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PHARMACOKINETICS OF KETOROLAC TROMETHAMINE IN HORSES AFTER INTRAVENOUS, INTRAMUSCULAR, AND ORAL SINGLE DOSE ADMINISTRATION

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PHARMACOKINETICS OF KETOROLAC TROMETHAMINE IN HORSES AFTER INTRAVENOUS,
INTRAMUSCULAR, AND ORAL SINGLE DOSE ADMINISTRATION

For the degree of Master of Science

Is approved by the final examining committee:

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PHARMACOKINETICS OF KETOROLAC TROMETHAMINE IN HORSES AFTER
INTRAVENOUS, INTRAMUSCULAR, AND ORAL SINGLE DOSE ADMINISTRATION

A Thesis

Submitted to the Faculty

of

Purdue University

by

Alexandra Walsh Bianco

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

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For my parents, who took my early aspirations seriously and have been endlessly supportive in my long journey. I love you.

For Hugo, who has provided endless emotional support; I love you too.

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LIST OF ABBREVIATIONS

ABBREVIATION	DESCRIPTION
KT	Ketorolac tromethamine
NSAID	Non-steroidal anti-inflammatory drug
IV	Intravenous
IM	Intramuscular
PO	Per os (oral)
COX	Cyclooxygenase enzyme
LPS	Lipopolysaccharide
PGE ₂	Prostaglandin E ₂
TXA ₂	Thromboxane A ₂
TNF _α	Tumor necrosis factor α
IL-1	Interleukin 1
CRI	Constant rate infusion
TER	Transepithelial resistance
t _{1/2}	Half life
HPLC-MS-MS	High performance liquid chromatography with tandem mass spectrometry
CBC	Complete blood count
SBA	Serum biochemical analysis
C ₀	Initial concentration
λ _z	Slope of the terminal linear phase
AUC _∞ (h × μg/mL)	Area under the curve
AUMC _∞ (h ² × μg/mL)	Area under the first moment curve
MRT	Mean residence time
MAT	Mean absorption time
Cl _p ({ml/min}/kg)	Plasma clearance
V _{d(ss)} (L/kg)	Volume of distribution at steady state
C _{max} (μg/mL)	Maximum plasma concentration
T _{max} (hour)	Time to reach maximum concentration
ANOVA	Analysis of variance
F (%)	Bioavailability
ESI	Electrospray ionization

ABBREVIATION	DESCRIPTION
MRM	Multiple reaction monitoring
CE	Collision energy
RSD	Relative standard deviation
IL-6	Interleukin 6

ABSTRACT

Bianco, Alexandra W. M.S., Purdue University, August 2015. Pharmacokinetics of ketorolac tromethamine in horses after intravenous, intramuscular and oral single dose administration. Major Professor: Sandra D. Taylor.

Non-steroidal anti-inflammatory drugs (NSAIDs) are an integral component of equine analgesia, yet currently available NSAIDs are both limited in their analgesic efficacy and have adverse effects. The NSAID ketorolac tromethamine (KT) is widely used in humans as a potent morphine-sparing analgesic drug but has not been fully evaluated in horses. The purpose of this study was to determine the pharmacokinetic profile of KT in horses after intravenous (IV), intramuscular (IM), and oral (PO) administration. Nine healthy adult horses received a single 0.5 mg/kg dose of KT via each route of administration. Plasma was collected up to 48 h post-administration and analyzed for KT concentration using HPLC-MS-MS. Non-compartmental analysis of IV dosage indicated a mean plasma clearance of 8.4 (mL/min)/kg and an estimated mean volume of distribution at steady state of 0.77 L/kg. Non-compartmental analysis of IV, IM and PO dosages indicated mean residence times of 2.0, 2.6, and 7.1 h, respectively. The drug was rapidly absorbed after IM and PO administration and mean bioavailability was 71 and 57% for IM and PO administration, respectively. Adverse effects were not observed after IV, IM and PO administration. More studies are needed to evaluate the analgesic and anti-inflammatory properties of KT in horses.

CHAPTER 1. INTRODUCTION

According to the United States Department of Agriculture 2012 census, there are approximately 3.6 million horses currently living in the United States. The majority of these horses are between the ages of 5-20 years old (56.7%).¹ The most recent report on equine morbidity in 1997 found that the leading causes of morbidity among adult horses in the United States (as reported by horse owners) were injury/trauma (6.6%), colic/gastrointestinal disease (5.6%), and lameness (4.8%).² The leading causes of death for horses older than 6 months of age in the United States in 2005 were “old age” (30.4%), injury/trauma (16%), and colic (15.2%).³ While the specific diagnoses of horses who died due to “old age” are unknown, a recent paper out of the United Kingdom found that chronic lameness was the most common reason for horses over 15 years old to be euthanized (24%).⁴ The role of horses in the United States has also evolved from primarily being used for work to that of a companion animal, likely in response to agricultural advancements. In 2005, 45% of horses in the United States were primarily classified as used for pleasure, with 24.8% used for farm or ranch work.³

With the paradigm of horse ownership shifting, the field of equine veterinary care has also shifted. One area of medicine in particular that has come to the forefront is the recognition and management of animal pain. While specific statistics on equine veterinary care in the United States are lacking, it can be assumed that given the demographic information on morbidity and mortality and the population of older, active horses in the United States, it is likely that a horse will need veterinary care for a potentially painful condition at some point in their lifetime.

In horses, non-steroidal anti-inflammatory drugs (NSAIDs) are the primary focus of pain research as they are the mainstay of equine analgesic therapy. However, there are few NSAIDs currently used in horses, and the same drugs and dosages are used for a variety of conditions ranging from minor injury to major abdominal or orthopedic surgery. Given these limitations, NSAIDs are not always sufficient at providing adequate analgesia in certain patients. In order to increase patient comfort, adjunctive analgesic drugs such as opioids or lidocaine are used. However, the side effects and/or limitations of these drugs may negate their use, resulting in animals continuing to experience pain.

Ketorolac tromethamine (KT) is a NSAID that was approved for short-term analgesia in humans in 1989. Since its approval, KT has been widely used in human medicine primarily as a post-operative analgesic for moderate to severe pain. Though only labeled for oral (PO) and intramuscular (IM) use in humans, KT is also used intravenously (IV) or as a constant rate infusion (CRI). Numerous studies in human medicine have evaluated KT as a morphine-sparing analgesic in post-operative patients and demonstrated significant reduction in morphine consumption (22-44%) when patients were concurrently treated with KT for the first 24 hours following abdominal or orthopedic surgery versus morphine analgesia alone.⁵⁻¹⁰

Though KT is commonly used as a potent analgesic in human medicine, there has been little research regarding its use in animals. A single dose pharmacokinetic profile of KT has been evaluated in dogs,^{11,12} cats,¹³ sheep,¹⁴ calves,¹⁵ and goats.¹⁶ While there have also been two publications regarding the pharmacokinetics of KT in horses, the first was in 1994 and the horses in the study only received a single 300 mg IV or IM dose without a randomized crossover design.¹⁷ The second, in 2014, examined the pharmacokinetics of KT when given IV to colts undergoing general anesthesia with concurrent administration of multiple other drugs.¹⁸

The goal of the research performed for this thesis was to better characterize the pharmacokinetic profile of KT in the horse in order to lay the foundation for future clinical use of the drug. Specifically, the aims of the study were to establish the pharmacokinetic profile of KT in the horse after a single IV, IM, and PO dose, establish the bioavailability of

KT when delivered IM or PO, and to document any adverse effects noted after a single IV, IM or PO dose of KT in the horse.

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CHAPTER 2. LITERATURE REVIEW

2.1 Assessing Pain in Horses

Whether or not animals experience pain is a surprisingly difficult question to answer in an objective way and one that has been historically debated. Early research models designed to detect pain in animals largely consisted of assessment of withdrawal response to noxious stimuli, or nociception. Nociception refers to the complex neural pathways that serve to preserve the life of the animal by preventing physical damage to the tissues.

Because nociception lacks an emotional component, it can be argued that response to noxious stimuli does not correlate to pain, as “pain” refers to the actual *feeling* of suffering. With this definition of pain, it was historically argued that animals *could not* experience pain as they lack the ability to communicate the feeling of suffering. Once it was accepted that even humans that cannot communicate verbally (e.g. infants) can clearly experience pain and suffering, more focus was given as to how to recognize pain in those who lack the ability to communicate. This has been a crucial turning point in the area of animal research, and current research has focused on how physical suffering can be better recognized in both animals used as research subjects and in veterinary patients. Prey species, such as horses, provide an even greater challenge in pain recognition due to the natural disadvantage of displaying pain as it implies vulnerability.^{1,2}

In addition to nociceptive responses that are the rudimentary signals of pain, animals have been shown to exhibit more advanced pain responses that reflect cognitive function, such as the ability to anticipate physical discomfort. A familiar example of this is when an animal is confronted with an action, object, location, or person that previously

caused pain or discomfort and displays aversive or even aggressive behavior to avoid repetition of an unpleasant experience.^{1,2}

As reactive or aversive behaviors are evolutionarily beneficial to the survival of an animal, they do not necessarily support a conscious feeling of suffering that goes beyond simple instinct. Regardless of the emotional depth of animal pain, however, it is generally accepted by the veterinary community that animals experience physical suffering and benefit from analgesic therapies.

Given the need for objectivity in assessing animal pain, several pain scales have been developed for use in horses in the past decade. One challenge, however, is that there is no “gold standard,” and animal pain scales are typically validated with physical and behavioral parameters correlating mainly to the sympathetic nervous system and hypothalamic-pituitary-adrenal axis.³⁻⁷ This validation is less than ideal, as observer bias and concurrent illness may influence behavioral and physical data. Furthermore, it has been demonstrated that breed and personality are closely related to the expression of pain in horses,⁸ a factor that can be confounding when clinical cases are compared with scoring systems.

Even when using a standardized method, relying on subjective human interpretation of animal pain is difficult. This was demonstrated in a 2012 clinical trial in which owners of dogs with hip osteoarthritis were asked to evaluate the clinical response to treatment with a given analgesic drug. The results indicated a clear placebo effect for all outcome variables of efficacy.⁹

2.2 Nonsteroidal Anti-Inflammatory Drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory effects via inhibition of cyclooxygenase (COX) enzymes in the arachidonic acid pathway. The activity of COX enzymes are rate-limiting steps of the cascade; therefore COX inhibition effectively reduces the conversion of arachidonic acid into several families of eicosanoids, including prostaglandins, prostacyclins, and thromboxane.

Tissue expression of COX and the products of the arachidonic acid pathway are integral to the healthy function of many biologic processes within the body, including maintenance of the immune system, gastrointestinal tract, reproductive tract, cardiovascular system, and renal system.¹⁰

While there are three isoforms of the COX enzyme, two are of interest in drug therapy: COX-1 and COX-2. The 3rd, COX-3, has not been evaluated in horses but its significance is debated in humans.¹⁰ The COX-1 isoform is expressed throughout the body and plays a vital role in maintaining the integrity of mucosal tissue through production of prostaglandins. These prostaglandins, namely prostaglandin E₂ (PGE₂), are involved in the regulation of gastric acid and mucous production in the stomach, and local vasodilation to the stomach and kidney to maintain adequate blood flow. Thromboxane A₂ is also an important product of COX-1 activity and is involved in inhibition of platelet aggregation.^{10,11}

The COX-2 isoform is also constitutively expressed by select tissues throughout the body. While expression of COX-2 also leads to increased production of prostanoids such as PGE₂, it is thought to more specifically promote the inflammatory functions of PGE₂.¹⁰ In horses, low-level expression of COX-2 has been demonstrated in healthy tissues including the glandular mucosa of the stomach,^{12,13} mucosa of the urinary bladder,¹³ jejunum^{14,15} and left dorsal colon.¹⁶ Higher COX-2 expression in these tissues is likely beneficial as PGE₂ promotes local inflammation and is protective in its inhibition of cytotoxic immune response to pathogen entry.¹⁷

In states of inflammation, global COX-2 expression is up-regulated by growth factors and inflammatory mediators such as interleukin-1 (IL-1), tumor necrosis factor alpha (TNF- α) and lipopolysaccharide (LPS).¹⁸ In horses, increased COX-2 expression has been demonstrated in cases of gastric ulceration of both the glandular and squamous portions of the stomach^{12,19} as well as after ischemia and reperfusion injury to the colon¹⁶ and jejunum.¹⁴ Increased COX-2 expression has also been demonstrated in the lamina cells of horses after induction of laminitis using the black walnut extract model.²⁰

Non-steroidal anti-inflammatory drugs are analgesic, anti-inflammatory, and anti-pyretic primarily due to inhibition of prostanoid production. Prostanoids reduce the activation threshold of sodium channels on sensory neurons, effectively producing an hyperalgesic effect.¹⁰ When vascular endothelial cells are exposed to pyrogens such as LPS, PGE₂ is released, activating the hypothalamus and inducing subsequent hyperthermia. With inhibition of PGE₂, NSAIDs therefore act as anti-pyretic drugs.

Given the role of COX enzymes in the pathways of pain and inflammation, NSAIDs, or COX inhibitors, are highly effective drugs in alleviating pain in both humans and animals. Furthermore, unlike sedatives or opioids that simply dull the transmission of pain, NSAIDs actually reduce inflammation and the underlying cause of discomfort.

2.3 Adverse Effects and COX Selectivity

As NSAIDs target the same mechanisms which in health are protective, NSAID use may lead to unwanted adverse effects, namely to the gastrointestinal and renal systems. As in other species, there is a risk of adverse effects (AE) when using NSAIDs in horses. Several studies have compared the relative risk of AE between different NSAIDs.

A study by MacAllister et al. in 1993 was one of the first to compare the risk of adverse effects between nonselective NSAIDs in healthy adult horses (n=16). In this study, horses either received the label dose of phenylbutazone (4.4 mg/kg), flunixin meglumine (1.1 mg/kg), ketoprofen (2.2 mg/kg), or saline, IV every 8 hours for 12 days. Postmortem examination revealed histologic erosions of the glandular mucosa of the stomach in all but one of the NSAID-treated horses. A treatment effect of phenylbutazone was negatively correlated to total protein and albumin levels, and only horses that received phenylbutazone had evidence of renal papillary necrosis or intestinal (non-gastric) lesions. With the clinicopathologic and histologic grading of this study, the adverse effects were most severe after treatment with phenylbutazone, followed by flunixin meglumine and ketoprofen, respectively.²¹

A more recent study by Mozaffari et al. in 2013 employed the same basic design as MacAllister and examined the same drugs in apparently healthy donkeys (n=20). In this

study, the phenylbutazone, flunixin meglumine, and ketoprofen were administered at 4.4 mg/kg, 1.0 mg/kg, 2.2 mg/kg, respectively, IV every 12 hours. During the course of treatment, the liver enzymes of all NSAID-treated animals significantly increased, and there was a significant increase in creatinine of phenylbutazone-treated animals. At post-mortem exam, all NSAID treated donkeys had ulcerations of the glandular mucosa of the stomach as well as histopathologic changes to the liver and kidney. While all NSAIDs were documented to have adverse effects, the results echoed MacAllister et al. in ranking of severity of adverse effects from phenylbutazone being most severe followed by flunixin meglumine and ketoprofen.²²

In horses, the severity of adverse effects when comparing different NSAIDs has also correlated to the dosage of the NSAIDs. As COX inhibition is known to be dose-dependent, the difference in severity of adverse effects may be related to the relative potency of each drug. If a drug lacks potency, a larger amount of drug is needed to produce the desired effect and may increase the severity of adverse effects.

Recently, in both human and animal medicine, there has been a focus on COX-selective NSAIDs, with the idea that selective inhibition of COX-2 would result in less adverse effects due to the maintenance of COX-1 activity. However, while COX-1 has a vital role in maintaining integrity of the gastrointestinal tract, selective inhibition of COX-2 would not spare potential toxicity to the kidneys or liver as both COX-1 and COX-2 are important in maintenance of blood flow.²³ This has been demonstrated in horses in a 2014 study where phenylbutazone, a nonselective COX inhibitor, and meloxicam, a COX-2 selective inhibitor, were found to have similar risk of adverse effects in volume-depleted horses.²⁴

In humans, the COX-2 specific NSAIDs have been shown to be as effective as non-selective NSAIDs at analgesia, with little change in risk of adverse effects.²⁵ However, few analgesic trials in human medicine evaluate patients receiving NSAIDs alone without the addition of supplemental narcotics.

There have been very few, if any, randomized controlled trials that compare the safety and efficacy of COX-2 vs. nonselective COX inhibitors in clinical equine patients.^{26,27}

Several experimental studies have compared the nonselective COX inhibitor phenylbutazone to the COX-2 selective NSAID meloxicam and found evidence of adverse gastrointestinal and renal effects when phenylbutazone was used at the recommended label dose, but also when meloxicam was used at greater than the recommended 0.6 mg/kg every 24 hours for a 14-day period.^{28,29}

There has been some question that NSAID use can affect not only the mucosal healing of the gastrointestinal tract but also motility. This is especially relevant in horses given the widespread use of NSAID therapy after major abdominal surgery in horses and the consequences of post-operative ileus.³⁰ In the ischemia-reperfusion model of equine colic, transepithelial electrical resistance (TER) is used to assess recovery of intestinal barrier function. A decreased TER is expected after ischemic damage as there is increase permeability of sodium and chloride ions; TER should increase as the intestine heals. Flunixin meglumine has been found to prevent increases in TER after ischemic damage to the jejunum and potentially may inhibit intestinal recovery in cases of ischemia and reperfusion.^{31,32} In comparison to the nonselective flunixin meglumine, the COX-2 specific deracoxib had similar results,³¹ whereas meloxicam did not prevent TER increase.³²

A 2009 study by Menozzi et al. used electrical field stimulation to test the effects of non-selective NSAIDs (indomethacin, flunixin meglumine) vs. COX-2 selective (celecoxib) on motility on postmortem equine ileal samples. Results of this study indicated that nonselective NSAIDs had minimal effects on tonic and phasic contractions of samples, while COX-2 selective inhibition was associated with concentration-dependent effects on both tonic and phasic motility.³³

2.4 Adjunctive Therapy

2.4.1 Opiates

Adjunct medications such as opiates or local anesthetics (e.g. lidocaine) are often used in conjunction with NSAIDs to provide additional pain control when NSAIDs alone are insufficient. In humans, narcotic analgesia, such as morphine sulfate delivered by a patient-controlled analgesia device, is frequently employed during the immediate post-

operative period following orthopedic and soft tissue procedures, with or without concurrent NSAID use.³⁴

Given their place in human analgesic therapy, opioids have also been evaluated for their analgesic properties in horses with mixed results. In experimental pain models of single-dose analgesic efficacy, opiates have shown varying degrees of analgesia in horses and are complicated by potentially confounding sedative effects.^{35,36} In a model of LPS-induced carpal synovitis in the horse, continuous morphine or methadone infusion produced analgesic effects while infusions of butorphanol or tramadol were ineffective at analgesia.³⁷

There have been few clinical trials evaluating the use of opiates as analgesics in horses. In 2004, Sellon et al. reported less surgical stress, more normal behavior, reduced weight loss and quicker recovery in horses treated with a combination of flunixin meglumine and butorphanol in clinical patients following colic surgery compared to control horses treated with flunixin meglumine alone.³⁸

Regardless of their potential analgesic effects, opiates have well established effects on behavior and gastrointestinal motility. Reported behavioral effects of opiates in horses include restlessness, increased appetite, ataxia, head tossing, and increased locomotion.^{35,39} The negative effects of opiate therapy on gastrointestinal motility are also well documented in the equine patient and may contraindicate their use in high-risk patients regardless of degree of perceived pain.³⁸⁻⁴¹

2.4.2 Lidocaine

In two human meta-analyses of 22 randomized controlled trials including over 850 post-operative human patients, IV lidocaine was associated with decreased opiate use, improved pain scores, and reduced incidence of nausea and vomiting.^{42,43} Similar results have been found in horses; in cases of postoperative colic, lidocaine use was significantly associated with decreased incidence of ileus as well as an increase in short term survival.⁴⁴

A 2005 study evaluated the anti-nociceptive properties of lidocaine using models of thermal pain as well as duodenal and colorectal distension. When delivered as a CRI,

lidocaine was effective at increasing the pain threshold to thermal stimulation; however, lidocaine failed to significantly alter tolerance to colorectal distension.⁴⁵

Interestingly, both flunixin meglumine and lidocaine have been shown to increase neutrophil migration and adhesion *in vitro*.⁴⁶ In an *in vivo* model of ischemia and reperfusion injury to the jejunum meant to mimic equine colic, immunohistochemistry demonstrated a significant increase in jejunal neutrophils 18 hours after ischemia in horses treated with flunixin meglumine compared to saline controls.¹⁵ Treatment with lidocaine alone or a combination of lidocaine and flunixin meglumine dampened this increase in neutrophils.¹⁵ While this was an *in vivo* model of equine gastrointestinal disease, all horses received perioperative butorphanol and no discussion was given as to analgesic efficacy of lidocaine when used alone or in combination with flunixin meglumine.

2.5 Current Analgesic Drugs are Limited

Given their unique ability to treat both pain and inflammation, NSAIDs play an integral role in equine drug therapy, and continued research and refinement is warranted. There are currently only three NSAIDs specifically labeled for systemic use in horses in the United States: phenylbutazone, flunixin meglumine, and firocoxib. These medications are used at the labeled dosages to treat a variety of conditions ranging from those associated with a mild degree of discomfort (e.g. lacerations, soft tissue injury, corneal ulcer, chronic osteoarthritis) to those assumed to have a larger potential for suffering (e.g. fracture, abdominal or orthopedic surgery, laminitis).

While the pharmacokinetics and safety of different NSAIDs have been established in horses, there have been few randomized controlled studies that have specifically focused on the analgesic potency and efficacy of NSAIDs in the treatment of acutely painful conditions. A reversible model of equine lameness has been developed by Foreman et al. and has been used to assess the analgesic efficacy of flunixin meglumine and phenylbutazone after induction of lameness. Using this method, flunixin meglumine or phenylbutazone at the label dose (1.1 mg/kg IV and 4.4 mg/kg IV, respectively) have been shown to effectively reduce heart rate and lameness score in horses up to 4 hours

post administration.^{47,48,49} There is one randomized, controlled, trial that compared the analgesic efficacy of two NSAIDs in horses experiencing moderate-severe pain. Naylor et al. in 2014 compared the efficacy of flunixin meglumine (1.1 mg/kg IV every 12 hours) or meloxicam (0.6 mg/kg IV every 12 hours) in 60 horses undergoing abdominal surgery for a strangulating small intestinal lesion. While there was no difference of treatment on horse survival, post-operative pain scores were significantly higher in horses who received meloxicam.⁵⁰ While not significant between treatments, 58 of the 60 horses also received lidocaine as a CRI.

The recognition of pain is an active area of research in equine medicine, especially in regards to outcome following gastrointestinal surgery. In 2005, a retrospective study was performed using 300 cases of surgical colic evaluated with a simple behavioral pain score. Results of this study indicated 81/253 (32.1%) of all horses experienced postoperative pain in, including 53/123 (43.1%) of horses with small intestinal lesions.^{51,52} Furthermore, the authors reported that post-operative pain following colic surgery was the most common reason for euthanasia. Though not specified, it is presumed that NSAID therapy was used in the immediate postoperative period as it is considered standard of care after colic surgery.

Using a multi-dimensional pain scale based on physiological and behavioral parameters, termed the post abdominal surgery pain assessment scale (PASPAS), Graubner et al. in 2011 demonstrated that in 34 cases of surgical colic, 35.3% of horses demonstrated low pain, 38.2% moderate pain, and 26.5% severe pain at 8 hours following surgery.⁵³ The majority of horses retained at least a low level of pain for at least 24 hours postoperatively. The horses in this study all received flunixin meglumine at 0.5 mg/kg every 4 hours IV immediately following surgery. Lidocaine CRI was used as deemed necessary, though these cases were not differentiated in the results.

One recent study utilized two behavior-based pain scoring systems to assess horses after surgery for gastrointestinal disease. Forty-eight horses were followed over 3 days post-operatively, during which time they received standard of care analgesic therapy consisting of flunixin meglumine (1.1 mg/kg IV every 12-24 hours) and lidocaine

infusions as deemed necessary. In this population, pain scores using both scoring systems were significantly and consistently higher in non-survivors (9/48) vs. survivors (39/48); however, all animals experienced some degree of postoperative pain.⁷

The use of facial expression as an indicator of pain has also been recently examined in horses when the “Horse Grimace Scale” was compared to a Composite Pain Score system. While it was found that the two pain scales were highly correlated to each other, it was also evident that horses undergoing routine castration experienced pain postoperatively. Furthermore, there was no difference in pain scores of horses who received a single perioperative dose of flunixin meglumine (1.1 mg/kg IV) or those who received an additional dose postoperatively.⁵

Despite the recent focus on pain recognition, there has been little discussion on the fact that many horses continue to experience pain despite “standard-of-care” analgesic therapy postoperatively. Current therapy primarily consists of a NSAID with adjunctive opioid or lidocaine use as deemed indicated by the attending veterinarian.

There is a need for a potent analgesic that can be safely administered to equine patients in situations associated with high degrees of pain, such as following orthopedic or abdominal surgery.

2.6 Ketorolac Tromethamine

2.6.1 Overview

Ketorolac tromethamine (KT) is a pyrrolizine carboxylic acid derivative, non-selective cyclooxygenase inhibitor that is administered clinically in its tromethamine salt form.^{54,55} The drug is actually a racemic mix of (S) and (R) enantiomers, with the (S) form being pharmacologically active.⁵⁶

Since its approval for use in humans in 1989, KT has been widely used in human medicine primarily as a post-operative analgesic for moderate to severe pain, though like other NSAIDs it is anti-inflammatory and anti-pyretic.^{55,57,58} Though only labeled for oral and intramuscular use, KT is often used IV or as a CRI.⁵⁹⁻⁶⁴

Ketorolac, like other NSAIDs, indirectly causes analgesia by dampening the hyperalgesic effect of prostaglandins. This means that KT does not alter pain responses in non-inflamed tissues, unlike a centrally-acting narcotic analgesic such as morphine.^{54,55,57,65} Because it is ineffective in a non-inflamed state, KT is non habit-forming; while this is typically not a concern for veterinary patients, it becomes relevant when considering the humans involved in veterinary care.

2.6.2 Metabolism and Excretion

Ketorolac conjugation with glucuronide is a major metabolic pathway in the human, monkey, and mouse and is thought to occur in the kidney, as conjugates have been detected in the urine but not the plasma.⁶⁶ Metabolites of KT do not have any analgesic activity.⁵⁷ In humans, monkeys, and rabbits, KT has been shown to be primarily excreted in the urine, whereas mice and rats have a higher percentage of fecal excretion.⁶⁶

2.6.3 Analgesic Efficacy of Ketorolac Tromethamine

Because KT is a non-specific COX inhibitor, the drug induces analgesia by the same mechanism as other NSAIDs. However, KT has been shown to have increased analgesic potency, or the relative amount of drug needed to exert a physiological or clinical response relative to other NSAIDs.^{54,65} While it is not known why KT is more potent than other NSAIDs, it may be due to its low distribution into adipose tissue.^{66,67}

Initial analgesic evaluations consisted of established rodent laboratory tests performed by a pharmaceutical laboratory for the purpose of drug approval. In the writhing model for visceral pain, mice receive an intraperitoneal injection of a chemical irritant (such as phenylquinone) and are assessed for “writhing,” or specific abdominal contraction and hind leg extension.⁶⁸ When administered orally, ketorolac was found to be over 350 times more potent than aspirin and phenylbutazone using a phenylquinone-induced writhing model in mice.^{54,65} In rats, ketorolac has been shown to have

significantly greater potency in reducing writhing than celecoxib, a COX-2 specific NSAID.⁶⁷

One common animal model of inflammation-induced pain is the carrageenan-induced model of paw edema. In this model, carrageenan yeast is injected in the plantar aspect of a hind rat paw to induce inflammation and pain; response is measured by time until foot withdrawal or escape after application of pressure.⁶⁹ After pre-treatment with oral ketorolac, rats have been shown to exhibit a dose-dependent increase in pain threshold of the affected paw.^{54,67} Importantly, there was no change in pain threshold of the non-inflamed paw, supporting a lack of central activity.

A lack of central activity was supported by an additional animal model of analgesia, the hot-plate method, in which mice are assessed for tolerance to heat before and after drug administration. Importantly, this method does not induce inflammation; therefore, NSAID administration should have little effect using this model. When tested, an intraperitoneal injection of ketorolac did not alter the response of mice using this method, whereas administration of morphine did have a significant effect.⁵⁴

2.6.4 Anti-Inflammatory Efficacy of Ketorolac

Ketorolac has been shown *in vitro* to exhibit dose-dependent inhibition of neutrophil chemotaxis, adherence, and degranulation with release of myeloperoxidase.^{70,71}

Using the carrageenan-induced model of inflammation described in section 1.6.3, the inflamed and uninflamed paws are compared for degree of inflammation as determined by comparing the weight of equal sized punch biopsies from both back feet.⁶⁶ In rats pre-treated with NSAIDs, the potency of KT was 36 times that of phenylbutazone and 3 times as potent naproxen at reducing paw edema.^{54,65}

One study evaluated the anti-inflammatory effects of ketorolac using a model of pulmonary embolism in the rat. In this model, ketorolac was shown to inhibit the upregulation of COX-2 as well as expression of the adhesion molecules intracellular

adhesion molecule-1 and selectin E. Ketorolac was also associated with significant reduction in cardiac tissue myeloperoxidase.⁷¹

In one of the first studies of ketorolac in non-laboratory animals, ketorolac was evaluated for its ability to ameliorate clinical and physiologic responses to LPS infusion in calves. In this study, KT (1.1 mg/kg IV) was compared to the other nonselective NSAIDs flunixin meglumine (1.1 mg/kg IV) and ketoprofen (2.2 mg/kg IV). All three NSAIDs were equally effective at reducing the clinical signs of endotoxemia as well as preventing increases in thromboxane A₂ and prostacyclin.⁷² In dogs, ketorolac has also been demonstrated to significantly reduce plasma PGE₂ levels for at least 24 hours after a single 0.5 mg/kg IV dose.⁷³

2.6.5 Safety

As stated in section 1.3, NSAID use in all species is associated with potential gastrointestinal, renal, or coagulation side effects due to the inhibition of COX-1. The safety of ketorolac has been evaluated extensively in humans with mixed results. While several studies report that the risk of adverse effects is not higher than other NSAIDs,^{35,74} other studies have cited KT has having a higher relative risk of upper gastrointestinal bleeding in comparison to other human NSAIDs.⁷⁵ The risk of adverse effects in humans has been shown to be increased in certain patients with preexisting conditions such as renal or gastrointestinal disease, coagulopathy, or those receiving concurrent administration of other NSAIDs. Given these potential risks in the human population, use of KT in the United States is restricted to no more than 5 days of treatment.⁷⁶

2.7 Clinical Trials of Ketorolac Tromethamine in Humans

Early studies in rats and mice indicated that the 50% inhibitory dose (ID₅₀) at which KT is analgesic is 0.1-0.3 mg/kg vs. ≥0.3 mg/kg for anti-inflammatory effects.⁶⁵ Given the analgesic potency of ketorolac, it is primarily used in human medicine for its analgesic rather than its anti-inflammatory effects.

There have been very few randomized controlled trials that have directly compared KT to other NSAIDs without the confounding use of opioids. Two studies have compared a single dose of oral ibuprofen or intramuscular KT in an emergency room setting. In both studies, the drugs were equal in efficacy, though neither provided complete analgesia.^{77,78} However, patients were only monitored for 2 hours, and the recorded pain intensity scores had not yet plateaued. In another study utilizing emergency room patients, KT was shown to be equally effective as acetaminophen at reducing fever.⁷⁹

The vast majority of randomized controlled clinical trials in humans have been regarding the efficacy of KT as a “morphine-sparing” analgesic, especially in post-operative patients using patient-controlled analgesia systems with the ability to self-administer morphine. The results of these studies showed significant reduction in morphine consumption (22-44%) when patients were concurrently treated with KT for the first 24 hours following abdominal or orthopedic surgery vs. morphine analgesia alone.^{34,59,60,61,80,81}

The ability of KT to reduce opioid use is significant in humans as it results in fewer opioid-related adverse. In a randomized controlled clinical trial, oral KT was compared to acetaminophen-codeine in terms of efficacy and incidence of adverse effects in 123 patients presenting to the emergency room for acute back pain. The patients were instructed to take the medication as needed every 4-6 hours for 1 week. Results indicated that while both drugs were effective analgesics, patients receiving acetaminophen-codeine had significantly greater incidence of adverse effects and were more likely to drop out of the trial than those receiving KT.⁵⁸ In a 2012 meta-analysis, a single preoperative dose of KT demonstrated not only a positive effect on reduction of postoperative pain, but also a decrease in incidence of nausea and vomiting.⁸²

2.8 Ketorolac Tromethamine in Veterinary Species

2.8.1 Pharmacokinetics

The single dose pharmacokinetic profile of KT has been evaluated in dogs,^{83,84} cats,⁸⁵ sheep,⁸⁶ calves,⁸⁷ and goats⁸⁸ (Table 1-1). In non-anesthetized dogs, Pasloske et al. in 2002 found KT to have a similar pharmacokinetic profile to that in humans when administered IV or PO at a dose of 0.5 mg/kg. The oral bioavailability of KT was variable. The authors recommend a dosing regimen of 0.5 mg/kg every 8 hours in dogs.⁸³ In a more recent study, the pharmacokinetics of a single IV dose of KT was examined in dogs undergoing routine castration under general anesthesia.⁸⁴ In these dogs, drug clearance was similar to that found by Pasloske et al., but the $V_{(dss)}$ was markedly increased, resulting in an elimination half-life ($t_{1/2}$) of approximately 10 hours rather than the 4 hours found in conscious dogs.^{83,84}

In cats, the single dose pharmacokinetic profile of KT was determined after a 0.5 mg/kg IV dose was given 20 minutes prior to general anesthesia and either a neuter or ovariohysterectomy. The pharmacokinetic profile was similar to that in conscious dogs and humans, with a $t_{1/2}$ of approximately 4 hours.⁸⁵

Pharmacokinetic evaluation of KT in ruminants has been performed using higher drug dosages than those used in humans and simple stomached animals. In goats, calves, and sheep, each animal received a single intravenous (2 mg/kg) dose. Goats and calves were also given KT orally (2 mg/kg in goats, 8 mg/kg in calves), and sheep received an additional dose IM (2 mg/kg). Overall, the clearance of KT was found to be much greater in sheep and goats, with serum $t_{1/2}$ of 0.4 and 1.05 hours, respectively.^{86,88} The serum $t_{1/2}$ in calves was found to be similar to that in dogs and humans, at 5.9 hours.⁸⁷ The difference in clearance rates are thought to be due to differences in protein binding.

There have been two publications regarding the pharmacokinetics of ketorolac in horses. Plånborg, et al. in 1994 described the pharmacokinetic profile of ketorolac in the horse; however, the horses in the study only received a single 300 mg IV or IM dose without a randomized crossover design.⁸⁹ Furthermore, the authors acknowledged the

need for further pharmacokinetic evaluation. In a 2014 study, Ferraresi et al. assessed the pharmacokinetics of a single perioperative dose of ketorolac (0.5 mg/kg IV) before routine castration in colts. These horses were also under general anesthesia and results indicated a short $t_{1/2}$ in horses of approximately 40 minutes.⁹⁰

2.8.2 Clinical Trials

Despite widespread use of ketorolac as an analgesic in human medicine, there has been little research regarding its clinical use in animals. Mathews et al. in 1996 is currently the only randomized controlled study investigating use of ketorolac in clinical veterinary use. In this study, ketorolac was compared to flunixin meglumine, butorphanol and oxymorphone after exploratory laparotomy and shoulder arthrotomy in dogs. The results of this randomized controlled trial found that ketorolac given at a dose of 0.5 mg/kg IM was as effective as flunixin meglumine and superior to both butorphanol and oxymorphone in providing consistent post-operative analgesia.⁹¹

Table 2-1: Various pharmacokinetic parameters of different species after a single dose of ketorolac tromethamine

Author	Year	Species	Number of Animals	Route of Administration			Dose (mg/kg)	C _{max} (ug/mL)	t _{1/2} (hour)	V _d (L/kg)	Cl (ml/kg/min)	F (%)	MRT (hour)	Protein binding (%)
Nagilla	2007	Calf	5	IV	-	-	2		5.86	0.2 ± 0.074	0.79 ± 0.61		8.4 ± 8.2	
Nagilla	2008	Calf	5	-	-	PO	8	5.2 ± 3.0				86.5 ± 20.6		
Villa	2015	Cat	12	IV	-	-	0.5	3.1 ± 1.04	4.14 ± 1.18	0.18 ± 0.06	0.95		6.47 ± 2.86	98.7
Pasloske	1999	Dog	6	IV	-	-	0.5		4.55	0.33 ± 0.1	1.3 ± 1.1			
Pasloske	2000	Dog	6	-	-	PO	0.5	1.6 ± 0.3	4.07			100.9 ± 46.7		
Cagnardi	2013	Dog	10	IV	-	-	0.5	2.5 ± 1.1	10.9	1.03 ± 0.62	1.5 ± 1.4		5.6 ± 2.1	98.9
Nagilla	2008	Goat	5	IV	-	-	2		1.05	0.42 ± 0.42	8.7 ± 3.9		0.86 ± 0.68	
Nagilla	2008	Goat	5	-	-	PO	6	0.73 ± 0.59	34		6.8 ± 3.07	132 ± 23.2	44.8 ± 11.3	
Plånborg	1994	Horse	3	IV	-	-	0.5		6.7	0.28	2		1.95	
Plånborg	1994	Horse	3	-	IM	-	0.5					69		
Ferraresi	2014	Horse	6	IV	-	-	0.5	4.74 ± 5.38	0.69 ± 0.61	0.21 ± 0.13	5.5 ± 2.2			75.8 ± 2.9
Jung	1988	Human	15	IV	-	-	10 mg	2.39 ± 1.3	5.09 ± 2.01	0.11 ± 0.02	0.35 ± 0.07		5.34 ± 1.02	
Jung	1988	Human	15	-	IM	-	10 mg	0.77 ± 0.12	4.99 ± 1.88			108.7 ± 22.3	6.27 ± 1.12	
Jung	1988	Human	15	-	-	PO	10 mg	0.81 ± 0.25	5.07 ± 0.97			100.0 ± 19.8	6.32 ± 1.06	
Santos	2001	Sheep	5	IV	-	-	2		0.3 ± 0.15	0.25 (0.08)	12.4 ± 2.8		0.37 ± 0.18	
Santos	2001	Sheep	5	-	IM	-	2	4.1 (1.07)	0.25 ± 0.05	0.31 (0.10)	13.3 ± 1.3	97.6 ± 10.15	0.5 ± 0.15	

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CHAPTER 3. PHARMACOKINETICS OF KETOROLAC TROMETHAMINE IN HORSES AFTER INTRAVENOUS, INTRAMUSCULAR, AND ORAL SINGLE DOSE ADMINISTRATION

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3.2 Introduction

Pain management is an active area of investigation in veterinary medicine. Managing pain in equine patients relies primarily on drug intervention, as other aspects of multimodal therapy are often impractical or cost-prohibitive. Non-steroidal anti-inflammatory drugs (NSAIDs) are an integral component of equine pain management as they demonstrate both anti-inflammatory and analgesic effects. The currently available NSAIDs labeled for use in horses are used to treat a wide variety of conditions associated with varying degrees of pain. Despite standard analgesic therapy, horses may still experience moderate to severe pain associated with certain conditions such as acute laminitis or following surgery. In a recent evaluation of 34 horses undergoing exploratory laparotomy, 65% of horses experienced moderate to severe pain following surgery despite “standard of care” analgesic therapy.¹ The horses in the Graubner et al. study received flunixin meglumine (0.5 mg/kg IV) every 8 hours and were evaluated for pain using a multidimensional pain scoring system based on physiological and behavioral parameters. In a retrospective study of 300 equine cases of surgical colic, post-operative pain was reported in 32% of horses that received flunixin meglumine (0.25 mg/kg IV) every 8 h after recovery from anesthesia.² Pain was the most common reason recorded for euthanasia in these horses.² Opiate drugs, such as morphine and butorphanol, have been used to provide adjunctive analgesia in horses receiving NSAIDs;³ however, their use is associated with significant side effects in horses including behavior changes and gastrointestinal hypomotility that often limit their use in high-risk patients, regardless of pain score.³⁻⁵

Ketorolac tromethamine (KT) is a pyrrolizine carboxylic acid derivative NSAID and non-selective cyclooxygenase (COX) inhibitor. While it is widely used in human medicine, primarily as a post-operative analgesic for moderate to severe pain, KT has not been thoroughly evaluated in horses⁶⁻¹² Ketorolac tromethamine is commonly administered intravenously (IV) or as a constant rate infusion (CRI) in humans, even though it is labeled for intramuscular (IM) and oral (PO) use⁶⁻⁸ Numerous studies in human medicine have

evaluated KT as a morphine-sparing analgesic in post-operative patients. The results of these studies indicated a 22 to 44% reduction in morphine consumption when patients were treated with morphine and ketorolac for the first 24 h following abdominal or orthopedic surgery versus morphine alone^{6,7,10} The adverse effects of KT in humans are similar to those associated with all NSAIDs, including gastrointestinal ulceration and increased risk of bleeding, but the overall incidence of adverse effects in post-operative patients is low.¹³ Ketorolac tromethamine has been shown to be equivalent in safety to the NSAIDs diclofenac and ketoprofen in post-operative human patients¹⁴ In rats, KT has demonstrated an anti-inflammatory potency up to 50 times that of phenylbutazone, and anti-pyretic properties 20 times that of aspirin.⁹ When administered orally in mice, KT was found to be more than 350 times as potent as phenylbutazone with respect to analgesia.^{9,15} Studies in mice and rats have indicated that the relative analgesic potency of KT is even greater than its anti-inflammatory potency.^{9,15}

We are aware of only two studies that have investigated the pharmacokinetic profile of KT in adult horses. A 1994 study reported pharmacokinetic data after a single 300 mg IV or IM dose of KT in six adult horses,¹¹ but the study failed to account for individual horse variability as it was not a crossover design. A more recent study evaluated KT in 6 colts (0.5 mg/kg IV) undergoing castration.¹² The colts in that study received several other medications concurrently and underwent general anesthesia, two factors that have the potential for confounding kinetic data as it is unknown how drug interactions or anesthesia affect metabolism of KT in the horse. The objective of the study reported here was therefore to evaluate the pharmacokinetic profile of KT in healthy adult horses after IV, IM, and PO single dose administration, and to investigate the potential for adverse effects following KT administration. A single dosage of 0.5 mg/kg was selected for investigation based on extrapolation from recommended human dosage protocols and previous pharmacokinetic studies in the horse and dog.^{11,16-18}

3.3 Materials and Methods

3.3.1 Animals and Experimental Design

Nine adult horses from the Purdue University teaching herd were utilized for the crossover pharmacokinetic study. The horses were determined to be healthy on the basis of physical examination, complete blood count (CBC) and serum biochemical analysis (SBA), and had no history of NSAID administration within two months prior to the start of the study. A repeated Latin square design was used to ensure that each horse received each route of drug administration (IV, IM, and PO). Horses were randomly assigned a number (1 through 9) and divided into groups of 3, and each trial was staggered over the course of 3 days. The first route of administration was randomly assigned to each horse. The horses consisted of 6 mares and 3 geldings that ranged in age from 6 to 24 years old, with a mean of 15 ± 6 years. Five breeds were represented, including 3 Standardbreds, 2 Thoroughbreds, 2 Warmbloods, 1 Saddlebred and 1 Quarter Horse. The horses weighed 460 – 650 kg and were weighed immediately prior to the start of each trial to ensure accurate dosing. All horses gained weight over the course of the study, with a mean weight gain of 21.6 ± 9.6 kg. This was attributed to access to lush forage as the study took place between April and June when the horses were housed on green pasture. All procedures in this study were approved by the Institutional Animal Care and Use Committee at Purdue University.

3.3.2 Drug Administration

A 0.5 mg/kg dosage of KT was used for IV, IM (30 mg/ml)^a and PO (10 mg/tablet)^b administration, with a 2 week washout period between each trial. The 0.5 mg/kg dose of KT was equivalent to a KT dose of 0.34 mg/kg. Injectable drug doses were rounded up to the nearest 0.1 mL; oral doses were rounded up to the nearest whole tablet (10 mg). The washout period was selected to exceed the anticipated elimination half-time by at least 10 times. For the first 24 h of each trial, the horses were housed in box stalls with free access to grass hay, alfalfa hay, and water. The horses were not fasted before or after PO

administration in order to mimic clinical administration. After the first 24 h of each trial and during the washout period the horses were housed on pasture.

Intravenous jugular catheters were aseptically placed the night before the start of each trial; horses undergoing IV drug administration had a catheter placed in each jugular vein. The catheter used to deliver the KT was removed after drug administration; the contralateral catheter was used for all blood collections. Intramuscular injection of KT was performed in the neck muscle opposite that of the jugular catheter. The oral dose of KT was delivered via nasogastric tube; the tablets were first dissolved in 60 mL of water, added to the nasogastric tube, and then followed by 3 L of water to ensure complete delivery of the drug.

Heparinized blood samples were collected from the jugular vein catheter immediately prior to drug administration ($t=0$) and at 5, 10, 15, 20, 30, 45, 60, 90 min and 2, 3, 4, 6, 8, 10, 12, 24 h and 48 h after drug administration. Plasma was harvested by centrifugation at 1,300 g for 5 minutes within 6 h of collection and stored at -80°C until analyzed. Ketorolac tromethamine is stable in plasma for at least 30 days when stored at -20°C ¹⁹ or -80°C ²⁰.

3.3.3 Adverse Effects

Horses were continually monitored during the first 12 h of each trial and complete physical examinations were performed at $t=0$, 4, 12, 24 and 48 h. A CBC^c and SBA^d were performed at $t=0$ and $t=24$ h. Subjective assessments were made regarding changes in behavior, appetite, and fecal consistency, as well as evidence of inflammation at the IM injection site (characterized by the presence of heat, swelling, or pain).

3.3.4 Sample Analysis

Plasma samples were prepared as described prior to high performance liquid chromatography (HPLC) tandem mass spectrometry (MS-MS) analysis^{20,21}. Briefly, an internal standard (IS) solution containing 50 μL etodolac (500 ng/mL in 50% water:50% acetonitrile) was added to 200 μL plasma and vortexed.²² Protein precipitation was

performed by adding 800 μ L of a solution of 0.1% formic acid in acetonitrile. The mixture was vortexed and then centrifuged at 12,000 g for 5 minutes. Aliquots of 500 μ L were transferred to HPLC vials with 10 μ L submitted for HPLC/MS-MS analysis.

Ketorolac tromethamine plasma levels were quantitated by HPLC-MS-MS. Separation was performed on an Agilent Rapid Res 1200 HPLC system using an Agilent Zorbax XDB-C18 (2.1 x 50 mm, 3.5 μ m) column.^e Mobile phase A was H₂O with 0.1% formic acid and mobile phase B was ACN with 0.1% formic acid. A linear gradient elution was used as follows: initial conditions 35% B; 0 - 8 min: gradient to 70% B; 8 - 8.5 min: gradient to 90% B; 8.5 - 9.5 min: gradient held 90% B. During compound elution, a flow rate of 0.4 mL/min was used. Column re-equilibration was 9.5 - 10.5 min: gradient to 35% B; 10.5 - 13.5 min: gradient held 35% B. During re-equilibration, flow rate was increased to 0.6 mL/min and column flow was diverted to waste. Retention time for KT was 3.2 minutes and for etodolac was 6.9 minutes.

Analytes were quantified using MS/MS utilizing an Agilent 6460 Triple Quadrupole mass spectrometer with electrospray ionization (ESI).^f Quantitation was based on Multiple Reaction Monitoring (MRM). For KT, ESI positive mode was used with a transition of 256.1 to 104.9 and a collision energy (CE) of 18 V. For etodolac, ESI negative mode was used with a transition of 286.1 to 212.1 and a CE of 20 V. Both compounds used a fragmentor energy of 125 V and a dwell time of 300 ms. Source parameters were as follows: nitrogen gas temperature = 350°C and flow rate = 9 L/min, nebulizer pressure = 40 psi, sheath gas temperature = 250°C, sheath gas flow rate = 7 L/min, and capillary potential = 3500 V. All data were collected and analyzed with Agilent MassHunter B.03 software. Quantitation was based on a 6 point standard curve, with KT concentrations ranging from 2.5 to 5,000 ng/mL, using a diluent of acetonitrile and water (1:1, v/v). Standard curves were fit to a quadratic function, with a 1/x curve fit weighting. Correlation coefficients > 0.9997 were obtained. Curves were used if the standard concentration accuracy for each point was between 95 - 105%. Responses for KT were normalized against the internal standard (RR = response ratio).

The limit of quantitation was 1.2 ng/mL and the limit of detection was 0.5 ng/mL, as defined as a signal-to-noise ratio (RMS) of 10:1 and 3:1, respectively, determined using authentic standards. Matrix effects and extraction recoveries were assessed using the approach detailed by Trufelli et al.²³ Matrix effects were determined by ratioing the RR in matrix-matched standards to the RR in neat standards. The matrix effect was determined at 2.5 (low), 250 (middle), and 5,000 (high) ng/mL, which were 122%, 127%, and 99.2%, respectively (n=6). Extraction efficiencies were determined by ratioing the RR in pre-extraction spiked plasma to matrix-matched standards. The extraction efficiencies were determined at 2.5 (low), 250 (middle), and 5,000 (high) ng/mL, which were 84.3%, 89.0%, and 123%, respectively (n=6). Since no significant matrix effects or extraction efficiencies were observed, calibrants and control samples were prepared using a solvent of acetonitrile and water (1:1, v/v). At 2.5 (low), 250 (middle), and 5,000 (high) ng/mL, the precision (relative standard deviation, RSD) of the standard curves were 7.0%, 3.7%, and 1.7%, respectively (n=6). Based on a 100 ng/mL control sample, the intraday assay precision RSD ranged from 3.6% - 13.1%, and the interday precision (n=7) was 8.3%. The intraday accuracy RSD ranged from 95.8% - 103.9%, and the interday accuracy (n=7) was 98.8%.

3.3.5 Pharmacokinetic Analysis

Pharmacokinetics were characterized using standard compartmental and noncompartmental methods and a software program.^e A variety of weighting schemes were examined and the best model fit (inverse of concentration squared) was determined using Akaike's Information Criterion and the smallest sum of squared residuals.

Compartmental pharmacokinetic variables were attempted to be obtained for IV, IM and PO data from each horse by fitting the plasma KT concentration-time data to a two and three-compartment open model using various weighting schemes. Compartment models could not be satisfactorily fit to IV data for the majority of horses and to IM and PO data for any horse. Non-compartmental analysis for IV, IM and PO data from each

horse were fit using the last three or more data points from the semilog plasma concentration-time curve to calculate the elimination rate constant (λ_z , slope of the terminal linear phase). The area under the curve (AUC_∞) and the area under the first moment curve ($AUMC_\infty$) were calculated for each horse and treatment from the KT concentration-time relationship using the trapezoidal method, with the area from the last time point extrapolated to infinity using the last measured plasma KT concentration and λ_z . The elimination half-life was calculated as $0.693/\lambda_z$. Mean residence time (MRT) was calculated from the ratio of $AUMC_\infty$ to AUC_∞ . Total plasma clearance (Cl_p) was calculated as dose/AUC_∞ . The apparent steady-state volume of distribution ($V_{d(ss)}$) was calculated as $\text{dose} \times AUMC_\infty / AUC_\infty^2$. Mean absorption time (MAT) for IM or PO administration was calculated as $MAT_{IM} = MRT_{IM} - MRT_{IV}$ or $MRT_{PO} - MRT_{IV}$. Bioavailability was determined by dividing the IM or PO AUC_∞ by the IV AUC_∞ and multiplying by 100.

3.3.6 Statistical Analysis

Significance was set at $p < 0.05$. Data was expressed as mean $\pm$ SD and plasma concentration-time data was presented in a semi-logarithmic graph. A mixed model analysis of variance (ANOVA) with repeated measures was used to evaluate the CBC and SBA data in order to account for the crossover experimental design.^f

3.4 Results

3.4.1 Pharmacokinetics

The plasma concentration-time relationship of KT following a single 0.5 mg/kg IV dose is depicted in Figure 1 and relevant pharmacokinetic variables are summarized in Tables 2-1, 2-2, and 2-3. Plasma KT concentration was below the limit of detection for all horses and routes of administration at 48 h. Mean total plasma clearance (Cl_p) was rapid at 8.4 (mL/min)/kg and the estimated mean volume of distribution at steady state ($V_{d(ss)}$) was 0.77 L/kg.

The plasma concentration-time relationship of KT following 0.5 mg/kg IM or PO dosing is depicted in Figure 1 and relevant pharmacokinetic variables are summarized in Table 1. The mean observed time to maximum concentration ($t_{\max}$) after IM or PO administration was 25 and 19 min, respectively, indicating rapid absorption. The observed peak mean plasma concentrations ($C_{\max}$) after IM or PO administration were 0.58 and 0.31 $\mu\text{g/mL}$, respectively. The MRT was 2.0 h for IV, 2.6 h for IM and 7.1 h for PO administration. Calculated mean bioavailability (F) of KT after IM and PO delivery was 71% and 57%, respectively.

3.4.2 Adverse Effects

There were no significant changes in the physical examination or CBC of any horse during any trial period (Table 2-4). No subjective changes were noted in the behavior, appetite, or fecal consistency of any horse during any trial period. While none of the IM injection sites showed any clinical evidence of pain or inflammation, plasma creatinine kinase activity was increased at 24 h after IM injection ($p = 0.012$). The mean CK value pre-injection was 136 ± 34 IU/L versus a post-injection CK value of 162 ± 50 IU/L (Table 2-5).

3.5 Discussion

Similar to other species, the pharmacokinetic profile of KT after IV administration was characterized by a low volume of distribution and rapid clearance from the plasma compartment.^{23,24} The drug was rapidly absorbed after IM and PO administration and no clinical adverse effects were observed after single dose administration.

Mean total plasma clearance of KT was higher in the current study (8.4 mL/min/kg) than previously reported for horses by Plånborg et al. (2.8 mL/min/kg) and Ferraresi et al. (5.6 mL/min/kg).^{11,12} Differences in drug clearance after IV administration of a water soluble drug such as KT is primarily due to species differences in the rate of hepatic KT metabolism or renal blood flow, as KT is primarily metabolized by the liver to form glucuronide conjugates and excreted by the kidneys.¹⁹ Species differences in urine pH

may impact plasma clearance of acidic drugs such as KT, as alkaline urine has the potential to ion trap KT and thereby prevent reabsorption; however, this is considered an unlikely reason for the high clearance in horses, as KT has a pKa value of 3.5 which indicates effectively full dissociation even in acidic urine (pH = 5.5). Moreover, clearance of KT after IV administration is variable in ruminants that typically have an alkaline urine, being 12.4 (mL/min)/kg in adult sheep,²⁴ 8.8 (mL/min)/kg in adult goats,²³ but only 0.8 (mL/min)/kg in calves.²⁵ Differences in drug clearance may also be due to differences in plasma protein binding between species, as small changes in binding percentage for highly bound drugs such as KT can have a large impact on drug availability for hepatic metabolism or urinary excretion.²⁵ Additional studies to determine the rate of hepatic metabolism are indicated to confirm the supposition that rapid hepatic metabolism is the reason for the high plasma clearance of KT in horses.

It is not clear why Plånborg et al. found KT to have a much slower clearance than that reported in our study, but only 3 horses were used in that study to estimate clearance, and their assay was able to detect KT only up to 3 h post administration,¹¹ which was probably too short a time interval to provide an accurate estimate of clearance. The colts in the Ferraresi et al. study received acepromazine, detomidine, ketamine, and diazepam in addition to KT before undergoing general anesthesia with isoflurane; clearance of KT is expected to be decreased in horses receiving these drugs because of drug-induced decreases in cardiac output, mean arterial pressure, renal blood flow, and core body temperature.²⁶⁻²⁸ Given the rapid clearance of KT in the horse, the most useful application of the drug in horses is likely to be as a short term continuous rate IV infusion (CRI) or oral administration in horses with normal gastrointestinal motility. While there are no reports in the veterinary literature, CRI of NSAIDs has been described in human medicine for treatment of fever or pain due to surgery or cancer.²⁹⁻³¹ When administered as a CRI in humans, KT has been shown to be safe and effective at providing analgesia and reducing the use of opiates.^{32, 33}

The mean $V_{d(ss)}$ of KT in the current study (0.77 L/kg; median 0.49 L/kg) was similar to that for other NSAIDs¹⁹ and was most likely due to a moderately high degree of plasma protein binding. While not specifically evaluated in the current study, Ferraresi et al. reported a plasma binding of 76% in horse plasma.¹² Plasma binding of KT is 72.0% in mice,¹⁹ 92.1% in rats,¹⁹ 98.9% in dogs,¹⁷ and 99.2% in humans.¹⁹

The mean plasma elimination $t_{1/2}$ of KT in this study following IV administration was 8.7 h (median 5.8 h). For comparison, sheep have a much shorter $t_{1/2}$ (18 min) after IV administration,²⁴ whereas studies in dogs,¹⁸ calves,²⁵ and the 1994 study in horses¹¹ reported an elimination half-life similar to that observed in humans of approximately 4 to 6 h.

The pharmacokinetic profile of KT after IM injection could not be adequately characterized using a compartmental model; consequently, non-compartmental analysis was performed. Ketorolac tromethamine was rapidly absorbed after IM injection in horses, with a mean absorption time of 36 min (95% confidence interval for mean absorption time of -5.5 to 6.7 h) that was similar to that reported in sheep (11 min)²⁴ and humans (46 min).³⁴

Oral administration of KT resulted in a t_{max} of 19 min in horses, which is shorter than the t_{max} in dogs (51 min)¹⁸ and humans (53 min).³⁴ While the oral pharmacokinetics of KT have been evaluated in goats and calves, it is difficult to compare oral drug administration in ruminants vs. non-ruminant animals given the vast difference in drug absorption resulting in delayed t_{max} (6.5 h in calves and 8.9 h in goats).^{23,25} The differences in t_{max} between horses and dogs may be related to method of administration; the tablets in the current study were dissolved in 60 mL of water prior to administration, and then followed by 3 L of water which may have facilitated gastric emptying and therefore a faster rate of delivery to the small intestine. The oral formulation given to dogs was reported to be a capsule, which may have delayed drug absorption.¹⁸ Ketorolac tromethamine was administered via nasogastric tube in this study in order to ensure the horse received the entire drug amount and permit accurate estimation of oral

bioavailability; however, this method of administration did not permit evaluation of the potential influence of buccal drug absorption. While in a clinical setting KT would likely be given via oral dose syringe, given the moderate oral bioavailability of KT found in this study, and all other studies in a variety of species,^{18,19,25} it is unlikely that buccal absorption would have had an impact on the plasma KT concentration-time profile. *Per os* administration of drugs is not usually accompanied by water, and delivery likely results in partial loss of drug. Thus, intragastric administration of KT may have falsely increased oral bioavailability in this study. Interestingly, the plasma concentration-time relationship after oral KT administration appeared to be biphasic, with a slightly higher plasma KT concentration present than anticipated from approximately 4 to 12 h after administration. A more exaggerated pattern has been observed in dogs administered KT intravenously,¹⁷ leading to speculation that enterohepatic cycling of KT may have been present.

As reported in humans and other species,^{18 19,23,25,34} the mean bioavailability of KT after both IM and PO administration was moderate at 71% and 57%, respectively. This is similar to the IM bioavailability previously reported in horses (69%).¹¹

While the target plasma concentration of KT for effective analgesia in horses is not known, an EC_{50} of 0.37 $\mu\text{g/mL}$ for plasma KT concentration has been calculated for humans using pharmacodynamic modeling.³⁵ Interestingly, oral administration of KT failed to achieve a mean plasma KT concentration of 0.37 $\mu\text{g/mL}$, and IM administration resulted in plasma KT concentration exceeding 0.37 $\mu\text{g/mL}$ for less than 1 h.

Despite the extensive literature on analgesic properties of KT in humans, veterinary studies are sparse. Only one analgesic trial has been performed in horses, when 6 colts undergoing elective castration received KT pre-operatively (0.5 mg/kg IV).¹² All colts experienced adequate analgesia, as determined by a visual analog score, although there was no untreated control group and investigators were therefore not masked to treatment.¹² Several studies have evaluated the clinical efficacy of KT in providing post-operative analgesia in dogs. In a randomized controlled trial, KT given at a

dose of 0.5 mg/kg IM was as effective as flunixin meglumine (1.0 mg/kg IM) and superior to both butorphanol (0.4 mg/kg IM) and oxymorphone (0.05 mg/kg IM) in providing consistent post-operative analgesia.¹⁶ Similar findings were observed in a clinical trial during which 15 dogs received KT (0.5 mg/kg IV) for analgesia associated with elective castration, though no control group was used in that study¹⁷. Given the diversity of individual pain sensitivity and the variety of painful medical conditions, determining the effective therapeutic concentration of a drug is difficult, even when patients can verbally communicate their pain level. Furthermore, the target therapeutic concentration of a drug is likely to vary among species. Therefore, the appropriate dosage of a drug must be based on attaining the plasma concentration that has been shown to be both effective and safe.

While no adverse effects were noted in the current study, NSAID use in all species is associated with damage to the renal medulla and gastrointestinal mucosa due to inhibition of prostaglandin E₂ and vasoconstriction. The safety of KT has been evaluated extensively in humans with mixed results. While several studies report that the risk of adverse effects is not higher than other NSAIDs,^{13,14} other studies have cited KT has having a higher relative risk of upper gastrointestinal bleeding in comparison to other human NSAIDs.³⁶ The risk of adverse effects in humans has been shown to be increased in certain patients with preexisting conditions such as renal or gastrointestinal disease, coagulopathy, or those receiving concurrent administration of other NSAIDs. Given these potential risks in the human population, use of KT in the United States is restricted to no more than 5 days of treatment³⁷. While no adverse effects have been noted in any of the previous veterinary single-dose pharmacokinetic studies, only one study has specifically evaluated the safety of multiple doses of KT. In this study, no difference in adverse effects was found between KT and flunixin meglumine in dogs after 3 doses of either drug administered 6 h apart.¹⁶ Given the potentially severe adverse effects of NSAIDs in horses, the safety of KT must be evaluated further.

The increase in CK values noted after IM injection may or may not be clinically relevant. Creatine kinase is a sensitive indicator of muscle damage; a small amount of muscle damage can result in a detectable elevation in CK and does not necessarily result in clinically detectable pain or inflammation.³⁸ None of the horses experienced clinical evidence of injection site reaction in this study or had a CK value that was outside of the reference range; however, the post-injection samples were collected 24 h after injection and CK activity peaks 6-12 h after muscle injury.³⁹ While the KT administered to horses in this study was formulated for IM use in humans and is commonly used without significant adverse effects, the potential for muscle damage may become apparent with repeated or prolonged dosing in horses.

3.6 Conclusion

In conclusion, the pharmacokinetic profile indicates rapid absorption when KT is administered orally or by IM injection in healthy adult horses with no obvious adverse effects after a single 0.5 mg/kg dosage. Further studies are necessary to evaluate the analgesic and anti-inflammatory properties of a CRI of KT in the horse, as well as the drug's safety profile when administered as a CRI.

^a Ketorolac tromethamine (30 mg/mL), Wockhardt USA Inc., Parsippany, NJ

^b Ketorolac tromethamine (10 mg/tablet), Teva Pharmaceuticals, North Wales, PA

^c Abbott Cell-Dyn 3500 Hematology Analyzer, Abbott Park, IL

^d Johnson & Johnson Vitros 5,1 FS Chemistry Analyzer, Holliston, MA

^e Agilent ZORBAX Eclipse XDB column, Agilent Technologies, Santa Clara, CA

^f Agilent 6460 Triple Quadrupole mass spectrometer, Agilent Technologies, Santa Clara, CA

^g PKSolver doi:10.1016/j.cmpb.2010.01.007

^h SAS 9.3, SAS Inc, Cary, NC

Table 3-1: Non-compartmental pharmacokinetic parameters and body weight for ketorolac tromethamine in healthy adult horses (n=9) following a single (0.5 mg/kg) intravenous dose.

Intravenous	Horse											
Variable	1	2	3	4	5	6	7	8	9	Mean	SD	CV (%)
Body weight (kg)	565	511	546	585	541	564	461	492	634	544	52	9
C ₀ (µg/mL)	3.48	1.46	1.06	3.50	3.65	2.77	5.84	1.73	3.78	3.03	1.47	49
λ _z (h ⁻¹)	0.049	0.549	0.555	0.051	0.032	0.120	0.483	0.045	0.171	0.228	0.231	101
t _{1/2} (h)	14.1	1.3	1.2	13.5	21.9	5.8	1.4	15.4	4.1	8.7	7.6	87
AUC _∞ (h×µg/mL)	0.984	0.437	0.204	0.824	0.697	0.824	0.515	0.628	0.804	0.657	0.240	37
AUMC _∞ (h ² ×µg/mL)	3.171	0.196	0.075	2.131	3.614	0.802	0.095	2.641	0.577	1.478	1.415	96
MRT (h)	3.2	0.4	0.4	2.6	5.2	1.0	0.2	4.2	0.7	2.0	1.9	94
Cl _p ({ml/min}/kg)	4.8	9.7	22.3	5.9	6.5	5.7	7.5	6.5	6.6	8.4	5.4	64
V _{d(ss)} (L/kg)	0.93	0.26	0.49	0.92	2.01	0.33	0.08	1.65	0.28	0.77	0.67	87

C₀ = plasma concentration at time = 0 h; λ_z = elimination rate constant; t_{1/2} = elimination half-life; AUC_∞ = area under the plasma concentration vs. time curve; AUMC_∞ = area under the first moment curve; MRT = mean residence time; Cl_p = Plasma clearance; V_{d(ss)} = Volume of distribution at steady state; SD = standard deviation; CV = coefficient of variation.

Table 3-2: Non-compartmental pharmacokinetic parameters and body weight for ketorolac tromethamine in healthy adult horses (n=9) following a single (0.5 mg/kg) intramuscular dose.

Intramuscular	Horse											
Variable	1	2	3	4	5	6	7	8	9	Mean	SD	CV (%)
Body weight (kg)	554	504	578	563	529	579	460	493	650	546	57	10
C _{max} (µg/mL)	0.31	0.70	0.67	0.52	1.55	0.39	0.37	0.40	0.29	0.58	0.39	68
t _{max} (h)	0.50	0.25	0.17	0.25	0.17	0.75	0.33	0.33	1.00	0.42	0.29	69
λ _z (h ⁻¹)	0.238	0.057	0.080	0.430	0.204	0.055	0.065	0.178	0.060	0.152	0.126	83
t _{1/2} (h)	2.9	12.1	8.6	1.6	3.4	12.5	10.7	3.9	11.6	7.5	4.5	60
AUC _∞ (h×µg/mL)	0.621	0.836	0.633	0.659	1.019	0.662	0.647	0.488	0.626	0.688	0.152	22
AUMC _∞ (h ² ×µg/mL)	1.160	2.766	1.354	0.828	1.257	2.313	3.429	0.676	2.212	1.777	0.944	53
MRT (h)	1.9	3.3	2.1	1.3	1.3	3.5	5.3	1.4	3.5	2.6	1.4	53
MAT (h)	-1.4	2.9	1.8	-1.3	-3.9	2.5	5.1	-2.8	2.8	0.6	3.1	489
F (%)	65	84	64	68	110	67	65	52	63	71	17	24

C_{max} = maximum plasma concentration; t_{max} = time to observed maximum plasma concentration; λ_z = elimination rate constant; t_{1/2} = elimination half-life; AUC_∞ = area under the plasma concentration vs. time curve; AUMC_∞ = area under the first moment curve; MRT, mean residence time; MAT = mean absorption time; F = percent bioavailability. SD = standard deviation; CV = coefficient of variation.

Table 3-3: Non-compartmental pharmacokinetic parameters and body weight for ketorolac tromethamine in healthy adult horses (n=9) following a single (0.5 mg/kg) oral dose.

Oral	Horse											
Variable	1	2	3	4	5	6	7	8	9	Mean	SD	CV (%)
Body weight (kg)	572	491	581	599	506	584	467	477	662	549	66	12
C _{max} (µg/mL)	0.22	0.15	0.46	0.32	0.12	0.30	0.24	0.79	0.23	0.31	0.20	64
t _{max} (h)	0.25	0.33	0.25	0.25	0.50	0.17	0.17	0.50	0.33	0.31	0.13	41
λ _z (h ⁻¹)	0.083	0.133	0.152	0.077	0.100	0.098	0.092	0.100	0.117	0.106	0.024	23
t _{1/2} (h)	8.3	5.2	4.6	9.0	7.0	7.1	7.5	6.9	5.9	6.8	1.4	21
AUC _∞ (h×µg/mL)	0.576	0.313	0.775	0.562	0.611	0.424	0.379	0.931	0.342	0.546	0.208	38
AUMC _∞ (h ² ×µg/mL)	5.997	1.954	3.815	5.491	5.447	1.923	2.877	4.840	2.124	3.830	1.661	43
MRT (h)	10.4	6.2	4.9	9.8	8.9	4.5	7.6	5.2	6.2	7.1	2.2	31
MAT (h)	7.2	5.8	4.6	7.2	3.7	3.6	7.4	1.0	5.5	5.1	2.1	42
F (%)	60	31	78	58	66	43	38	99	35	57	22	40

C_{max} = maximum plasma concentration; t_{max} = time to observed maximum plasma concentration; λ_z = elimination rate constant; t_{1/2} = elimination half-life; AUC_∞ = area under the plasma concentration vs. time curve; AUMC_∞ = area under the first moment curve; MRT, mean residence time; MAT = mean absorption time; F = percent bioavailability. SD = standard deviation; CV = coefficient of variation.

Table 3-4: Mean $\pm$ SD of complete blood count parameters for intravenous (IV), intramuscular (IM) and oral (PO) before (pre) and 24 hours after (post) administration of ketorolac tromethamine (0.5 mg/kg) in 9 adult horses.

			IV pre		IV post		IM pre		IM post		PO pre		PO post	
Variable	Units	Reference range	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Total Plasma Protein	g/dl	5.7-8.1	6.9	0.4	7.0	0.3	6.8	0.3	6.9	0.3	6.9	0.5	7.0	0.4
RBC	M/ μ l	6.0-12.0	8.2	0.9	7.8	0.9	8.4	0.9	8.3	1.0	8.2	0.8	7.9	0.8
Hematocrit	%	35.0-50.0	40.7	4.0	38.8	3.7	41.9	4.1	40.9	4.1	40.6	3.5	39.3	2.7
Hemoglobin	g/dl	11.0-19.0	13.7	1.3	13.1	1.4	14.2	1.5	13.9	1.6	13.7	1.2	13.3	0.9
MCV	fL	35.0-55.0	49.9	2.2	50.0	2.2	49.8	2.0	49.5	1.6	49.8	2.2	49.9	2.3
MCHC	g/dl	30.0-36.0	33.7	0.5	33.8	0.7	33.8	0.5	33.9	0.8	33.8	0.6	33.9	0.4
White blood cells	K/ μ l	6.0-12.0	7.6	2.0	7.9	2.5	7.2	1.4	7.8	1.5	7.6	2.2	7.4	2.3
Neutrophil	K/ μ l	3.0-7.0	5.0	2.3	5.2	2.7	4.5	1.2	4.9	1.4	5.0	1.9	4.7	1.6
Lymphocyte	K/ μ l	1.5-5.5	2.3	0.6	2.4	0.9	2.5	0.9	2.5	0.5	2.3	0.7	2.3	1.0
Monocyte	K/ μ l	0.05-0.80	0.2	0.2	0.2	0.1	0.2	0.1	0.3	0.2	0.1	0.1	0.2	0.1
Eosinophil	K/ μ l	0.0-0.4	0.2	0.2	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Table 3-5: Mean $\pm$ SD of serum biochemical analysis parameters for intravenous (IV), intramuscular (IM) and oral (PO) before (pre) and 24 hours after (post) administration of ketorolac tromethamine (0.5 mg/kg) in 9 adult horses.

Variable	Units	Reference range	IV pre		IV post		IM pre		IM post		PO pre		PO post	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Glucose	mg/dl	73-124	98.3	6.2	98.8	9.6	96.6	7.0	95.1	4.8	96.8	10.0	98.8	9.7
BUN	mg/dl	8.0-27	18.4	3.5	17.3	4.4	18.2	4.4	18.3	4.9	18.8	3.8	18.0	4.3
Creatinine	mg/dl	0.6-1.8	1.0	0.1	1.0	0.2	1.0	0.1	1.0	0.1	1.0	0.1	1.0	0.1
Phosphorus	mg/dl	2.0-5.7	3.9	0.4	3.8	0.6	3.8	0.5	3.7	0.8	3.6	0.5	3.7	0.5
Calcium	mg/dl	10.7-13.4	12.2	0.8	12.1	0.7	11.0	3.5	12.2	0.4	12.2	0.6	12.3	0.5
Sodium	mmol/L	132-144	138.2	2.4	138.7	1.7	138.7	2.1	138.0	2.5	138.3	1.4	138.7	1.4
Potassium	mmol/L	2.7-4.8	3.5	0.7	3.5	0.4	3.6	0.6	3.5	0.7	3.5	0.5	3.4	0.6
Chloride	mmol/L	94-103	100.1	2.0	99.6	1.7	100.1	2.2	99.0	2.0	99.8	2.3	98.9	1.3
Carbon dioxide	mmol/L	23-31	29.3	1.4	29.8	2.0	29.7	1.8	29.6	1.6	29.7	1.9	29.9	2.0
Anion Gap	mmol/L	12.0-20	12.3	1.4	12.8	1.8	12.5	1.4	12.9	1.8	12.4	2.4	13.3	1.5
Total Protein	g/dl	4.7-7.5	6.7	0.4	6.6	0.3	6.6	0.4	6.7	0.4	6.6	0.5	6.8	0.4
Albumin	g/dl	2.5-3.8	3.0	0.2	3.0	0.1	3.0	0.1	3.0	0.2	3.0	0.2	3.0	0.2
Globulin	g/dl	2.2-3.8	3.7	0.4	3.7	0.3	3.7	0.3	3.7	0.3	3.7	0.3	3.7	0.2
AST	IU/L	206-810	379.9	101.8	368.2	75.0	363.2	73.2	376.3	69.5	370.9	48.3	369.9	43.9
ALP	IU/L	109-331	153.8	74.5	156.2	76.6	144.2	49.4	148.3	48.9	152.1	72.2	156.2	77.1
GGT	IU/L	12.0-46	31.7	9.4	32.3	9.7	31.7	8.9	32.8	8.1	32.7	10.3	33.3	10.3
Bilirubin	mg/dl	0.10-2.6	1.5	0.3	1.7	0.4	1.3	0.4	1.7	0.6	1.4	0.3	1.7	0.5
Magnesium	mg/dl	1.6-2.7	1.6	0.2	1.7	0.2	1.7	0.2	1.7	0.2	1.7	0.1	1.7	0.2
Creatinine Kinase	IU/L	88-453	161.4	67.3	159.0	40.8	135.7	33.5	162.1	50.2	135.8	39.2	153.9	65.2

Figure 3-1: Concentration vs. time curve for ketorolac tromethamine (0.5 mg/kg) in 9 adult horses after intravenous (IV), intramuscular (IM) and oral (PO) administration

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CHAPTER 4. CONCLUSIONS AND FUTURE DIRECTIONS

Ultimately, KT needs to be evaluated for its anti-inflammatory and analgesic efficacy, as well as safety, before being used in horses in a clinical setting. Specifically, KT should be compared to the NSAIDs currently approved for use in horses, including the nonspecific COX inhibitors flunixin meglumine and phenylbutazone, as well as the COX-2 selective inhibitor firocoxib.

The anti-inflammatory properties of KT have not yet been evaluated in horses. Initial studies may be performed *in vitro* and then *in vivo* using established equine models of gram negative sepsis, or endotoxemia.¹⁻⁶ Currently, we have obtained funding to study the anti-inflammatory properties of KT. Monocytes will be isolated and cultured from healthy horses and then exposed to LPS *in vitro* as previously described⁴ and then exposed to KT, a positive control (flunixin meglumine) or negative control. Cytokine (TNF α , IL-6, and IL-8) and eicosanoid (PGE₂ and TXB₂) production will be measured as a measurement of anti-inflammatory properties.

In order to evaluate KT's efficacy as an analgesic, a standardized pain model should be used before implementing a clinical trial. Foreman et al. have developed a model of reversible lameness that they have used to evaluate the analgesic efficacy of several NSAIDs.^{7,8} By using a standardized model, both the dose and efficacy of KT can be established.

In human medicine, KT is often used as a CRI to provide analgesia in certain situations such as postoperatively or for cancer pain.⁹⁻¹⁶ Other NSAIDs, such as diclofenac,^{17,18} ketoprofen,¹⁹⁻²¹ or ibuprofen²² have also been used in human clinical patients. While postoperative use of butorphanol²³ and lidocaine²⁴ CRIs have previously been evaluated in clinical equine patients, there has been no published report of an NSAID

given as a CRI in veterinary medicine. One future goal of this research is to explore the possibility that KT can be employed as a CRI to provide superior, continuous analgesia to horses with a high degree of pain, such as postoperatively.

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