PHARMACOKINETICS AND PHARMACODYNAMICS OF ORAL MELOXICAM TABLETS IN HEALTHY HORSES

by

KARIE VANDER WERF

B.S., University of Florida, 2000
D.V.M., Ross University School of Veterinary Medicine, 2005

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Clinical Sciences
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2013

Approved by:

Major Professor
Elizabeth Davis, DVM, PhD, DACVIM
Abstract

The first aim of the current study was to investigate the pharmacokinetics of oral meloxicam tablets and the gastrointestinal and renal effects after a 14-day treatment period. Meloxicam was orally administered to six adult horses once daily at a dosage of 0.6 mg/kg for 14 consecutive days. Blood was collected prior to each administration and at 20 and 40 min, and 1, 2, 4, 8, 12, and 24 hours after administration on days 1, 7, and 14 for the determination of meloxicam plasma concentrations by mass spectrometry. In addition, trough samples were taken on days 3 and 10. Complete blood count, serum biochemical analysis, urinalysis, and gastroscopy were performed at baseline and conclusion of the investigation.

Complete blood count, serum chemistry, and urinalysis results were unchanged through the study period. Gastroscopy scores were not significantly increased. The $C_{\text{max}}$ was $1.82 \pm 0.80 \mu g/mL$ at $T_{\text{max}}$ 3.48 ± 3.30 hr on day 1, $2.07 \pm 0.94 \mu g/mL$ at $T_{\text{max}}$ 1.24 ± 1.24 hr on day 7, and $1.81 \pm 0.76 \mu g/mL$ at 1.93 ± 1.30 h on day 14 ($p = 0.30$). The mean half-life was 4.99 ± 1.11 h.

The second aim of the study was to compare the analgesic effects and gastrointestinal and renal adverse effects of oral meloxicam tablets (0.6 mg/kg) to oral phenylbutazone tablets (4.4 mg/kg) orally once daily for 4 days in induced and naturally occurring lameness in adult horses. The study was performed on 4 healthy but lame adult horses. Complete blood count, serum biochemistry, urinalysis, and gastroscopy were performed prior to entrance to the study. Lameness was exacerbated in two horses using lipopolysaccharide (LPS; *E. coli* O55:B5) injected into the right metacarpophalangeal joint. The remaining two horses had Grade 3 or Grade 4 lameness due to naturally occurring laminitis. Meloxicam or phenylbutazone was administered to two horses each in a blinded, randomized manner once daily for four days. Lameness was evaluated using a pressure mat system and contact pressure, force, and stride length were evaluated at baseline and twice daily.

Complete blood count, serum chemistry, and urinalysis were unremarkable for all four horses except one horse with an increased GGT. This horse experienced hepatic rupture secondary to amyloidosis the final day of the study. Gastric ulcer scores did not change during the study period. Phenylbutazone administration resulted in a greater response (force and contact area) in the right front and left hind limbs compared to meloxicam administration. There were not enough data points to evaluate the other two limbs.
A third aim of the study was two-fold and first evaluated the effects of \textit{ex vivo} stimulation of \textbf{peripheral blood mononuclear cells (PBMCs)} with LPS on \textbf{cyclooxygenase (COX) messenger RNA (mRNA)} expression. The second portion documented the effects of LPS-induced joint inflammation and treatment with non-steroidal anti-inflammatory drugs on the mRNA and protein expression of COX-2 in PBMCs. The results indicate that LPS upregulates COX-2 gene expression in PBMCs. Additionally, injection of LPS into the metacarpophalangeal joint increases both COX-2 mRNA and protein expression in PBMCs at 24 hours after injection. The relative expression of COX-2 after treatment with meloxicam or phenylbutazone indicates a stronger inhibition with phenylbutazone; however, further study with additional horses is needed.

Pharmacokinetic analysis of the oral tablet formulation of meloxicam indicates the pharmacokinetics are similar to the oral suspension formulation. Meloxicam appears to be inferior to phenylbutazone in its analgesic properties for induced lameness and naturally occurring laminitis, however the small sample size used in the study makes interpretation difficult.
# Table of Contents

List of Figures ........................................................................................................ vi
List of Tables ........................................................................................................ vii
Acknowledgements ............................................................................................... viii
Dedication ............................................................................................................... ix

Chapter 1 - Introduction ....................................................................................... 1

Chapter 2 - Literature Review .............................................................................. 3
  Pharmacokinetics ................................................................................................ 3
  Introduction ......................................................................................................... 3
  Principles of pharmacokinetic analysis .............................................................. 5
  Pharmacokinetic parameters ............................................................................. 6

Non-steroidal anti-inflammatory drugs ................................................................. 9
Meloxicam ............................................................................................................. 11
Synovitis models .................................................................................................. 15
Lameness Grades ................................................................................................. 16
Figures and Tables ............................................................................................... 17

Chapter 3 - Materials and Methods.................................................................... 21
Aim 1 ................................................................................................................... 21
  Experimental design and sample collection ...................................................... 21
  Statistical analysis ........................................................................................... 22
Aim 2 ................................................................................................................... 23
  Statistical analysis ........................................................................................... 23
Aim 3 ................................................................................................................... 24

Chapter 4 - Results .............................................................................................. 25
Aim 1 ................................................................................................................... 25
Aim 2 ................................................................................................................... 26
Aim 3 ................................................................................................................... 29

Chapter 5 - Discussion ......................................................................................... 33

References ............................................................................................................ 36
List of Figures

Figure 2.1 One compartment open pharmacokinetic model with first-order absorption............. 18
Figure 2.2 Generalized open two-compartment pharmacokinetic model with first-order absorption ($K_{01}$) into and elimination ($K_{el}$) from the central compartment. $K_{12}$ and $K_{21}$ represent intercompartmental micro-rate constants................................................................. 18
Figure 2.3 Three-compartment pharmacokinetic model after intravenous administration........ 18
Figure 2.4 The arachidonic acid cascade. HPETE = hydroperoxyeicosatetraenoic acid; HETE = hydroxyeicosatetraenoic acid; LT = leukotriene........................................................................................................ 19
Figure 2.5 COX-1 and COX-2 substrate-binding channels. Schematic depiction of the structural differences between the substrate-binding channels of COX-1 and COX-2 that allowed the design of selective inhibitors. The amino acid residues Val434, Arg513, and Val523, form a side pocket in COX-2 that is absent in COX-1. A, Nonselective inhibitors have access to the binding channels of both isoforms. B, The more voluminous residues in COX-1, Ile434, His513, and Ile532, obstruct access of the bulky side chains of the coxibs. (From Grosser T, Fries S, FitzGerald GA: Biological basis for the cardiovascular consequences of COX-2 inhibition: Therapeutic challenges and opportunities. J Clin Invest 116:4-15, 2006.)........ 20
Figure 4.1 Pharmacokinetic profile of oral meloxicam tablets on Day 1, 7, and 14 after administration of 0.6 mg/kg PO every 24 hours for 14 days................................................................. 31
Figure 4.2 qRT-PCR for COX-2 gene expression in equine PBMCs incubated ex vivo with LPS for 6 hours. There is a 4-fold increase in COX-2 gene expression in LPS treated cells...... 31
Figure 4.3 qRT-PCR performed on mRNA isolated from PBMCs from a horse treated with meloxicam (0.6 mg/kg PO q 24 h). Cycle threshold (CT) values decreased from 30.8 to 26.5 within 24 hours of administration................................................................. 32
Figure 4.4 qRT-PCR performed on mRNA isolated from PBMCs collected from 2 horses treated with phenylbutazone (4.4 mg/kg PO q 24 h). Cycle threshold values decreased in both horses at 96 hours.................................................................................................................. 32
List of Tables

Table 2.1 Comparison of pharmacokinetic studies of intravenous and oral suspension formulations of meloxicam. .............................................................................................................. 17
Table 2.2 Comparison of half-lives of oral and intravenous meloxicam formulations in different studies. .............................................................................................................. 17
Table 3.1 Primers used for qRT-PCR for COX-2 and 18S gene expression from peripheral blood mononuclear cells in horses. ...................................................................................... 24
Table 4.1 Gastric ulcer score pre- and post-treatment with oral meloxicam tablets, 0.6 mg/kg, once daily for 14 days. ........................................................................................................ 30
Table 4.2 Gastric ulcer score pre- and post-treatment for horses in Aim 2 of the study. .......... 30
Table 4.3 Mean ± SD values for pharmacokinetic parameters for multiple doses of meloxicam (0.6 mg/kg) administered orally once daily to 7* horses for 14 consecutive days. .......... 30
Acknowledgements

I would like to express my gratitude to my supervisor, Dr. Elizabeth Davis, whose expertise, understanding, and patience, added considerably to my graduate experience. I appreciate her vast knowledge and skill and her assistance in writing reports (i.e., grant proposals, manuscripts, and this thesis). I would like to thank the other members of my committee, Dr. Butch KuKanich and Dr. Jim Lillich for the assistance they provided at all levels of the research project.

A very special thanks to Michelle Hubin, without whose help and encouragement I would not have been able to complete the research. Her organizational skills and enthusiasm were a great help in the crazy time that was the Summer of 2010.

Caroline Gillespie was essential in the analysis of the PBMCs and put together a wonderful poster with her research information.

I must also acknowledge Dr. David Anderson for providing the pressure mat and saving us with his microsurgery skills when it was damaged. I would also like to sincerely thank Dr. Hans Coetzee and Laura Kohake for their assistance with the pressure mat data.

I would also like to thank my family and friends for the support they have provided me through my entire life.

In conclusion, I recognize that this research would not have been possible without the financial assistance from the Kansas State University Mentored Clinical, Applied, or Translational Research Award and I express my gratitude for this opportunity.
Dedication

This manuscript is dedicated to Henry, Big Boy, Billie Jean, and Ginger – without their ultimate sacrifice, this work could not have been completed.
Chapter 1 - Introduction

As one of the most common and most troublesome of all equine ailments, lameness remains the primary reason why horses are unable to fulfill their potential and their owners’ expectations. More preparation time is lost, more competitions are missed, and more careers are ended prematurely because of lameness than any other condition. Osteoarthritis, tendon and ligament injuries, and sepsis of a joint are frequently diagnosed in the equine athlete and pasture pet. Medications and therapies such as non-steroidal anti-inflammatory drugs (NSAIDs), platelet rich plasma, mesenchymal stem cell therapy, glucosamine/chondroitin neutraceuticals, physical therapy, and extracorporeal shock wave treatment have all been evaluated as treatment options for lameness; however, NSAIDs remain the mainstay of therapy.

Non-steroidal anti-inflammatory drugs are the most commonly used medications in the equine medical world. They are used to treat pain and inflammation due to colic, lameness, surgical interventions, and other medical procedures. Additionally, their anti-thrombotic, anti-pyretic, and attenuation of eicosanoid production properties are favorable. Examples of NSAIDs that are often used in the horse include phenylbutazone (Bute®), flunixin meglumine (Banamine®), firocoxib (Equioxx®), and ketoprofen (Ketofen®). All of the above medications have labels supporting their usage in the treatment of osteoarthritis or other musculoskeletal disorders.

In the horse, the pharmacokinetics of the oral and intravenous formulations of the commonly used NSAIDs have been investigated thoroughly; however, the pharmacokinetics of meloxicam have only been investigated for the intravenous and oral suspension formulations of the drug. In the United States, both formulations would be financially prohibitive, resulting in costs of over $200.00 a day. Although off label, the use of oral tablets would result in a cost of approximately $2.00 per day. This is between the average cost of phenylbutazone and firocoxib (Equioxx®) (approximately $0.40 and $9.00 per day, respectively). In addition, due to its potential cyclooxygenase isoform selectivity, meloxicam may result in fewer gastrointestinal and renal adverse effects compared to the more commonly used NSAIDs.

It was the purpose of this investigation:

a. To determine the disposition and adverse effects of oral meloxicam tablets following administration of a single dose and daily doses over 14 days.
b. To ascertain the analgesic effects of meloxicam on lame horses and compare to the effect of phenylbutazone in natural lameness and lipopolysaccharide-induced synovitis models.

c.

a. To evaluate the effects of incubation of equine PBMCs with LPS \textit{ex vivo} to determine the level of cyclooxygenase-2 upregulation.

b. To evaluate the effects of local injection of LPS into the metacarpophalangeal joint on the level of cyclooxygenase-2 protein expression in equine PBMCs and effect of orally administered phenylbutazone or meloxicam using qRT-PCR.
Chapter 2 - Literature Review

Pharmacokinetics

Introduction

Pharmacokinetics deals with the mathematical description of the time course of drug absorption, distribution, and elimination by means of a suitable model (Levy 1972). This discipline has allowed dosages of drugs to be tailored to both individuals and groups to optimize therapeutic effectiveness and minimize toxicity and adverse events.

To facilitate the study of the pharmacokinetic behavior of drugs, the body is depicted as a system made up of one, two, or three distribution compartment open models (Figures 2.1, 2.2, 2.3). The principle adopted in analyzing the serum-concentration time profiles is to employ the pharmacokinetic model with the least number of compartments that can adequately describe the data.

If a drug distributes very rapidly relative to the rate of elimination, the disposition kinetics of the drug behaves as a single homogenous distribution and the one-compartment open model is applied (Figure 2.1). This model assumes that a change in the drug concentration in one tissue is accompanied by a corresponding change in drug concentration in all other tissues (including plasma) at the same time. The following expression satisfies the one-compartment open model (Baggot 1977):

\[ C_p = B e^{-\beta t} \]  

where \( C_p \) is the concentration of the drug in plasma at any time, \( t \); \( B \) (\( \mu \text{g/mL} \)) is the zero-time intercept extrapolated from the overall elimination rate constant, \( \beta \); and \( e \) is the base of the natural logarithm.

It is assumed that a drug that is introduced into the central compartment, which consists of blood plasma and the extracellular fluid of highly perfused organs such as kidneys, lungs, liver, and heart, distributes instantaneously and homogeneously and that it is eliminated exclusively from the central compartment. Distribution into the second or peripheral (tissue)
compartment, which consists of less perfused tissues such as muscle, skin, and body fat, occurs more slowly. A two-compartment open model (Figure 2.2) can be described by the following expression:

\[ C_p = Ae^{-\alpha t} + Be^{-\beta t} \]  

where \( C_p \) is the concentration of the drug in the plasma at any time, \( t \); \( A \) and \( B \) (\( \mu g/mL \)) are extrapolated intercepts; \( \alpha \) and \( \beta \) are the rate constants for the distribution and elimination phases respectively; \( e \) represents the base of the natural logarithm. The sum of \( A \) and \( B \) (\( \mu g/mL \)) gives the plasma concentration at time zero.

The three-compartment open model (Figure 2.3) can be described by the following expression:

\[ C_p = Pe^{-\pi t} + Ae^{-\alpha t} + Be^{-\beta t} \]  

where \( C_p \) is the concentration of the drug in the plasma at any time, \( t \); \( P \), \( A \), and \( B \) (\( \mu g/mL \)) are zero-time extrapolated intercepts respectively; \( \pi \) and \( \alpha \) are the rate constants of distribution phases; \( \beta \) is the rate constant of the elimination phase; and \( e \) is the base of the natural logarithm.

The rate constants of the absorption and elimination of the drug are assumed to follow first-order kinetics, i.e., a constant fraction of the drug present is eliminated per unit time (Goodman 1975). The elimination of most drugs is exponential since drug concentrations usually do not approach those required for saturation of the elimination process. Suitable dosage regimens for drugs exhibiting dose-dependent kinetics in the therapeutic range defy easy calculation and are only established by careful titration of drug level in the patient (Levy 1968).

In certain exceptional cases, the drug elimination processes may become saturated and the result is zero-order kinetics, i.e., a constant amount of the drug is eliminated per unit time. An increase in dosage results in a more prolonged half-life and a disproportionately greater accumulation of the drug in the body (Goodman 1975). The elimination of phenylbutazone in the dog (Dayton 1967) and in the horse (Piperno 1968) obeys zero-order kinetics. The rate of elimination of a drug may be influenced by extensive binding to plasma protein, the rate of
various metabolic pathways, and the efficiency of excretion processes, particularly glomerular filtration (Baggot 1977).

**Principles of pharmacokinetic analysis**

Intravenous (IV) injection is the most reliable method of administration of a drug for the purpose of pharmacokinetic analysis. The biexponential equation,

\[ C_p = Ae^{-\alpha t} + Be^{-\beta t} \]  

(4)

describes the decline of a drug concentration-time curve after IV of a single bolus dose. The biexponential expression of the two-compartment open model assumes that, when injected IV, the drug distributes instantaneously throughout the central compartment. The initial steep decline in plasma concentration of a drug is due to the distribution of the drug by passive diffusion from the central to the peripheral compartment. Once apparent distribution equilibrium is attained, the rate of decline in plasma drug concentration is decreased and the linear terminal phase is referred to as the \( \beta \) or the elimination phase. Iterative least squares linear regression analysis is used to find the best fit curve. The slope of the linear terminal portion of the curve may be defined as \(-\beta/2.303\) and an extrapolation of this line gives the zero-time intercept, \( B \) (\( \mu g/mL \)), which is an estimate of the drug concentration that would have been attained if the distribution were instantaneous.

The residual data points, representing \( \alpha \) (the distribution phase), are obtained by subtracting the extrapolated portion of \( \beta \) (the elimination phase) from the experimental data of the initial steep portion of the concentration-time curve. Least squares regression analysis for the residual data is used to obtain the best fit curve of the distribution phase (\( \alpha \)) and extrapolation of this line yields zero-time intercept, \( A \).

For the three-compartment open model, the second set of residual data points are obtained by subtracting the extrapolated portion of the first distribution phase from its steep portion. Least squares regression analysis for the second set of residual data is used to obtain the best fit curve of the second distribution phase, \( \pi \). The extrapolation yields the zero-time intercept, \( P \) (\( \mu g/mL \)).
**Pharmacokinetic parameters**

**Pharmacokinetic rate constants**

The plasma drug concentration-time curve provides the only accurate information for drug measurement in the body. The experimental constants (A, B, α, or β) are “hybrid” pharmacokinetic parameters and are used to calculate the actual pharmacokinetic rate constants (k_{12}, k_{21}, and k_{el}) associated with the two-compartment open model.

\[
k_{12} = \frac{A\beta + B\alpha}{A + B} \quad (5)
\]

\[
k_{21} = \alpha + \beta - k_{21} - k_{el} \quad (6)
\]

are the rate constants for distribution between central and peripheral compartments, and

\[
k_{el} = \frac{\alpha\beta}{k_{21}} \quad (7)
\]

is the rate constant for elimination. All of these expressions are given in units of reciprocal time (h^{-1}).

Determination of the microconstants allows an assessment of the relative contribution of distribution and elimination processes (which may be altered in diseased states) to the concentration-time profile of a drug (Baggot 1977). A computer program, using values of the individual rate constants of the model, can be used to predict curves which describe the levels of a drug (expressed as a fraction of a single dose) in the central and peripheral compartments as a function of time, as well as the elimination curve. Based on the hybrid and actual pharmacokinetic parameters, other parameters associated with the two-compartment model can be calculated.

**Volume of distribution**

The volume of distribution is defined as the volume of fluid which would be required to contain the amount of drug in the body if it was uniformly distributed at a concentration equal to
that in plasma. The body is assumed to behave as a single homogenous compartment with respect to the drug. The value of $V_d$ serves as a proportionality constant relating the plasma concentration of the drug to the amount in the body at any time after distribution equilibration has been attained. $V_d$ is expressed as,

$$V_d (\text{mL}) = \frac{\text{mass (mg)}}{\text{concentration (mg/mL)}}$$  \hspace{1cm} (8)

The calculation of its value will be dependent on the modeling scheme utilized. Multiple physiological and protein-binding properties change the nature of the concentration profile being modeled and can change the value of $V_d$ obtained.

**Half-life**

The half-life for the elimination phase or the biological half-life of a drug is defined as the time required for the body to eliminate half of a particular drug, i.e., the time it takes for the serum concentration of the drug to decrease by 50% during the elimination phase of the disposition curve. It is assumed that, once pseudodistribution equilibrium has been established, the ratio of the drug in peripheral to central compartments remains constant. The half-life value is obtained from the equation,

$$t_{1/2} = \frac{0.693}{\beta}$$  \hspace{1cm} (9)

Where $\beta$ is the overall elimination rate constant and $t_{1/2}$ is expressed in units of time (h). An estimate of the half-life may also be obtained graphically. A large value of $\beta$ corresponds to a short half-life and indicates rapid elimination (Baggot 1977).

For drugs that obey zero-order kinetics, their half-lives become progressively longer as the doses increase (Baggot 1978). Knowledge of the half-life is a very useful for design of rational dosage regimens.

The half-lives of most drugs are independent of the dose and route of administration. Intravenous injection of a single dose of the drug is the only satisfactory procedure to determine
the half-life value since the apparent overall rate of elimination following other routes may be influenced by the rate of absorption (Byron 1976).

**Mean absorption time**

Mean absorption time (MAT) is the mean of the arrival time of bioavailable drug into systemic circulation. It is the statistical moment theory equivalent of estimating $k_a$ and is a relatively straightforward method to characterize the rate of absorption in bioavailability studies.

$$\text{MAT} = \text{MRT}_{ni} - \text{MRT}_{IV}$$

MAT is the difference in mean residence time (MRT) following IV injection ($\text{MRT}_{IV}$) and the MRT by any other route ($\text{MRT}_{ni}$).

**Bioavailability**

The extent and rate of absorption after oral, topical, or inhalational drug administration is referred to as the bioavailability, $F$. To estimate the amount of drug that would normally be found if the entire dose were absorbed, an intravenous dose would be required, as this is the only route of administration that guarantees 100% of the dose is systemically available ($F = 1.0$) and the pattern of disposition and metabolism can be quantitated. For most drug studies, absorption is measured using blood concentrations. When drug concentrations in plasma are assessed, the measure of the area under the concentration-time curve (AUC) is used to estimate total absorption. Absolute systemic availability is calculated as

$$F(\%) = \frac{\text{AUC}_{\text{route}} \times \text{Dose}_{\text{iv}}}{\text{AUC}_{\text{iv}} \times \text{Dose}_{\text{route}}}$$

Calculation of $F$ only gives an estimate of the extent, not the rate, of absorption.

**Accumulation ratio**

When multiple doses are given, the drug accumulates until the plasma concentration is large enough for elimination to balance absorption. Accumulation from one dose to the next is significant when the next dose is given before most of the previous dose has been eliminated.
The accumulation ratio, AR, is stated as the ratio of the average concentration at steady state to the average concentration during the first dose interval and is calculated by the equations,

\[
AR = \frac{C_{av,ss}}{C_{av,1}}
\]  

(12)

\[
AR = \frac{\text{AUC}_{ss} \times CL}{\text{AUC}_{1} \times CL}
\]  

(13)

**Non-steroidal anti-inflammatory drugs**

Non-steroidal anti-inflammatory drugs exert their activity by blocking the cyclooxygenase-driven conversion of arachidonic acid to the prostanoids such as thromboxane and the prostaglandins (Figure 1). There are two commonly recognized isoforms of cyclooxygenase (formerly known as prostaglandin endoperoxide synthase (Vane 1998): **isoform 1 (COX-1)** and **isoform 2 (COX-2)**. COX-3 is a recently described isoform that is encoded by the same gene as COX-1; however, COX-3 retains an intron that is not retained in COX-1. It is not functional in humans and has not yet been studied in the equine species and therefore will not be discussed further.

The COX-1 isoform, discovered in 1976 (Hemler), is responsible for the production of **prostaglandin E\textsubscript{2} (PGE\textsubscript{2})**, **thromboxane A\textsubscript{2} (TxA\textsubscript{2})**, and **prostaglandin I\textsubscript{2} (PGI\textsubscript{2})**. It is constitutively produced in most tissues and produces prostanoids that are essential for normal physiologic processes, such as platelet aggregation, gastrointestinal protection, and perfusion and electrolyte balance in the kidney.

The COX-2 isoform was discovered in 1991 and it converts arachidonic acid into the prostanoids PGE\textsubscript{2} and PGI\textsubscript{2}. It is not expressed in most cells under normal conditions, but is rapidly inducible as a result of damaging stimuli from cytokines, growth factors, and bacterial toxins present at inflammatory sites, in infection, and in neoplasia. This isoform is responsible for producing prostaglandins that amplify shock, inflammation, and pain (Ferraz 1997).

In the first step of prostanoid synthesis, the cyclooxygenase activity of either COX-1 or COX-2 oxygenates and isomerizes arachidonic acid, creating the intermediate product **prostaglandin G\textsubscript{2} (PGG\textsubscript{2})** (Figure 2.4). In the second step, which occurs as a different
enzymatic site, peroxidase reduces PGG$_2$ to its hydroxyl analog, PGH$_2$. Distinct synthases or reductases then convert PGH$_2$ to one of several stable prostanoids: PGD$_2$, PGE$_2$, PGF$_{2\alpha}$, PGI$_2$, or TxA$_2$ (Smith 1996).

The molecular structures of COX-1 and COX-2 show that both isoforms are dimers, with the COX activation site located in a long, hydrophobic channel between the two subunits (Figure 2.5). A substitution of one amino acid in the activation site, from isoleucine in COX-1 to valine in COX-2, creates a second, larger NSAID-binding pocket; it is this additional binding site that confers selectivity of NSAIDs for one isoform or the other (Kurumbail 1996). Smaller NSAIDs can bind to the pocket in the activation sites of either COX-1 or COX-2; the larger COX-2-specific inhibitors only fit into the larger binding pocket found on the COX-2 isoform.

Selective inhibition of COX-1 leads to the gastrointestinal toxicity associated with the use of NSAIDs. Prostaglandins function to regulate motility, secretion, blood flow, and mucosal cytoprotection and inhibition of these functions leads to gastrointestinal ulceration. In small animal medicine, the use of the COX-2 selective inhibitors (carprofen, etodolac) has reduced the occurrence of GI side effects; however, there is a paucity of published data on these substances in horses. Drugs such as phenylbutazone and flunixin meglumine continue to be the mainstay of NSAID therapy. In one report, phenylbutazone was associated with greater toxicity than flunixin meglumine (MacAllister 1993). In a study combining flunixin meglumine and phenylbutazone at regular doses (1.1 mg/kg IV q 12 h for 5 days and 2.2 mg/kg PO q 12 h for 5 days, respectively), one horse died acutely due to acute necrotizing colitis, with lesions most severe in the right dorsal colon (Keegan 2008). The horse had recently completed the combination therapy portion of the study. The same group performed the study two years prior and, in that study, one horse was euthanized with severe hypoproteinemia, hypoalbuminemia, and colitis after only 5 days of combination therapy. The horse was euthanized on day 7 of the study for severe colic, unresponsive to analgesic medications (Reed 2006).

Renal complications of NSAIDs are also related to the inhibition of basal prostaglandin production. Hypovolemia, congestive heart failure, chronic renal insufficiency, sepsis, and anesthesia are common conditions that lead to increased vasoconstrictor activity in the kidney. Angiotensin, norepinephrine, and vasopressin lead to reduced renal blood flow and result in a feedback loop causing a release of prostaglandins. This causes vasodilation and a return to normal renal blood flow and glomerular filtration. Prostaglandins have regulatory functions at
the tubular level. NSAIDs lead to reduced blood flow, hyperkalemia secondary to suppression of renin and aldosterone secretion, increased sodium, chloride, and water reabsorption, and causes renal papillary necrosis secondary to medullary ischemia. Used at clinically relevant doses in healthy patients, the incidence of adverse events is low; however, even mild dehydration can result in dramatic detrimental effects.

Most NSAIDs block both COX-1 and COX-2 to some degree, but some of the newer drugs, such as meloxicam, are reported to be more selective against the COX-2 enzyme. As the use of non-selective NSAIDs (those that block both COX-1 and COX-2) has been associated with gastrointestinal ulceration (including right dorsal ulcerative colitis) (Karcher 1990, Galvin 2004, McConnico 2008, Marshall 2011) and renal papillary necrosis in equines (MacKay 1983, Gunson 1983a/b, Read 1983, Collins 1984, Faulkner 1984, Black 1986), use of selective inhibitors may be associated with a decreased incidence of these adverse effects. However, it is important to note that compounds that are highly selective COX-2 inhibitors in one species may not necessarily exert the same selectivity in other species. Highly selective COX-2 inhibitors in humans have been associated with severe liver and cardiovascular side effects, probably in relation to highly reduced anti-COX-1 activity (Ferreiro 2000; Schattner 2000; Topol 2004).

In 1998, a classification system was developed (Lipsky 1998). According to this system, COX inhibitors can be considered to belong to one of four groups: (1) COX-1-specific inhibitor: an agent with no measurable inhibition of COX-2. Low-dose aspirin is the only drug that fits into this category; (2) COX-nonspecific inhibitor: inhibits both COX-1 and COX-2, perhaps with small pharmacologic differences in activity between the two isoenzymes, but with no clinically relevant differences in specificity; (3) COX-2-preferential inhibitor: inhibits COX-2 with little COX-1 inhibition at therapeutic dosages; (4) COX-2-specific inhibitor: agents that produce no clinically significant inhibition of COX-1, even at the highest therapeutic dosages.

**Meloxicam**

Meloxicam, an enolic acid in the oxicam class, is similar in structure to sudoxicam and piroxicam. It is intended to be administered intravenously or orally to horses for the treatment of inflammatory conditions, particularly orthopedic disorders. It is approved for use in humans, dogs, and cats in the United States and is approved for use in the horse in Europe and other countries. There has been a recent boom in investigations of meloxicam as an analgesic and
anti-inflammatory in rats, goats, mice, dogs, and cats undergoing orthopedic procedures or injury (Bourque 2010; Goodman 2010; Ingvast-Larsson 2010; Keita 2010; Murison 2010; Gruet 2011; Hakan 2011; Hicks 2011; Morton 2011; Tubbs 2011). Currently, there is no approved formulation of meloxicam for use in the horse in the United States.

The pharmacokinetics of meloxicam were first investigated in ponies in 1991 (Lees 1991). In this study, ponies were injected with 0.6 mg/kg meloxicam intravenously (then named “miloxicam”) and pharmacokinetic variables were measured over 24 hours. In the same study, the actions of meloxicam were compared to placebo in a carrageenan-sponge model of inflammation. In five of the ponies, the plasma-time concentration curve was defined as a two-compartment model. In one pony, a three-compartment model was defined. The results of the pharmacokinetic investigation are listed in Table 1.1. In unpublished investigations by the manufacturer, the half-lives of meloxicam were evaluated in multiple species (Table 1.2). Another investigation evaluated the pharmacokinetics of the oral suspension of meloxicam (Metacam® oral suspension, Boehringer Ingelheim, Vetmedica) and compared the bioavailability between fed and non-fed horses (Toutain 2004a) and found that the bioavailability was not significantly different, however the mean absorption time was longer in fed horses. The pharmacokinetic-pharmacodynamic relationships and dose response was evaluated in horses using an induced arthritis model using Freund’s adjuvant (Toutain 2004b). In this study, increasing doses of meloxicam (from 0 to 2.0 mg/kg) were administered intravenously and stride lengths and clinical lameness scores were used to assess the effect of the drug. This study suggested that a dose of 0.6 mg/kg would be an effective dose for clinical use in horses. The pharmacokinetics of meloxicam was determined again in adult horses and donkeys using the intravenous formulation (Sinclair 2006). The investigators found an extremely short mean residence time (MRT) and total body clearance (CLT) in donkeys compared to horses, indicating that its use may be impractical in this species. The pharmacokinetic variables of the oral suspension of meloxicam have also been determined in foals less than 6 weeks of age and no adverse effects were noted (Raidal 2009).

Plasma pharmacokinetic data can be problematic when determining dosing intervals with non-steroidal anti-inflammatory drugs. For instance, phenylbutazone and flunixin meglumine block the synthesis of eicosanoids such as PGE₂ for 12-24 hours when administered intravenously, but their elimination half-lives are short (4.7-6.1 h and 1.6-1.9 h, respectively)
Studies that have examined pharmacodynamic features of meloxicam have indicated that, although the drug has a relatively short half-life and mean residence time, once daily dosing is feasible because it reversibly inhibits eicosanoids in inflammatory exudates between 4 and 24 hours after dose administration (Lees 1991). A short MRT and long clearance from inflammatory exudates is similar in the other acidic NSAIDs such as phenylbutazone and flunixin meglumine. Mean residence time is a non-compartmental variable that is based on statistical moment theory and is analogous to half-life calculations in compartmental analyses. However, pharmacokinetic and statistical studies have found that MRT is often greater than half-life, especially if the drug is defined by a two compartment model. Regardless, the clinical usefulness of the drug is often determined by its median effective dose (ED$_{50}$). The ED$_{50}$ is a variable related to formulation or route of administration. It is the dose of the drug at which 50% of E$_{max}$ is achieved and is a measure of drug potency.

The median effective dose of the drug was calculated in one study using a Freund’s adjuvant-induced synovitis (Toutain 2004b). The overall ED$_{50}$ for lameness score at three selected time points (4, 6, and 8 hours) was 0.265 mg/kg. However, 0.617 mg/kg was calculated as the overall dose at 70% of the maximum possible effect for clinical lameness scores. This coincides with other studies that have determined that the appropriate dose is 0.6 mg/kg. Because the study concluded that intravenous meloxicam was an efficacious anti-inflammatory and analgesic drug in horses with experimentally induced arthritis, a similar model was chosen in this study to evaluate the effect of the oral formulation.

The EC$_{50}$, or median effective concentration, was also calculated by Toutain, et al (2004b). In that study, the overall EC$_{50}$ for lameness score was 195 ng/mL and was calculated at the point in which 70% of the lameness was suppressed. This EC$_{50}$ can be used for any meloxicam formulation and other routes of administration by transforming into the corresponding ED$_{50}$ by multiplying the EC$_{50}$ by the plasma clearance for the IV route or an apparent plasma clearance (i.e., clearance divided by bioavailability) for an extravascular route of administration.

Mean absorption time (MAT) was calculated in one study (Toutain 2004a) and found to vary between 2.07 to 6.32 hours (mean 3.62 ± 1.39 hours). The MAT after the first dose for 6 of 8 fed horses was significantly higher than the MAT obtained in non-fed horses. This would
suggest that the physiologic status of the gastrointestinal tract may slow the rate of absorption after oral administration but, in this study, did not affect the bioavailability.

Meloxicam is intended for multiple administrations. With respect to bioavailability, Toutain, et al (2004b), found that there was no difference between the first and last dose in a 14 day study. The accumulation ratio was low in that study (1.08 ± 0.111), which suggests that steady-state concentration was reached immediately.

Meloxicam has been evaluated for its effectiveness in lameness models, both in vitro and in vivo. The efficacy of NSAIDs in inflammatory joint disease is largely attributed to their inhibition of COX-mediated production of PGE₂ within the joint space. Additionally, NSAIDs have demonstrated effects on cytokine release (Rainsford 1997; Bianchi 2007), proteoglycan turnover (Rainsford 1997; Blot 2000), as well as on matrix metalloproteinase (MMP)-activity (Chu 2008). In vitro suppression of MMP activity has also been reported for meloxicam (Barracchini 1998, 1999; Sadowski 2001). In vitro, synovial explants have been used to evaluate the anti-inflammatory effects of meloxicam in an LPS challenge model (Moses 2001). Synovial explants were prepared and treated with several NSAIDs including phenylbutazone, flunixin meglumine, meloxicam, etc. In this study, meloxicam was shown to significantly decrease PGE₂ concentrations when compared to the other NSAIDs in LPS-challenged explants. This was confirmed in vivo in 6 horses with LPS-induced synovitis (deGrauw 2009a). Prostaglandins, especially PGE₂, are potent mediators of joint inflammation and occur naturally in osteoarthritic joints and are responsible for the clinical signs associated with osteoarthritis (Dayer 1986; Tawara 1991; May 1992; Knott 1993; Knott 1994). Early use of meloxicam has been shown to ameliorate clinical signs and inflammation in acute synovitis and may also limit inflammation-induced cartilage catabolism (deGrauw 2009a).

There has been much discussion about whether meloxicam is a selective inhibitor of COX-2. In the rat, using pleurisy and peritonitis models, meloxicam significantly reduced PGE₂ concentrations over piroxicam, diclofenac, and tenidap (Engelhardt 1996). The concentration required to inhibit 50% of COX activity (IC₅₀) has been determined in dogs by the use of a whole blood assay. The ratio of IC₅₀ for COX-1 to the IC₅₀ of COX-2 for meloxicam was 10 (Brideau 2001). Another study was performed to compare the effects of phenylbutazone, flunixin meglumine, meloxicam, and carprofen on thromboxane B₂ (TxB₂) and PGE₂ concentrations in blood incubated with LPS (Beretta 2005). The values of TxB₂ were used as a
measure of COX-1 activity and the values of PGE₂ were used as a measure of COX-2 activity. Using concentration response curves, concentrations producing 50% (IC₅₀) and 80% (IC₈₀) inhibition for each NSAID were evaluated. Selectivity at 50% and 80% inhibition was calculated as IC COX-1/IC COX-2. At 50% inhibition, flunixin had the strongest anti-COX-1 activity while meloxicam had the strongest anti-COX-2 activity. When the four NSAIDs were examined for COX-1 inhibition at concentrations producing 80% inhibition of COX-2, COX-1 inhibition was 70.97% for meloxicam, 72.58% for carprofen, 79.59% for phenylbutazone, and 83.81% for flunixin meglumine. Unfortunately, the debate cannot be settled using *in vitro* inhibition assays as, even in human whole blood assays, minor variations in conditions leads to different IC₅₀ ratios of 2.71 and 24.78 in a single laboratory (Warner 1999).

Evidence for the COX-2 selectiveness of meloxicam was further suggested in a study evaluating the effect of meloxicam on ischemic jejunum as compared to the effect of flunixin meglumine (Little 2007). In this study, 18 horses were subjected to general anesthesia and the jejunum placed under ischemic conditions for 2 hours. The horses were administered saline solution (control), flunixin meglumine, or meloxicam (0.6 mg/kg IV) one hour prior to the ischemic event. After recovery from anesthesia, the horses were monitored for 18 hours then euthanized and samples of the jejunum were submitted for histopathology and Ussing chamber studies. COX-1-elaborated prostaglandins are necessary for proper recovery of intestinal barrier function and meloxicam appears to mainly inhibit the detrimental effects of COX-2-elaborated prostaglandins. The results of the study indicate that meloxicam did not impede recovery of ischemia-injured jejunum and may be considered for analgesia and endotoxemia associated with colic.

**Synovitis models**

In horses, intra-articular lipopolysaccharide (LPS) injection is an established model for induction of sterile, transient synovitis and allows the study of clinical symptoms and therapeutic efficacy (Firth 1987; Palmer 1994; Morton 2005; Meulyzer 2008; deGrauw 2009a). LPS allows for a reversible, non-septic inflammation of a joint sufficient to cause temporary lameness without severe systemic or long-term side effects. The inflammatory markers and cartilage biomarkers in synovial fluid were evaluated after a single dose of intra-articular LPS (deGrauw 2009b). The LPS injection led to a sharp rise in PGE₂ at 8 hours post-injection, and substance P,
bradykinin, and MMP activity were increased at 8 and 24 hours after injection. Equine articular chondrocytes, when exposed to inflammatory stimuli, increase PGE$_2$ concentrations and upregulate COX-2 expression (Farley 2005). Meloxicam has been shown to influence synovial fluid prostaglandin E$_2$, substance P, bradykinin, and MMP activity in the first 24 hours after injection of LPS (deGrauw 2009a). In addition to attenuation of synovial fluid PGE$_2$, substance P, and bradykinin concentrations, meloxicam significantly improved lameness scores compared with those of placebo.

The penetration of NSAIDs into synovial compartments depends on the chemical characteristics of the compound but usually reaches approximately 60% of the mean plasma concentration because of low protein concentrations within synovial fluid. Penetration may increase in the presence of synovitis and synovial fluid concentration in inflamed joints often exceeds plasma concentrations because of accumulation and binding of proteins. In one study, intra-articular ketoprofen concentrations in horses with acute synovitis were 6.5 times higher than those in healthy horses (Owens 1995).

**Lameness Grades**

The American Association of Equine Practitioners (AAEP) has developed a grading system to standardize the evaluation of lame horses. The grading scheme is as follows:

- **0:** Lameness not perceptible under any circumstances.
- **1:** Lameness is difficult to observe and is not consistently apparent, regardless of circumstances (e.g. under saddle, circling, inclines, hard surface, etc.).
- **2:** Lameness is difficult to observe at a walk or when trotting in a straight line but consistently apparent under certain circumstances (e.g. weight-carrying, circling, inclines, hard surface, etc.).
- **3:** Lameness is consistently observable at a trot under all circumstances.
- **4:** Lameness is obvious at a walk.
- **5:** Lameness produces minimal weight bearing in motion and/or at rest or a complete inability to move.
Figures and Tables

Table 2.1 Comparison of pharmacokinetic studies of intravenous and oral suspension formulations of meloxicam.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation</td>
<td>IV</td>
<td>Oral suspension</td>
<td>IV</td>
<td>IV</td>
</tr>
<tr>
<td>V_d</td>
<td>0.158 L/kg</td>
<td>0.12 ± 0.018</td>
<td>0.23</td>
<td>0.24 ± 0.16</td>
</tr>
<tr>
<td>Clβ</td>
<td>41.87 mL/kg/h</td>
<td>34 ± 5.7</td>
<td>81.0</td>
<td>34.7 ± 9.21</td>
</tr>
<tr>
<td>AUC</td>
<td>14.53 ± 0.8 μg/mL/h</td>
<td></td>
<td></td>
<td>18.8 ± 7.31</td>
</tr>
<tr>
<td>Bioavailability</td>
<td></td>
<td>98 ± 12% fed</td>
<td>85 ± 19% non-fed</td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2 Comparison of half-lives of oral and intravenous meloxicam formulations in different studies.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose</th>
<th>Half-life</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cow</td>
<td>0.2, 0.5 mg/kg IM</td>
<td>13 h</td>
<td>Boehringer-Ingelheim, unpublished</td>
</tr>
<tr>
<td>Rat</td>
<td>0.3 mg/kg IV</td>
<td>11.3 h</td>
<td>Boehringer-Ingelheim, unpublished</td>
</tr>
<tr>
<td></td>
<td>30.0 mg/kg IV CRI</td>
<td>9.4 h</td>
<td>Boehringer-Ingelheim, unpublished</td>
</tr>
<tr>
<td>Miniature pig</td>
<td>Unknown</td>
<td>4 h</td>
<td>Boehringer-Ingelheim, unpublished</td>
</tr>
<tr>
<td>Man</td>
<td>Variable</td>
<td>20-50 h</td>
<td>Boehringer-Ingelheim, unpublished</td>
</tr>
<tr>
<td>Dog</td>
<td>Variable</td>
<td>12-36 h</td>
<td>Boehringer-Ingelheim, unpublished</td>
</tr>
<tr>
<td>Horse</td>
<td>0.2 mg/kg IV</td>
<td>1.9 h</td>
<td>Boehringer-Ingelheim, unpublished</td>
</tr>
<tr>
<td></td>
<td>0.5 mg/kg IV</td>
<td>2.1 h</td>
<td>Boehringer-Ingelheim, unpublished</td>
</tr>
<tr>
<td></td>
<td>0.6 mg/kg IV</td>
<td>2.7 h</td>
<td>Lees 1991</td>
</tr>
<tr>
<td></td>
<td>0.6 mg/kg oral susp</td>
<td>8.54 ± 3.02 h</td>
<td>Toutain 2004a</td>
</tr>
<tr>
<td></td>
<td>0.6 mg/kg IV</td>
<td>5.15 h</td>
<td>Toutain 2004b</td>
</tr>
<tr>
<td></td>
<td>0.6 mg/kg IV</td>
<td>9.6 ± 9.24 h*</td>
<td>Sinclair 2006</td>
</tr>
</tbody>
</table>

*Mean Residence Time
Figure 2.1 One compartment open pharmacokinetic model with first-order absorption.

Figure 2.2 Generalized open two-compartment pharmacokinetic model with first-order absorption ($K_{01}$) into and elimination ($K_{el}$) from the central compartment. $K_{12}$ and $K_{21}$ represent intercompartmental micro-rate constants.

Figure 2.3 Three-compartment pharmacokinetic model after intravenous administration.
Figure 2.4 The arachidonic acid cascade. HPETE = hydroperoxyeicosatetraenoic acid; HETE = hydroxyeicosatetraenoic acid; LT = leukotriene.
Figure 2.5 COX-1 and COX-2 substrate-binding channels. Schematic depiction of the structural differences between the substrate-binding channels of COX-1 and COX-2 that allowed the design of selective inhibitors. The amino acid residues Val434, Arg513, and Val523, form a side pocket in COX-2 that is absent in COX-1. A, Nonselective inhibitors have access to the binding channels of both isoforms. B, The more voluminous residues in COX-1, Ile434, His513, and Ile532, obstruct access of the bulky side chains of the coxibs. (From Grosser T, Fries S, FitzGerald GA: Biological basis for the cardiovascular consequences of COX-2 inhibition: Therapeutic challenges and opportunities. J Clin Invest 116:4-15, 2006.)
Chapter 3 - Materials and Methods

All procedures were approved by Kansas State University Institutional Animal Care and Use Committee.

Aim 1

Experimental design and sample collection

Sixteen horses of mixed breeds, 14 owned by the University and 2 client-owned animals, were evaluated via gastroscopy for the presence of gastric ulcers prior to entrance to the study. The horses were fasted for 12 hours prior to the examination and water was withheld for 3 hours. The horses were sedated with xylazine hydrochloride (0.5 mg/kg IV) and butorphanol (0.01 mg/kg IV) or with detomidine (0.01 mg/kg IV) and butorphanol (0.01 mg/kg IV), depending on the nature and temperament of the horse. Each was placed in stocks and restrained normally. Gastric ulcer scoring was performed by two investigators. If the horses had grade 2 ulcers or below, the horse was included in the study and complete blood count, serum biochemistry, and urinalysis was performed the following day.

The animals that participated in the study were housed in 12 x 12 stalls in a climate controlled environment. They were fed free choice brome and alfalfa hay and 3 pounds of equine Strategy or equine Senior (Purina) twice daily, depending on age. Free choice fresh water was available at all times. Each horse was weighed with a commercial scale prior to the beginning of the study and an intravenous catheter (14 g, 5.25 inch Abbocath™, Abbott Laboratories, Abbott Park, IL) was placed in the left jugular vein using aseptic technique. A baseline blood sample was taken via the jugular catheter and placed into a heparinized tube (green top). Meloxicam (15 mg tablets, Caraco Pharmaceutical Labs, Detroit, MI) was administered to each horse at 0.6 mg/kg orally once daily for fourteen days. The tablets were crushed and mixed with 15 mL of molasses and given via dosing syringe in the morning. Blood sampling for pharmacokinetic analysis was performed at 20 and 40 minutes, and at 1, 2, 4, 8, 12, and 24 hours after administration of the tablets. The intravenous catheter was removed after 24 hours and the horses were returned to their normal environment. This was repeated and blood samples were taken on days 7 and 14. Trough samples were taken via direct venipuncture on days 4 and 10 while in their natural environment. All blood samples were centrifuged for 5
minutes at 3700 g and the plasma was removed and frozen at -80°C until analysis could be performed.

At the conclusion of the study, repeat complete blood count, serum biochemistry, urinalysis, and body weight was performed. Additionally, the horses were fasted and a gastroscopy under IV sedation as noted above was performed to evaluate gastric ulcer scores.

**Sample analysis**

Plasma concentrations of meloxicam (m/z 352.09→114.90) were determined with high-pressure liquid chromatography (Shimadzu Prominence, Shimadzu Scientific Instruments, Columbia, MD, USA) and mass spectrometry (API 2000, Applied Biosystems, Foster City, CA, USA). Plasma samples or standards (100 µL) were added to 100 µL of internal standard (piroxicam 0.5 µg/mL in methanol, m/z 332.12→95.10) and 300 µL of methanol with 0.1% formic acid to precipitate the proteins. The samples were vortexed for 5 seconds and centrifuged for 10 minutes at 10,000 x g. The supernatant, 200 µL, was transferred to an injection vial with the injection volume set to 10 µL. The mobile phase consisted of A: acetonitrile and B: 0.1% formic acid at a flow rate of 0.4 mL/min. The mobile phase consisted of 85% B from 0 – 0.5 minutes with a linear gradient to 50% B at 2.5 minutes which was maintained until 3 minutes, followed by a linear gradient to 85% B at 4 minutes with a total run time of 5 minutes. Separation was achieved with a C8 column (Supelco Discovery C8, 50 mm x 2.1 mm x 5 µm, Sigma-Aldrich, St. Louis, MO, USA) maintained at 40° C. The standard curve was linear from 0.01 µg/mL to 10 µg/mL and was accepted if the correlation coefficient exceeded 0.99 and predicted values were within 15% of the actual values.

**Statistical analysis**

Pharmacokinetic analyses were performed with computer software (WinNonlin 5.2, Pharsight Corporation, Mountain View, CA, USA). The variables calculated included the area under the curve (AUC) using the linear trapezoidal rule, maximum serum concentration (C\text{MAX}) and time to maximum serum concentration (T\text{MAX}) obtained directly from the plasma concentration data. Pharmacokinetic parameters on days 1, 7, 14 were compared for difference using a repeated measures analysis of variance. Paired t tests were used to compare pre- and post-administration white blood cell counts, total serum protein measurements, creatinine levels, and gastric ulcer scoring. Repeated measures ANOVA was used to evaluate differences between
days 0, 7, and 14 for volume of distribution, clearance, area under the curve, half-life, and mean residence time. All tests were considered statistically significant with a P value <0.05.

**Aim 2**

For the second aim of the study, four horses were evaluated via physical examination, body weight, complete blood count, serum biochemistry, and urinalysis. Gastroscopy was performed as described above but there were no restrictions on level of gastric ulceration for inclusion in the study. The only restriction placed on the acquisition of the horses was that they had not received NSAID medications within the last 6 months. The horses were donated to the University because of their lameness, attributed to chronic laminitis as evidenced by characteristic abnormalities of their hoof walls (dished dorsal hoof wall, concentric rings). For cost purposes, the feet were not radiographed and, due to the temperament of the horses and level of lameness, the exact cause of the lameness was not investigated. Each was evaluated by an equine surgeon and lameness was graded on a 0-5 scale (AAEP). For the horses graded 2 or less, to ensure statistically significant differences in pressure mat data, lameness was further exacerbated by injection of 3 μg lipopolysaccharide (LPS) from E. coli O55:B5 (Sigma-Aldrich, St Louis MO) into the right metacarpophalangeal joint after aseptic preparation. The horses were then evaluated via pressure mat (MatScan®, TekScan, Inc. Boston, MA). The horses were then randomly assigned to either receive phenylbutazone (4.4 mg/kg orally once daily) or meloxicam (0.6 mg/kg orally once daily) for four days. The investigators were blinded to the treatment drug. Pressure mat data was obtained at baseline and at 12 hour intervals for four days. At the conclusion of the study, the horses were humanely euthanized with sodium pentobarbital after sedation with 1 mg/kg xylazine IV. The horses were sent to a board certified pathologist for histopathologic analysis of the kidneys and gastric mucosa and to evaluate for any other adverse effects of the drugs. The pathologists were also blinded to the treatment group.

**Statistical analysis**

Pressure mat data was evaluated with computer software (MANUFACTURER). The variables calculated included force, contact area, and stride length.
**Aim 3**

Equine PBMCs were harvested from 3 normal horses and 3 of the horses used in Aim 2 of the study. Sixty mL of blood was collected via jugular venipuncture into a syringe containing 3000 u sodium heparin (50 u heparin/mL of blood) once on normal horses and at 0 (baseline prior to treatment with NSAID) and 24 hours on Horse 4, and at 0, 24, and 96 hours on Horse 1 and 2. To each 50 mL conical tube, 15 mL of complete medium (RPMI 1640 with 5% FBS and 2% Pen/Strep) was added to 15 mL of whole blood. The media-diluted blood (30 mL) was layered over 10 mL of Ficoll 1.083. The samples were then centrifuged at 1800-2200 rpm for 30 minutes at room temperature. The PBMCs were then collected and placed into a 15 mL conical tube and washed twice with 10 mL sterile PBS and centrifuged at 2800-3300 rpm for 10 minutes. The cells were then resuspended in 5 mL of complete medium. The PBMCs obtained from the normal horses were treated with 100 ng/mL of LPS for 6 hours. Messenger RNA was isolated from these cells using a commercial kit (Qiagen, Valencia, CA) and analyzed for relative concentrations of COX-2 mRNA in the presence or absence of LPS using qRT-PCR (18S expression was used as the loading control). The primers used for PCR are listed in Table 3.1.

Both PBMCs and protein (whole cell lysates using a commercial lysis buffer, Santa Cruz Biotechnology, Santa Cruz, CA) were isolated from treated horses. The RNA was analyzed using qRT-PCR for COX-2 expression while the whole cell lysates were assayed for relative protein expression of COX-2 using standard protein electrophoresis and western blotting procedures. Actin served as the loading control. Protein expression was visualized using chemiluminescence (Pierce, Rockford, IL). Densitometry was used to determine relative protein expression (Cell Biosciences, Santa Clara, CA).

**Table 3.1 Primers used for qRT-PCR for COX-2 and 18S gene expression from peripheral blood mononuclear cells in horses.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward Sequence</th>
<th>Reverse Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>COX-2</td>
<td>5'-TGGCGAGGTGTATCCGCCCA-3’</td>
<td>5’-GGTGTCAAGGCACAAGGGGATGC-3’</td>
</tr>
<tr>
<td>18S</td>
<td>5'-TCGCTCCACCAACTAAGAAC-3’</td>
<td>5’-GAGGTTCGAAGACGATCAGA-3’</td>
</tr>
</tbody>
</table>
Chapter 4 - Results

Aim 1

Seven of the 16 horses initially evaluated had gastric ulcer scores of Grade 2 or less and were entered into the study. Of the seven horses included in the study, there were 3 mares, 3 geldings, and 1 stallion. The mean age was mean 20.3 ± 7.95 y (10 to 31 y, median: 21 y) and mean weight was 508.4 ± 62.8 kg (median: 483 kg). The weights of the horses did not significantly change during the study period (p = 0.95). Breeds represented included Quarter Horse (n = 3), Thoroughbred (n = 2), Arabian (n = 1), and Hanoverian (n = 1), and were representative of the regional population.

Initial and final complete blood count, serum chemistry, and urinalysis were within normal limits on all horses. The changes in white blood cell count, total protein, and creatinine concentration were not significantly different over the 14 day period (p = 0.70, p = 0.58, p = 0.56, respectively).

The results of the gastric ulcer scores are noted below (Table 4.1). There was no significant difference between pre-treatment gastric ulcer scores and post-drug administration ulcer scores (p = 0.1465, 95% CI = -2.40 – 0.40), however two horses had an increase from Grade 0 to Grade 3 during the experimental period.

One horse experienced severe insect hypersensitivity beginning on day 8 of meloxicam administration. Urticaria with ulceration and necrosis of the skin was noted. Meloxicam administration was discontinued and punch biopsies were taken of the affected areas. Histopathology revealed severe dermatitis with dermoepidermal clefting, early epidermal necrosis, and mild lymphocytic, neutrophilic, and eosinophilic dermal inflammation. Dexamethasone was administered at 0.05 mg/kg IV once daily for 3 days and then as a tapering dose over 2 weeks. The lesions healed without incident. The horse was not re-entered into the study and pharmacokinetic data for day 14 was not included in the final analysis. The CBC, serum chemistry, urinalysis, and gastroscopy for this horse were performed on day 12.

A second horse did not experience any adverse events during meloxicam administration but the trough concentration on day 15 of the study was double the amount of the previous trough concentrations on days 4 and 10 and those of cohort animals on day 15. The sample was
re-evaluated but the same results were obtained. The data for this horse was removed from the final outcome.

The calculated pharmacokinetic parameters for meloxicam are presented in Table 4.3 and the plasma concentrations are presented in Table 4.4. The model describes a one-compartment model. Dosage for oral administration ranged from 0.60 to 0.61 mg/kg (mean, 0.61 ± 0.008 mg/kg). Plasma clearance was 51.2 ± 11.01 mL/kg/h (range: 44.7 to 55.3 mL/kg/h). Mean steady state volume of distribution over 14 days was 0.37 ± 0.11 L/kg (range: 0.32 to 0.41 L/kg) and there was no difference from day 0 to day 14 (p = 0.28). The MRT ranged from 6.66 to 7.51 h (mean, 7.18 ± 1.41 h) and was not statistically different from day 0 to day 14 (p = 0.57). Terminal half-life was 4.99 ± 1.11 h (range: 4.72 to 5.25 h). The C_max (range: 0.91 to 2.78 µg/mL; mean, 1.82 ± 0.80 µg/mL) was detected 3.48 ± 3.3 h, 1.24 ± 1.24 h, and 1.93 ± 1.30 h after meloxicam administration on days 0, 7, and 14, respectively. There was a statistically significant difference between the T_max on the sample days (p = 0.04).

Trough concentrations on days 0, 4, 7, 10, and 14 were similar (p = 0.45), which indicate steady-state conditions. This was confirmed with the accumulation ratio of meloxicam during the 14 days of administration (range, 0.64 to 1.78; mean, 1.17 ± 0.39).

**Aim 2**

Mean age of the horses included in this portion of the study was 11 ± 3.16 y (9 to 15 y, median: 10.5 y). All four horses were Quarter Horses and their mean weight was 539.4 ± 40.8 kg (median: 538.3 kg). The weights did not significantly change over the experimental period (p = 0.95). There were two geldings and two mares.

Gastric ulcer scores for all of the horses are shown in Table 4.2. There was no significant difference between the gastric ulcer scoring prior to the start of the experiment and the ulcer scores at post-mortem (p = 0.17), however the methods used to evaluate gastric ulcer number and severity was different.

Initial CBC for **Horse 1** (meloxicam, LPS) revealed a mild stress leukogram. This was confirmed by the presence of mild hyperglycemia (blood glucose: 145 mg/dL; ref. range: 70-116 mg/dL). The urinalysis was within normal limits except for a mild increase in protein (3+ on strip, approximately 5 mg/dL on sulfasalicylic acid). The horse was determined to be Grade II lame in the right front limb prior to LPS injection. After LPS injection, Grade IV lameness was
noted. The horse tolerated the administration of meloxicam with no adverse events noted. The laboratory analysis on day 4 of treatment showed resolution of the stress leukogram and hyperglycemia. The urinalysis revealed a 3+ protein on the strip but negative on the sulfasalicylic acid test. At post-mortem, rotation of the distal phalanx was noted in both front feet. Histopathologic evaluation of the stomach revealed mild degenerative changes in the epithelium of the stomach but this was suspected to be due to post-mortem changes as no inflammatory cells were noted. In the right ventral colon, crypt epithelial loss with infiltration of *Balantidium coli* was shown in the lamina propria. There were no gross or histopathologic changes in the right dorsal colon, the most common location for NSAID-induced changes in the large intestine. In the kidneys, mild multifocal tubular mineralization was found. This appeared to be a chronic change and not associated with NSAID administration.

A stress leukogram was noted in **Horse 2** (phenylbutazone, LPS) and this was also accompanied by mild hyperglycemia (blood glucose: 159 mg/dL; ref. range: 70-116 mg/dL). Increased protein (3+ on strip, negative on SSA) was noted on the urinalysis. Baseline lameness was Grade II in both front limbs. After LPS was injected into the right carpus, lameness increased to Grade IV. At the conclusion of the study, leukopenia (white blood cell count: 4.8 K/µL; ref. range: 6-14 K/µL) due to lymphopenia (0.9 K/µL; ref. range: 1.5-7.7 K/µL) was found. Mild anemia (PCV: 30%; decreased from the initial 40%) was also noted. The increased protein in the urine was resolved. At post-mortem examination, both forefeet had evidence of laminitis with rotation of the third phalanx. In the cecum, there were large numbers of lymphocytes, plasma cells, and eosinophils in the lamina propria. The enterocytes that line the crypts stained more basophilic than normal and goblet cells appeared to be decreased. There were no histopathologic changes in the stomach, right dorsal colon, or kidneys.

**Horse 3** (meloxicam, no LPS) had a stress leukogram and significant hyperglycemia (blood glucose: 246 mg/dL). This horse was more feral than the other 3 horses and handling likely induced this response. The behavior of the horse precluded gathering of a urine sample at the beginning and end of the study. Baseline lameness in this horse was Grade IV in both forelimbs. The laboratory values were within normal limits at the conclusion of the study. At post-mortem examination, the only significant lesion was laminitis in both forelimbs with rotation of the third phalanx.
**Horse 4** (phenylbutazone, no LPS) had a mild stress leukogram, moderate anemia (packed cell volume: 25%; ref. range: 32-50%), hyperglycemia (178 mg/dL), increased total protein (8.2 g/dL; ref. range: 5.8-7.5 g/dL) due to a hyperglobulinemia (5.4 g/dL; 2.5-4.7 g/dL), increased γ-glutamyltransferase (GGT) (66 U/L; ref. range: 6-24 U/L), and mild increase in alkaline phosphatase (263 U/L; ref. range: 76-249 U/L) at initial examination. Due to cost limitations, further examination of the liver was not performed. Increased protein was noted on urinalysis (3+ on strip, approximately 5 mg/dL on sulfasalicylic acid). Baseline lameness was Grade III in the left front limb. Lymphopenia was noted on the CBC at the conclusion of the study (0.9 K/µL). The anemia was significantly worse (19%). On serum chemistry, hyperglycemia (198 mg/dL), decreased total protein (5.3 g/dL) due to hypoalbuminemia (2.3 g/dL; ref. range: 2.7-3.7 g/dL), decreased total calcium (9.6 mg/dL; ref. range: 11.7-14.8 mg/dL), and mildly increased GGT (35 U/L) were noted. The urinalysis revealed a normal protein level. At post-mortem examination, hepatic and vascular amyloidosis with hepatic rupture was noted.

Overall, there was a statistically significant decrease in the total white blood cell count between the pre- and post-experimental period (p = 0.01), but stress may have induced an increase in the total white blood cell count (as indicated by increased glucose concentrations and behavior of the horses) and, once the horses settled into their new environments, the glucose concentration and white blood cell count became more normal. No significant difference was found in the pre- and post-administration total protein and creatinine measurements (p = 0.2159 and 0.5945, respectively). All horses tolerated the administration of the medications in molasses.

When evaluating force and contact area of each limb and comparing the effect of the treatments to baseline measurements, there was a statistically significant increase in both force and contact area for the right front limb and left hind limb after treatment with phenylbutazone when compared to treatment with meloxicam. Due to the low number of horses in each group, mean and standard error could not be calculated. Additionally, the data was pooled across time points and therefore violates the assumption of independence required for statistical tests to be robust. Evaluation of left front and right hind limb force and contact area could not be performed because of insufficient data points secondary to malfunction of the pressure mat.
Aim 3

Cyclooxygenase-2 expression increased four-fold in PBMCs incubated *ex vivo* with LPS (Figure 4.2). In PBMCs collected from Horse 1 (meloxicam, LPS), there was a decrease in the cycle threshold (CT) value from 30.8 at 0 hours to 26.5 at 24 hours (Figure 4.3). In Horse 2 (phenylbutazone, LPS) and Horse 4 (phenylbutazone), there was a 200-fold increase in COX-2 expression at 96 hours compared to baseline (Figure 4.4).

Western Blot analysis of the PBMCs collected from Horse 1 (meloxicam, LPS) revealed a decrease in COX-2 protein expression at 96 hours compared to baseline. This is in contrast to the results from the qRT-PCR analysis in which an increase in COX-2 gene expression was seen at 96 hours. In Horse 2 (phenylbutazone, LPS), there was a 10% decrease in protein expression levels by 24 hours, but a 25% increase in protein levels by 96 hours. In Horse 4 (phenylbutazone), there was a 50% decrease in COX-2 protein expression at 96 hours compared to baseline.
Table 4.1 Gastric ulcer score pre- and post-treatment with oral meloxicam tablets, 0.6 mg/kg, once daily for 14 days.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Sex</th>
<th>Age</th>
<th>Breed</th>
<th>Ulcer Score Pre-Treatment</th>
<th>Ulcer Score 14 days Post-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse 1</td>
<td>Gelding</td>
<td>10 y</td>
<td>QH</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Horse 2</td>
<td>Stallion</td>
<td>21 y</td>
<td>QH</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Horse 3</td>
<td>Mare</td>
<td>23 y</td>
<td>QH</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Horse 4</td>
<td>Gelding</td>
<td>31 y</td>
<td>Arab</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Horse 5</td>
<td>Mare</td>
<td>27 y</td>
<td>TB</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Horse 6</td>
<td>Mare</td>
<td>20 y</td>
<td>Hanoverian</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Horse 7</td>
<td>Gelding</td>
<td>10 y</td>
<td>TB</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Gastric ulceration scores were performed via histopathology at post-mortem examination, rather than gastroscopy prior to death.

Table 4.2 Gastric ulcer score pre- and post-treatment for horses in Aim 2 of the study.

<table>
<thead>
<tr>
<th>Horse</th>
<th>Treatment</th>
<th>Gastric Ulcer Score Pre</th>
<th>Gastric Ulcer Score Post*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse 1</td>
<td>Meloxicam</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Horse 2</td>
<td>Phenylbutazone</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Horse 3</td>
<td>Meloxicam</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Horse 4</td>
<td>Phenylbutazone</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 4.3 Mean ± SD values for pharmacokinetic parameters for multiple doses of meloxicam (0.6 mg/kg) administered orally once daily to 7* horses for 14 consecutive days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Multiple doses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 1st dose</td>
</tr>
<tr>
<td>Plasma t1/2 (h)</td>
<td>5.25 ± 1.40</td>
</tr>
<tr>
<td>T_max (h)</td>
<td>3.48 ± 3.30</td>
</tr>
<tr>
<td>C_max (µg/mL)</td>
<td>1.58 ± 0.71</td>
</tr>
<tr>
<td>AUC (h*µg/mL)</td>
<td>11.22 ± 2.00</td>
</tr>
<tr>
<td>Accumulation ratio</td>
<td>NA</td>
</tr>
<tr>
<td>Clearance (mL/h/kg)</td>
<td>55.33 ± 9.10</td>
</tr>
<tr>
<td>Vdss (L/kg)</td>
<td>0.41 ± 0.10</td>
</tr>
</tbody>
</table>

*Parameters for the 14th day were calculated for 5 horses.
Figure 4.1 Pharmacokinetic profile of oral meloxicam tablets on Day 1, 7, and 14 after administration of 0.6 mg/kg PO every 24 hours for 14 days.

Figure 4.2 qRT-PCR for COX-2 gene expression in equine PBMCs incubated ex vivo with LPS for 6 hours. There is a 4-fold increase in COX-2 gene expression in LPS treated cells.
Figure 4.3 qRT-PCR performed on mRNA isolated from PBMCs from a horse treated with meloxicam (0.6 mg/kg PO q 24 h). Cycle threshold (CT) values decreased from 30.8 to 26.5 within 24 hours of administration.

Figure 4.4 qRT-PCR performed on mRNA isolated from PBMCs collected from 2 horses treated with phenylbutazone (4.4 mg/kg PO q 24 h). Cycle threshold values decreased in both horses at 96 hours.
Chapter 5 - Discussion

Oral meloxicam tablets were well tolerated in all horses. A limitation of this study included small sample size and this was a result of the inability to find a suitable horse without gastric ulceration. As gastric ulceration is prevalent in horses of all disciplines, this was not unexpected. Although there was not a statistically significant increase in gastric ulcer scoring over the 14 day period, three horses did experience an increase in gastric ulcer grade and two of these horses had clinical signs associated with the ulceration (inappetance). These two horses were not part of the teaching herd and were transported to the testing site the morning of sampling and returned home the following day on three occasions. Transport stress, a new and changing environment, and handling of these horses may have contributed to the increase in gastric ulcer score. Ideally, two control horses that were transported in the same manner but not administered medications would be required to determine whether the medications or the changing environments resulted in the worsening of gastric ulceration. Previous studies using label doses of phenylbutazone (2.2 mg/kg) given orally twice daily for 14 days indicate that the drug is safe over this period of time (Andrews 2009). As there were no changes in total protein, urinalysis, or creatinine measurements over the 14 day period, it does not appear that meloxicam affects the kidneys or results in right dorsal colon ulceration when given in this manner.

Plasma clearance obtained in this study was slightly higher (mean, 51.2 ± 11.01 mL/kg/h) than that obtained in previous studies using intravenous and oral suspension formulations (Lees 1991, Toutain 2004a, Sinclair 2006) but lower than that obtained in one study using the intravenous form (mean, 81 mL/kg/h; Toutain 2004b). The origin of these differences is unclear; however, differences in analysis and population may affect results.

The terminal half-lives in the previous studies reported are highly variable. This is likely due to the methods of analysis, where the limit of quantification may have not been sufficiently low enough to monitor the decrease of plasma concentrations for a long enough period of time to determine the true terminal portion of the curve. A terminal half-life of approximately 5 hours is sufficient to justify a once-daily dosing regimen for an NSAID and yet avoid any accumulation as indicated by the accumulation ratio which was close to 1 (1.17 ± 0.39). The plasma patterns of meloxicam should be nearly identical after each administration and should preclude the use of
a loading dose. The steady state volume of distribution was limited (0.37 ± 0.11 L/kg), likely a result of extensive protein binding.

The decision to allow the horses to be fed during our study allowed this to be a more practical and clinically applicable study. The bioavailability of the drug is not changed with feeding (Toutain 2004b); however, MRT, MAT, and T_{max} are significantly greater and C_{max} significantly lower in fed than in nonfed horses. Feeding substantially lowers the rate of drug absorption but does not appear to affect bioavailability. The bioavailability could not be evaluated in this study because intravenous data was not obtained. The C_{max} obtained in our study (1.82 ± 0.8 µg/mL) was very similar to that found in fed horses in another study (1.73 ± 0.61 µg/mL; Toutain 2004a); however, the T_{max} was slightly shorter (mean: 2.22 ± 1.95 µg/mL) than in the fed horses of the Toutain study (3.4 ± 1.19 h (fed), 1.5 ± 1.07 h (non-fed)).

Four horses were used in the second aim of the study. This was due to limits in budget and availability of horses that had not been administered NSAIDs in the last 6 months. The horses that were obtained had been previously used as serum producing horses. The behavior of the horses (semi-feral) and budget limitations restricted our evaluation of lameness to pressure mat analysis only. The behavior of the horses also affected the evaluation of the pressure mat data as one of the horses damaged the pressure mat during the evaluation. Budget limitations also affected the ability to evaluate gastric ulcer scoring via gastroscopy at the end of the study. However, endoscopic scoring of gastric ulceration tends to underestimate the number, severity, and depth of gastric ulcers compared to histopathology (Andrews 2002) and therefore, scoring at post-mortem was the more sensitive test.

One horse experienced leukopenia and evidence of typhlocolitis on histology at post-mortem, although no clinical evidence was noted. It is likely that this was a result of systemic absorption of LPS from the carpus joint. Another horse had acute hepatic rupture noted at post-mortem, secondary to hepatic amyloidosis. A large amount of blood filled the abdomen and this explained the significant anemia and hypoproteinemia. All of the horses had been used as hyperimmunized serum donors and amyloidosis has been associated with the repeated injections of the endotoxin-releasing bacteria Escherichia coli and Pasteurella multocida (Abdelkader 1991). Occasionally, horses will die with no clinical signs; the only clinical sign this horse showed was a decreased appetite the morning of the day of euthanasia. In addition to the hepatic findings, there was chronic rotation of the third phalanx of both front feet and chronic
tubulointerstitial nephritis. There were no histopathologic changes in the right dorsal colon except for vascular amyloidosis.

Due to pressure mat malfunction, a full analysis of the data obtained could not be performed. However, the data suggests that meloxicam is not as potent as phenylbutazone for lameness pain. Induction of lameness with LPS presents a challenge in evaluating daily lameness changes due to the steady improvement in lameness over time. A more consistent method of lameness induction may have included a shoe that allows for a reversible model of lameness (Foreman 2010). This was a pilot study and the aim was to determine if there were any improvements noted with meloxicam administration. Although the horses were not critically evaluated for lameness grade, each horse appeared clinically more comfortable and able to mobilize around the stall easier after administration of both medications.

Other parameters that could have been measured during the second aim of the study included serum concentrations of meloxicam during the administration period and comparing the concentration at which 50% of the lameness was reduced (EC$_{50}$ and ED$_{50}$). Additionally, the circumference of the carpal joint, the angle of the carpus at rest, maximal forced flexion angle of the carpus, and pedometry may have been used to evaluate additional parameters of lameness pain.

Incubation of PBMCs with LPS resulted in an increase in gene expression of COX-2 in normal horses. Systemically, injection of LPS into the metacarpophalangeal joint resulted in an increase in both gene and protein expression of COX-2 and this increase was variably attenuated by meloxicam and phenylbutazone. With the very small sample size used in this study, it is impossible to interpret the clinical usefulness of these results, but rather indicates the need for further research to explain the variability found in the results.

In conclusion, oral meloxicam tablets administered at 0.6 mg/kg orally once daily to horses are well-tolerated and we found similar pharmacokinetics to the oral suspension formulation. Additionally, administration of this medication over 14 days did not result in evidence of renal or gastrointestinal changes. Although inferior to phenylbutazone for induced and naturally occurring lameness, it may be considered an alternative due to its selective or preferential COX-2 inhibition.
References


Gunson DE. Renal papillary necrosis in horses. JAVMA 1983a Feb 1;182(3):263-6.


Keegan KG, Messer NT, Reed SK, Wilson DA, Kramer J. Effectiveness of administration of phenylbutazone alone or concurrent administration of phenylbutazone and flunixin meglumine to alleviate lameness in horses. AJVR 2008;69(2):167-173.


MacAllister CG, Morgan SJ, Borne AT, Pollett RA. Comparison on adverse effects of phenylbutazone, flunixin meglumine, and ketoprofen in horses. JAVMA 1993;202:71-77.


McConnico RS, Morgan TW, Williams CC, Hubert JD, Moore RM. Pathophysiologic effects of phenylbutazone on the right dorsal colon in horses. AJVR 2008 Nov;69(11):1496-505.


Raidal SL, Pippia J, Noble G. Pharmacokinetics of single and multiple oral doses of meloxicam in foals less than 6 weeks of age. AAEP Proceedings, Las Vegas, NV 2009.

Rainsford KS, Ying C, Smith FC. Effects of meloxicam, compared with other NSAIDs, on cartilage proteoglycan metabolism, synovial prostaglandin E2, and production of interleukins 1, 6, and 8 in human and porcine explants in organ culture. J Pharm Pharmacol 1997;49:991-998.


Reed SK, Messer NT, Tessman RK, Keegan KG. Effects of phenylbutazone alone or in combination with flunixin meglumine on blood protein concentrations in horses. AJVR 2006;67(3):398-402.


Toutain PL, Cester CC. Pharmacokinetic-pharmacodynamic relationships and dose response to meloxicam in horses with induced arthritis in the right carpal joint. AJVR 2004b;65(11):1533-1541.


Warner TD, Giuliano F, Vojnovic I, Bukasa A, Mitchell JA, Vane JR. Nonsteroid drug selectivities for cyclooxygenase-1 rather than cyclooxygenase-2 are associated with human