the curve (AUC) for zero to 24, ( $AUC_{0-24}$ ), and zero to infinity, ( $AUC_{0-\infty}$ ), was established. Descriptive values were reported as Mean  $\pm$  SD.

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<sup>9</sup> Microsoft Office Excel 2003, Microsoft, Redmond, WA, USA

<sup>10</sup> WinNonlin® version 5.0.1, Pharsight Corporation, Mountain View, CA, USA

## Results

### Pharmacokinetics

Following oral administration to neonatal foals, firocoxib (0.1mg/kg) was rapidly absorbed. After the initial dose, an average peak serum concentration ( $C_{max}$ ) of  $89.50 \pm 53.36$  ng/mL (mean  $\pm$  SD) was achieved ( $T_{max}$ ) in  $0.54 \pm 0.65$  hours. Figure 1 shows the average concentration-versus-time curve for neonatal foals ( $n = 7$ ) treated with nine consecutive oral daily doses of firocoxib (0.1mg/kg). There was minimal accumulation after repeat dosing, with an average maximum concentration ( $C_{avg}$ ) in serum of  $39.1 \pm 8.4$  ng/mL and steady state was achieved after either three or four doses in all the foals.

Following the ninth dose of firocoxib, the harmonic mean of the elimination half-life ( $T_{1/2\lambda}$ ) was  $10.46 \pm 4.97$  hours. Bioavailability could not be determined as there is no accompanying intravenous dose of firocoxib for this age group to permit the calculation. Table 3 displays the calculated mean  $\pm$  SD after single (day 1) and multiple (days 5 and 9) consecutive oral daily doses of firocoxib. The final dose of firocoxib was administered at 192 hours. Drug was detected in six out of seven foals for 36 hours, and in four out of seven foals for 48 hours after the final dose. Drug was not detected in any foals at 72 hours after the final dose. Figure 2 shows the individual concentration versus time curve from dose 9 through elimination samples.

### Physical Examinations and Body Weight Monitoring

Twice daily physical examinations remained within normal limits for all study subjects, other than the complications discussed below in two of the foals. No oral ulceration was observed during the course of the study. All foals continued to gain appropriate weight over the study period, with most foals gaining at least one kilogram per day.

### Gastroscopy

No abnormalities were observed on initial gastroscopy. On day 11, only Foal E displayed mild hyperkeratosis of the margo plicatus. All other evaluations of esophageal, gastric and pyloric mucosa were unremarkable.

#### Hematologic and Biochemical Parameters

All of the foals had mild changes consistent with stress at one or both of the sampling periods. This was evidenced in all foals by either mild leucopenias due to a lymphopenia or mild lymphopenia without changes to the total white cell count. Three of seven foals had a mild neutrophilia and lymphopenia on either day 1 or 11. Foal E had evidence of inflammation on day 11, which is discussed below. None of the blood urea nitrogen (BUN) or serum creatinine values were elevated at either of the sampling periods. In all foals, BUN was below published reference intervals on day 1 and on day 11 in four of the foals. On day 11 only, five of the foals had mild to moderately elevated serum GGT, with two also displaying mildly elevated AST.

Urinalyses were within reference intervals for 3 out of 7 foals throughout the study period. Four of the seven foals had traces of blood and/or protein in the urine on day 1. One foal (foal C) displayed trace proteinuria on day 11. Urine specific gravity measure remained low throughout the study, with the highest reading in any foal being 1.007 (foal G on day 8). Urinary GGT-to-creatinine ratios were determined for each of the foals on either three, four or five days across the study period, pending when free catch urine samples were obtained. Values for urinary GGT ranged from 1U/L to 20 U/L and urinary creatinine from 14mg/dL to 176.2mg/dL. Urinary GGT-to-creatinine ratios ranged from 5.59 to 31.06. Urinary enzyme values were highly variable before and after firocoxib administration.

Though likely unrelated to firocoxib administration, minor complications developed in three of the seven foals. On day 11 of the study, Foal E was lethargic, pyrexia (103.4°F) and lame in the left hind limb. The foal had a total white cell count of  $19.370 \times 10^3/\text{UL}$ , (reference interval  $9075 \pm 2200$ ; mean  $\pm$  SD), a segmented neutrophilia of  $17.046 \times$

$10^3/\text{UL}$ , (reference interval  $6528 \pm 2000$ ) and monocytosis of  $0.581 \times 10^3/\text{UL}$ , (reference interval  $305 \pm 145$ ). This was accompanied by a mild hyperfibrinogenemia (500 mg/dL, reference interval  $310 \pm 90$ ). Further diagnostics identified mild cellulitis at the catheter site, a patent urachus and inflammation of the left metatarsophalangeal joint. The foal was treated with medical and surgical management including umbilical resection and joint lavage. These complications were unlikely due to firocoxib administration and samples continued to be collected and evaluated until the end of the study period. The foal remained bright, continued to gain weight and was healthy at discharge on day 22.

One mare (Foal F) died 60 hours after foaling due to uterine artery rupture, despite aggressive medical therapy. The foal then was provided 25% of its body weight per day as a formulated mare's milk replacer<sup>11</sup>, offered free choice. Freshly prepared milk replacer was offered every six hours. In addition, the foal was dosed with omeprazole (1mg/kg per os q24h). No adverse effects were noted in the foal's attitude, demeanor or hydration status throughout the study period.

Foal G dislodged its intravenous catheter on day five, then developed mild cellulitis at its second catheter site on day ten, for which it received oral antimicrobials and topical therapy. The cellulitis resolved without complications.

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<sup>11</sup> Mare's Match®, Land O' Lakes inc., Saint Paul's, MN, USA

## Discussion

Firocoxib, given per os to neonatal foals at the standard adult therapeutic dose, achieves detectable concentrations in plasma without inducing clinical or laboratory evidence of toxicity.

Firocoxib is absorbed and eliminated more rapidly in neonatal foals than adults. The time to peak serum concentration ( $C_{\max}$ ) after a single oral dose of the drug is considerably shorter in foals at  $0.54 \pm 0.65$  hours (mean  $\pm$  SD) compared to adults at  $3.90 \pm 4.40$  hours (62). Another study evaluating the pharmacokinetics of firocoxib after multiple daily oral doses obtained a value for  $C_{\max}$  of  $7.80 \pm 4.80$  hours after the first dose in adults (70). The difference in results obtained from each of these studies in adult horses could be due to different methods of sample analysis, as the initial study used HPLC with UV detection and the second study utilized liquid chromatography-mass spectrometry-mass spectrometry. The elimination half-life is shorter in neonates at  $10.46 \pm 4.97$  hours when compared to adults at  $29.6 \pm 7.50$  hours (62). Additionally, the average maximum serum concentration following a single oral dose of firocoxib is higher in neonates ( $89.5 \pm 53.36$  ng/mL) compared to adults ( $45.0 \pm 11.3$  ng/mL), (70). Adult  $C_{\max}$  has also been reported as  $75.0 \pm 33.0$  (62, 70). After multiple daily oral doses, the average maximum serum concentration is lower in foals ( $71.2 \pm 21.52$  ng/mL) compared to adults ( $173 \pm 44.0$  ng/mL), (70). This finding is due to a lack of accumulation of firocoxib in foals versus adults, from faster hepatic metabolism and renal excretion. Firocoxib is metabolized via hepatic oxidation, probably by the cytochrome P450 2C subgroup, to inactive metabolites which are excreted in urine (68%) and feces (15%) (62). Greater activity of liver enzymes in neonatal animals could account for the faster metabolism and increased urine flow compared to adults for firocoxib's faster excretion. It appears that firocoxib displays the phenomenon of flip flop pharmacokinetics in foals, with the rate of absorption being slower than the rate of elimination.

Other NSAIDs, such as ketoprofen and flunixin, have longer half-lives and reduced elimination in foals (80, 81). This could result in more frequent and higher dosing of



firocoxib being required to maintain the same serum concentrations as in adults. However, whether similar serum concentrations in equine neonates would provide clinically appropriate anti-inflammatory, antipyretic and analgesic therapy needs to be established. Currently, there is no published pharmacodynamic or efficacy data for firocoxib for equine neonates.

In adult horses, bioavailability is 79% (62). Bioavailability could not be determined in the present study, as an intravenous dose was not administered.

Toxicity evaluation in adults (NADA 141-253) showed toxicity was not induced until treatment at the recommended dose exceeded 30 days. At 5 times the recommended dose, toxic effects including prolonged buccal mucosal bleeding times, mild increases in creatinine and papillary necrosis were observed in some of the horses. In another target animal safety study utilizing higher doses of up to 1.25 mg/kg q24 hours (or 12.5 times the recommended dose) for a longer period (92 days), treatment related adverse effects were seen in all groups. These included tubulointerstitial nephropathy, papillary necrosis, oral ulceration, gastric ulceration and erosion of skin on the mandible and head. Several horses in the 12.5 times group displayed elevations in liver enzymes (GGT, AST, SDH and ALT) though no clinical effects of hepatopathy were described. Ulceration improved but no recovery from tubulointerstitial nephropathy was observed by day 149. Therefore firocoxib shows a relatively high safety margin in adults but adverse effects become apparent at higher doses and with long dosing periods. This may also occur in foals and requires further investigation.

Although no gastric ulceration was evident after nine consecutive days of oral dosing in any of the foals, one foal was receiving omeprazole (foal F), which may have masked the development of lesions in this foal.

Flunixin meglumine, (the current primary NSAID used in equine neonates), administered at the recommended therapeutic dose (1.1 mg/kg) resulted in oral ulceration in all foals on days 10 or 11 and these increased in size and number over the 30 day study (42). In

the current study, firocoxib did not show any oral ulceration and only one foal had mild thickening of the margo plicatus on day 10. Therefore, firocoxib may be safer therapeutic alternative in foals at risk for gastric and oral ulceration.

Proteinuria and/or hematuria were observed in four of the seven foals on day 1. These are considered a normal finding in healthy neonatal foals during the first 48 hours of life, (84), and likely consistent with closure of the urachus and umbilical trauma at foaling.

Published reference intervals for urinary GGT-to-creatinine ratios for equine neonates are variable (85, 86). Mild elevations observed in the urinary GGT-to-creatinine ratios from the foals are within normal limits according to the most recent report in adult horses (87). Established reference intervals for urinary GGT-to-creatinine ratios for the equine neonate were determined in 96 hour old horse and pony foals (85). Four of the six healthy neonates in the current study had values above the findings in the Brewer et al. 1991 study prior to administration of firocoxib, indicating the high variability in urinary GGT-to-creatinine ratios in healthy foals. The values displayed a downward trend by day 11 in all four of the foals. Other renal biochemical parameters (creatinine and BUN) were within or below established reference intervals and no clinically apparent renal effects were observed. The low BUN values may be due to the foals remaining well hydrated by frequent suckling, as evidenced by low urine specific gravity measures throughout the study. Firocoxib did not have any clinically adverse effects on the kidneys of healthy foals in the current study.

Some of the foals displayed mild to moderately elevated GGT values during the study. Although biochemical profiles were not re-evaluated following the study, these foals remained part of a teaching herd at the VMRCVM that was closely observed over the following two years and no long term changes relating to a hepatopathy were reported. None of the other clinicopathological changes from the study were considered significant to the administration of firocoxib.

As no clinically apparent adverse side effects were observed during the course of this study, it appears firocoxib can be safely administered per os to healthy neonatal foals for at least nine consecutive doses at the labeled dose (0.1 mg/kg q24 hours).

At the labeled dose (0.1 mg/kg q24 hours), firocoxib provides substantial analgesia, without causing adverse effects, in experimentally induced lameness and in naturally occurring osteoarthritis in horse, with an efficacy that is comparable to phenylbutazone (72, 88). In a 96 horse field study, firocoxib was more effective at improving chronic degenerative joint disease pain than vedaprofen (73). Although firocoxib's clinical effectiveness for musculoskeletal pain in foals has not been evaluated in published material, it is possible that the anti-inflammatory and analgesic effects would be comparable.

Another frequent use of NSAIDs in neonates is for visceral inflammation and pain. In adult horses with experimentally induced jejunal ischemia, intravenous firocoxib administration provided effective visceral analgesia and improved recovery of mucosal barrier function in vitro faster than flunixin meglumine or the saline control (74). This was attributed to firocoxib's selectivity for COX-2, which is upregulated after ischemic injury. The current anti-inflammatory of choice for visceral pain in neonates is flunixin meglumine. As a non-selective COX-inhibitor, flunixin also inhibits the production of homeostatic COX-1, which is required for prostaglandin-mediated intestinal repair. As a highly selective COX-2 inhibitor, with a COX-1/COX-2 IC<sub>50</sub> ratio of 263-643 in the horse, (62), it appears that firocoxib could be a more appropriate choice for visceral pain in neonates.

Due to its clinical effectiveness in adults when compared to other NSAIDs and reduced side effects as reported in toxicity studies, firocoxib provides promise as an effective and safer anti-inflammatory choice than the frequently utilized non-selective COX inhibitors, including flunixin meglumine, ketoprofen and phenylbutazone, for neonatal foals. This study provides initial evidence for the safety and absorption of oral firocoxib in the neonate. However, more information is needed to determine the appropriate anti-

inflammatory and analgesic dose in the neonate, due to differences in the pharmacokinetic profile of firocoxib in neonates when compared to adults.

## Chapter 3

### Conclusions

This study was conducted to evaluate the pharmacokinetic profile and safety of multiple oral doses of firocoxib to neonatal foals. As the only COX-2 selective NSAID labeled for use in horses, firocoxib should provide analgesic and anti-inflammatory effects by inhibiting COX-2 without disrupting the homeostatic effects of COX-1. This makes it a promising drug for use in neonatal patients. Currently, there are no published pharmacodynamic or efficacy data for firocoxib for equine neonates.

The study's focus was to establish whether the labeled dose of firocoxib given per os to adult horses would be absorbed and achieve similar plasma concentrations in neonatal foals. Additionally, the occurrence of adverse effects was monitored for in the foals.

There were considerable differences in the pharmacokinetics of firocoxib observed between adults and neonates. Firocoxib was rapidly absorbed following oral administration with minimal accumulation after repeat dosing. Clearance was more rapid than reported for adults. Peak plasma concentrations were higher than in adult horses after a single dose, but lower after repeated dosing. Steady state was achieved after three or four doses in all foals.

Adverse effects were monitored for by serial physical examinations, complete blood count and serum chemistries, urinalyses, urinary GGT-to-creatinine ratios and by gastroscopy. There were minor deviations outside the reference intervals in some of the safety monitoring parameters but none were considered clinically significant.

As no clinically apparent adverse side effects were observed during the course of this study, it appears firocoxib can be safely administered to neonatal foals for at least nine consecutive doses at the recommended labeled dose (0.1 mg/kg q24 hours).

This pilot study, evaluating the pharmacokinetics and safety of firocoxib in equine neonates, provided new information for the use of this COX-2 selective NSAID in foals. However, many questions remain unanswered for firocoxib's use in horses, and particularly, neonatal foals.

Further investigation is required to establish a suitable, and therefore therapeutically effective, dosing interval in the foal. This would warrant a more extensive study with a larger study population to evaluate different dosing regimens and the associated pharmacokinetics.

Additionally, there is no pharmacodynamic data published for foals, so the therapeutic effectiveness of firocoxib for various inflammatory conditions remains to be determined. Currently, the use of firocoxib in the clinical setting is hindered by a lack of information on its efficacy and reluctance of clinicians to use a drug for which they do not have personal or anecdotal experience with.

Further, it is important to collect epidemiological data for a large number of equine neonates in various stages of health (including animals that are dehydrated, septic and inflicted with a variety of inflammatory conditions), that receive firocoxib to detect any possible unforeseen complications associated with selective inhibition of COX-2 and use of this drug.

Appendix A

Table 1. Target tissues of some of the main prostanoids and leukotrienes.

<b>Prostanoid or Leukotriene</b>	<b>Tissues Affected</b>
<b>TXA<sub>2</sub></b>	Bronchi
	Kidney
	Platelets
	Vascular Smooth Muscle Cells
<b>PGE<sub>2</sub></b>	Central Nervous System
	Kidney
	Mucosa
	Vascular Smooth Muscle Cells
<b>PGI<sub>2</sub></b>	Endothelium
	Kidney
	Mucosa
<b>PGE<sub>2</sub></b>	Bone
	Chondrocytes
	Central Nervous System
	Kidney
	Nociceptors
	Synovium
<b>PGI<sub>2</sub></b>	Central Nervous System
	Endothelium
	Kidney
	Nociceptors
	Platelets
	Uterus
<b>LTB<sub>4</sub></b>	Bronchi
	Macrophages
	Neutrophils
	Synovium
<b>LTC<sub>4</sub>, LTD<sub>4</sub>, LTE<sub>4</sub></b>	Bronchi
	Synovium

Table 2. Classes of NSAIDs used in Veterinary Medicine. Some of these drugs, marked with an asterisk (\*), are highly toxic and not commonly used for anti-inflammatory therapy in animals.

<p><b>Salicylates</b>            Acetylsalicyclic acid (aspirin)            Salicylate</p>	<p><b>Oxicams</b>            Piroxicam</p>
<p><b>Propionic Acid Derivatives</b>            Carprofen            Flurbiprofen (ophthalmic)            Ibuprofen*            Ketoprofen            Naproxen            Vedaprofen</p>	<p><b>Phenylacetic Acids</b>            Diclofenac sodium (topical)</p>
<p><b>Acetic Acids</b>            Etodolac            Eltenac</p>	<p><b>Pyrazole Derivatives</b>            Phenylbutazone            Dipyrone</p>
<p><b>Fenamic Acids</b>            Flunixin meglumine            Meclofenamic acid</p>	<p><b>Pyranocarboxylic Acid</b>            Etodolac</p>
<p><b>Indoles</b>            Indomethacin*            Sulindac</p>	<p><b>Semiselective COX-2 Inhibitors</b>            Meloxicam</p>
	<p><b>Selective COX-2 Inhibitors</b>            Celecoxib            Deracoxib            Firocoxib            Rofecoxib            Valdecoxib</p>



Table 3. Mean +/- SD values for pharmacokinetic parameters for single (day 1) and multiple oral doses of firocoxib (0.1mg/kg) to neonatal foals.

Parameter	Day 1 (Mean ± SD)	Day 5 (Mean ± SD)	Day 9 (Mean ± SD)
$\lambda_2$ (1/h)	0.08 ± 0.03	0.07 ± 0.02	0.07 ± 0.03
T 1/2 $\lambda_2$ (h)	10.46 ± 4.97	10.46 ± 2.87	11.04 ± 3.23
T <sub>max</sub> (h)	0.54 ± 0.65	0.43 ± 0.28	1.46 ± 1.75
C <sub>max</sub> (ng/mL)	89.50 ± 53.36	94.07 ± 61.23	71.17 ± 21.41
AUC <sub>0-24</sub> (h*ng/mL)	629.16 ± 178.09	794.44 ± 187.10	1162.45 ± 326.98
AUC <sub>0-∞</sub> (h*ng/mL)	-	-	1255.68 ± 372.76
AUC % extrapolated	-	-	7.01 ± 3.42
MRT <sub>0-∞</sub> (h)	14.66 ± 6.93	14.72 ± 4.2	16.22 ± 4.09
C <sub>avg</sub> (ng/mL)	-	-	39.1 ± 8.4
% Fluctuation	-	-	147 ± 72
Accumulation index	-	-	1.29 ± 0.15

Appendix B

Figure 1. Average plasma concentration-time curve for doses 1, 5 and 9 from neonatal foals treated with nine consecutive oral daily doses of firocoxib (0.1mg/kg).

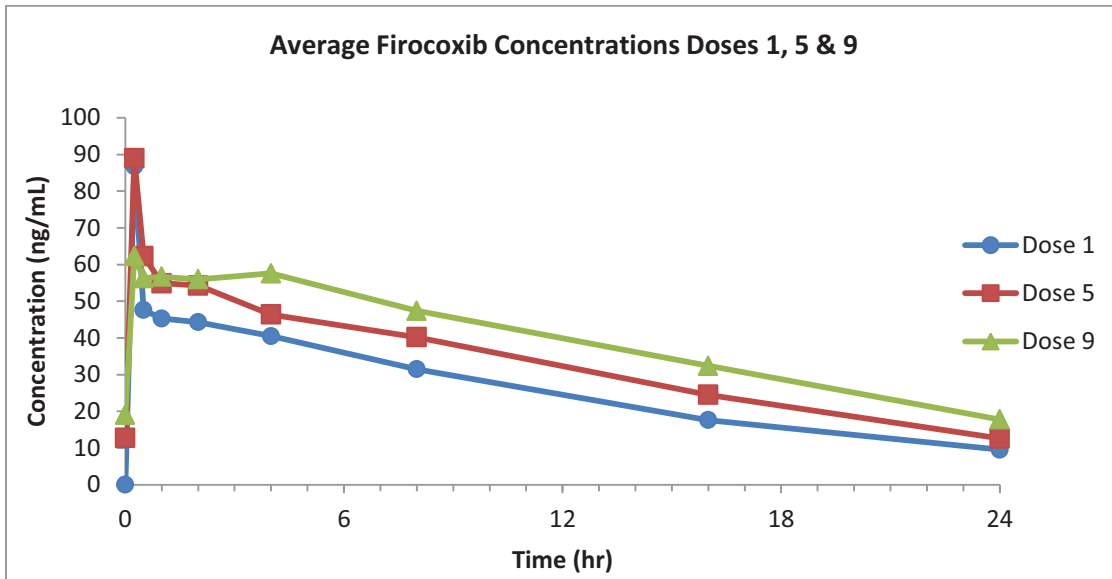
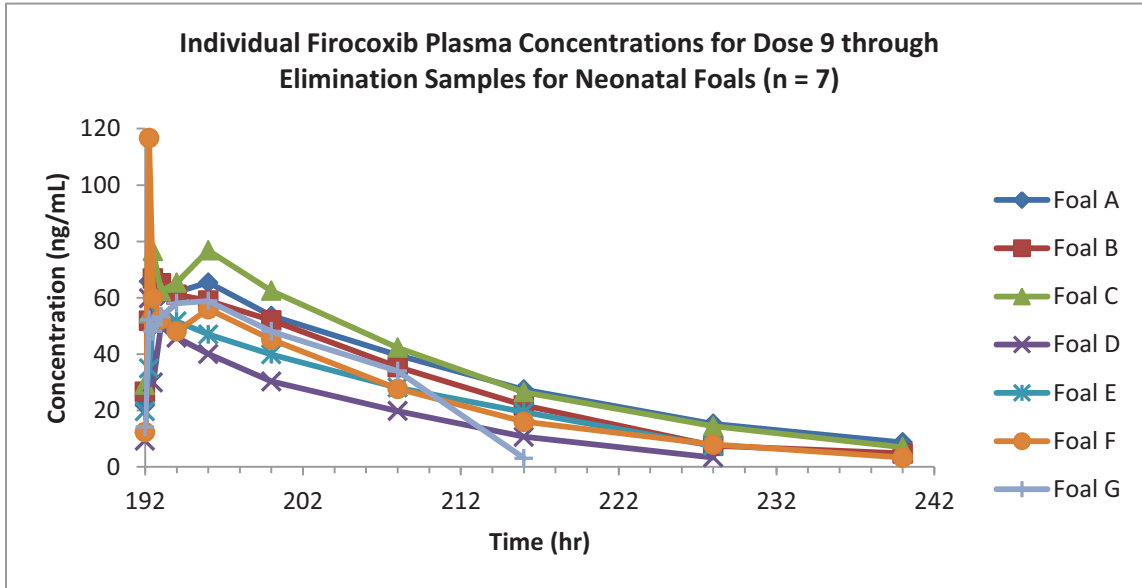


Figure 2. Individual plasma concentration-time curve from dose 9 through elimination samples. In foal G, firocoxib was no longer detectable past 216 hours from the initial dose.



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