Pharmacokinetics of mavacoxib in the New Zealand white rabbit

by

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B.S., University of Massachusetts – Dartmouth, 2016
D.V.M., Kansas State University, 2020

A REPORT

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Clinical Science
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2022

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Abstract

Mavacoxib, a selective COX-2 non-steroidal anti-inflammatory drug used for management of osteoarthritis and other inflammatory conditions in dogs, could be very advantageous in zoological medicine patients because of its long plasma half-life (Cox 2010, Lees 2015). The purpose of this study was to characterize the pharmacokinetics of mavacoxib in rabbits and to describe any clinicopathologic effects seen with this dosing. Six healthy, 4-month-old New Zealand white rabbits (3 male, 3 female) were administered 6 mg/kg mavacoxib orally (PO) once. Before drug administration, clinicopathologic samples were collected for baseline data (CBC, biochemistry, and urinalysis), as well as at set time intervals to compare to baseline. Plasma mavacoxib concentrations were determined using liquid chromatography with mass spectrometry, and pharmacokinetic analysis was performed using noncompartmental methods. Following a single PO dose, mean peak plasma concentration (C_{max}) was (mean; range) 854.6 (713.2-1040.7) ng/mL; mean time to peak plasma concentration (T_{max}) was 0.36 (0.17-0.50) d; mean area under the curve (AUC_{all}) was 2000 (1765-2307) d*ng/ml; mean terminal half-life ($T_{1/2}$) was 1.63 (1.30-2.26) d; and mean terminal rate constant (L_z) was 0.42 (0.31-0.53) d. Complete blood count, biochemistry, urinalyses, and urine protein:creatinine all remained within published normal reference intervals. Further research is needed to make a dosing recommendation, including a pharmacodynamics study and investigating pharmacokinetics at different doses and multiple doses. Based on this study, it was determined that plasma levels reached target levels of 400ng/mL for 48 hours in 3/6 rabbits, with 3/6 being 10.8-56.8 ng/mL under the target level at 48 hours, when given 6 mg/kg orally once.

Key words: Analgesia, mavacoxib, nonsteroidal anti-inflammatory drug, *Oryctolagus cuniculus*, rabbit, pharmacokinetics

Table of Contents

List of Figures

List of Tables

Pharmacokinetics of mavacoxib in the New Zealand white rabbit

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Objectives

To characterize the pharmacokinetics of a single oral dose (6 mg/kg) of mavacoxib in the New Zealand white rabbit and to describe any clinicopathologic effects seen with this dosing.

Animals

6 healthy, 4-month-old New Zealand white rabbits (3 male, 3 female).

Procedures

Before drug administration, clinicopathologic samples were collected for baseline data (CBC, biochemistry, and urinalysis including UPC). All 6 rabbits received a single oral dose (6 mg/kg) of mavacoxib. Clinicopathologic samples were collected at set time intervals to compare to baseline. Plasma mavacoxib concentrations were determined using liquid chromatography with mass spectrometry, and pharmacokinetic analysis was performed using noncompartmental methods.

Results

Following a single oral dose, mean C_{max} was (mean; range) 854.6 (713.2-1040.7) ng/mL; mean T_{max} was 0.36 (0.17-0.50) d; mean AUC_{all} was 2000 (1765-2307) d*ng/mL; mean $T_{1/2}$ was 1.63 (1.30-2.26) d; and mean L_z was 0.42 (0.31-0.53) d. Complete blood count, biochemistry, urinalyses, and urine protein:creatinine all remained within published normal reference intervals.

Conclusions and Clinical Relevance

This study determined that plasma levels reached target levels of 400 ng/mL for 48 hours in 3/6 rabbits, with 3/6 being 10.8-56.8 ng/mL under the target level at 48 hours, when given 6 mg/kg orally once. Further research is needed to make a dosing recommendation, including a pharmacodynamics study and investigating pharmacokinetics at different doses and multiple doses.

ABBREVIATIONS

AUC_{all}	Area under the curve from 0 to the last measured time point
AUC_{inf}	Area under the curve extrapolated to infinity
AUC $\%_{extrap}$	Percent of the AUC_{inf} extrapolated
C_{max}	Peak plasma concentration
L_z	Terminal rate constant
$T_{1/2}$	Terminal half-life
T_{max}	Time to C_{max}
UPC	Urine protein:creatinine ratio

Introduction

Providing proper pain management in nontraditional mammalian species can be challenging due to a general lack of pharmacologic studies for analgesic medications. The most common medications for managing pain in these species are non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, however, these drugs often require frequent dosing to achieve adequate pain control. Due to significant variation in the pharmacokinetics and pharmacodynamics of many drugs across mammalian species, extrapolating doses and dosing frequency can be detrimental, due to either toxic doses resulting in adverse effects or sub therapeutic doses resulting in low efficacy.[1]

Mavacoxib is a selective COX-2 inhibitor NSAID that is approved in Europe and the UK for pain associated with degenerative joint disease in dogs. A notable benefit of mavacoxib is the infrequent dosing interval, administered orally every 30 days in dogs, due to a long duration of action.[2,3] It has been used to manage pain in other chronic conditions, as well. This infrequent dosing interval could be especially useful in prey species, such as rabbits, where daily administration of medications by owners may not be feasible due to the stress of handling and patient noncompliance. The only pharmacokinetic studies of NSAIDs in rabbits currently are for meloxicam, which was found to have a short half-life and therefore requires a frequent dosing interval.[4,5]

Previous pharmacokinetic studies of mavacoxib in mammalian species have been limited to the domestic dog, where one study showed that a single 2 mg/kg PO dose reached therapeutic plasma concentrations for management of osteoarthritis.[3] Mavacoxib has also been studied in a limited number of avian species, including the cockatiel (*Nymphicus hollandicus*),[6,7] the flamingo (*Phoenicopterus ruber ruber*),[8] and the African grey parrot (*Psittacus erithacus*).[9] In a

study on mavacoxib in cockatiels, a 4 mg/kg PO dose resulted in plasma concentrations of the drug equivalent to therapeutic concentrations in dogs, as well as a prolonged half-life compared to other NSAIDs.[6] In flamingos dosed at 6 mg/kg PO once, plasma concentrations of drug reached a concentration higher than canine therapeutic levels for 5-7 days.[8] Another study in African grey parrots concluded that a single dose of mavacoxib dosed at 4 mg/kg PO showed a high volume of distribution and low clearance levels, leading to a less frequent dosing recommendation in this species.[9] Currently, there are no other pharmacodynamics or pharmacokinetic studies reported on mavacoxib in mammalian species outside of domestic dogs. Additionally, the effective and toxic concentrations of mavacoxib are unknown in other species, and as such, they are extrapolated from dogs.

The purpose of this study is to obtain pharmacokinetic data for an oral (PO) dose of mavacoxib in the domestic rabbit (*Oryctolagus cuniculus*), administered at 6 mg/kg once, via liquid chromatography with tandem mass spectrometry (LC/MS/MS). The data obtained from this pharmacokinetic study will be used to determine if this dose of mavacoxib in the rabbit provides plasma concentrations associated with therapeutic plasma levels in the dog, assess the adverse effects of this dose of mavacoxib, and determine the recommended dosing frequency for this species.

Methods and Materials

Animals

Six 4-month-old, 3 male and 3 female, New Zealand white rabbits (specific-pathogen free *Pasteurella* sp.) were used in this study. They were deemed clinically normal after undergoing a

physical exam and obtaining a packed cell volume, total plasma protein, and plasma biochemical analysis immediately prior to the study. They were housed in indoor runs in the research facilities of the Kansas State University College of Veterinary Medicine and were fed a diet consisting of a pelleted diet[c] and timothy hay.[f] Each rabbit received a single oral dose (6 mg/kg) of compounded mavacoxib.[e] This study was approved by the Institutional Animal Care and Use Committee of Kansas State University.

Experimental design

One 75 mg tablet of mavacoxib was crushed using a mortar and pestle. The powder was added to 11.6 mL of ORA-Sweet,[d] 11.6 mL ORA-Plus,[d] and 0.23 mL of strawberry flavoring. The resulting concentration was 3 mg/mL of mavacoxib. The formations were made approximately 48 hours prior to dosing and stored in a refrigerator until administration.

Blood samples were collected from the lateral saphenous vein using heparinized syringes and a 23 or 25-gauge needle prior to drug administration and at 0, 4, 8, 12, and 24 hours, and 2, 3, 5, 7, 11, 14, 17, and 21 days after mavacoxib administration. The blood samples were centrifuged and the plasma supernatant harvested and stored at -70°C until analysis via LC/MS/MS. Additional blood samples and free catch urine samples were collected for each rabbit on days 0, 7, 14, and 21 and were submitted for a packed cell volume, total plasma protein, and plasma biochemical analysis, and urinalysis with UPC in order to monitor for adverse effects that may result from mavacoxib.

Plasma drug and pharmacokinetic analysis

Plasma drug analysis was performed with liquid chromatography with mass spectrometry[b] after protein precipitation. Plasma samples, standards in rabbit plasma, and quality

control samples in rabbit plasma were processed in an identical manner. To a 1.5 mL microcentrifuge tube, 0.05 mL plasma, standard or quality control sample was added to 0.15 mL methanol containing 1% formic acid and 500 ng/mL of the internal standard, celecoxib. The mixture was vortexed for 5 seconds, followed by centrifugation at 15000 x G for 10 minutes. The clear supernatant was transferred to a 96 well plate and placed in the autosampler. The injection volume was 0.01 mL, separation was achieved with a column[a] maintained at 50°C and the mobile phase was A: 0.1% formic acid in deionized water and B: 0.1% formic acid in methanol. The mobile phase flow was 0.6 mL/min with a gradient starting at 90% A with a linear gradient to 20% A at 1.5 minutes, then a linear gradient starting at 2 minutes from 20% A to 90% A at 2.1 minutes with a total run time of 3 minutes. The retention times for mavacoxib and celecoxib were 2.1 and 2.2 minutes, respectively. The qualifying ions for mavacoxib and celecoxib were m/z 385.88 and 381.4, respectively. The quantifying ions for mavacoxib and celecoxib were m/z 365.96 and 361.99, respectively. The standard curve in rabbit plasma was linear from 50 – 10,000 ng/mL. The accuracy and precision (coefficient of variation) of the assay were determined on replicates of 3 at each of the following concentrations: 50, 1000, and 10,000 ng/mL. The mean accuracy was 87% of the actual concentration while the mean precision was 9%.

Pharmacokinetics analysis was performed with computer software (PK plug in for Excel) using noncompartmental methods. The AUC_{all} (AUC 0 – 6 days), C_{max}, and T_{max} were determined directly from the data. The $T_{½}$ and L_z were determined by log-linear regression of the last 4 time points on the terminal portion of the plasma curve. The AUC_{inf} was determined by extrapolation from the last time point using the L_z. The percent of the AUC $\%_{extrap}$ was

determined by dividing the AUC_{inf} by AUC_{all}. Summary pharmacokinetic data are presented as geometric mean and range.

Results

The six rabbits in this study remained clinically healthy throughout the duration of the study. All pre-study baseline clinicopathological data were within published reference ranges. No adverse effects or changes in behavior, attitude, mentation, appetite, urination, or defecation was noted throughout the study. Mavacoxib was present in all plasma samples from days 1 to 6, but none was detected on or after day 7. The geometric means and ranges of the pharmacokinetics parameters measured for mavacoxib are summarized in Table 1. The mean C_{max} was 854 (range, 713-1040) ng/mL and the mean T_{max} was 0.36 (range, 0.17-0.50) days. The mean AUC_{all} for days 1-6 was 2000 (range, 1765-2307) d*ng/mL. The AUC_{inf} was 2196 (range, 2019-2443) days. The mean terminal half-life ($T_{1/2}$) was 1.63 (range, 1.30-2.26) days, and mean terminal rate constant (L_z) was 0.42 (range, 0.31-0.53) days.

Three of the 6 rabbits achieved the targeted 400 ng/mL plasma concentration of mavacoxib for 48 h. The remaining 3/6 rabbits were below 400 ng/mL (range 343 – 389 ng/mL). Clinicopathologic data for all rabbits, which were performed on days 7, 14, and 21 remained within published reference ranges.[10-12]

Discussion

The purpose of this study was to determine pharmacokinetic parameters of a single oral dose of mavacoxib at 6 mg/kg. The results showed that mavacoxib plasma concentrations

associated with efficacy in dogs (400 ng/mL) for 48 hours in 3/6 rabbits and were 343 – 389 ng/mL in the remaining 3/6 rabbits.

Previous mavacoxib pharmacokinetic studies have been conducted in cockatiels, African grey parrots, and flamingos, as well as in dogs. In cockatiels, a 4 mg/kg oral dose achieved 0.4 ug/mL for less than 5 days in some birds,[6] whereas in flamingos a 6 mg/kg oral dose exceeded this concentration in all birds for 5-7 days.[8] Although the effective plasma concentration for pain control in dogs has been established, it has not been established in any other species, including rabbits.

The C_{max}, $T_{max,}$ and $T_{1/2}$ were all lower or shorter in rabbits (854 ng/mL, 0.36 d, 1.63 d, respectively) than in fasted dogs (1040 ng/mL, 2.81 d, 19.3 d) at 4 mg/kg orally[2] and flamingos (2970 ng/mL, 0.78 d, 3.10 d) at 6 mg/kg orally.[8] The C_{max} for rabbits at 6 mg/kg orally was higher than cockatiels (584 ng/mL) administered 4 mg/kg orally, however the T_{max} and $T_{1/2}$ were longer in cockatiels (0.60 d, 5.64 d).[6] The results of these parameters in this study were lower than expected. While the reason for this remains unclear at this time, it is possible that there could be differences in protein binding, metabolic pathways, or bioavailability when compared to the other species with known pharmacokinetic parameters of mavacoxib. Studies have shown a significant difference in the oral bioavailability of mavacoxib in dogs that have been fed versus dogs that have been fasted,[2,3] which may warrant further studies in rabbits, as well. Additionally, NSAIDs are known for having high protein binding, making it difficult to interpret plasma concentration in relation to physiologic and pharmacologic activity since there are currently no data available on mavacoxib protein binding in rabbits.[13]

The most common adverse effect associated with mavacoxib in dogs include gastrointestinal issues such as diarrhea, vomiting, and hypo- or anorexia. NSAIDs in general

have also been reported to be associated with gastrointestinal ulcerations and acute kidney injuries in dogs, although these have not been reported to be associated with mavacoxib at this time.[14] All 6 rabbits in this study remained healthy throughout the entire duration of the study, with no abnormal behavior noted and all clinicopathologic data remaining within normal reported reference intervals. However, it is important to note that chemistry panels and urinalyses are relatively insensitive measures of acute kidney injury.

Limitations of this study include a small sample size consisting of a fairly homogenous population. Having a larger population made up of various breeds, ages, and sex, including both intact and altered, would make the results more applicable to the range of patients treated clinically. Another limitation is having no intravenous administration of mavacoxib. If an IV administration was performed, a more complete pharmacokinetic profile could be obtained, including determination of the oral bioavailability

Further studies are needed to make dose frequency recommendations, and while none of the rabbits experienced any adverse effects, further safety studies are warranted. These studies would include evaluating a higher dose, repeated doses, and a pharmacodynamics study. Repeated doses would allow the steady-state pharmacokinetics to be evaluated. A pharmacodynamics study is needed to determine a therapeutic plasma concentration and efficacious dose. This study could be done in vitro or ex vivo via COX inhibition or in vivo using various induced inflammation models. A study to evaluate IV mavacoxib pharmacokinetics is also important, as it would help expand the known pharmacokinetic profile by determining bioavailability, volume of distribution, average absorption time, and clearance of the oral administration.

Footnotes

a. Acquity UPLC HSS, T3, 1.8µM, 2.1x50mm, Waters Corp, Milford, MA 01757, USA
b. Acquity UPLC, TQD, Waters Corp, Milford, MA 01757, USA
c. Bunny Basics 15/23, Oxbow Pet Products, Murdock, Nebraska 68407, USA
d. Paddock Laboratories, LLC, Minneapolis, MN 55427, USA
e. Trocoxil, Zoetis, Marino del Tronto, Italy
f. Western Timothy Hay, Oxbow Pet Products, Murdock, Nebraska 68407, USA

Acknowledgements

This study was supported by a research grant from the Clinical Sciences Research Committee at Kansas State University's College of Veterinary Medicine. Funding sources did not have any involvement in the study design, data analysis and interpretation, or writing and publication of the manuscript. The authors would like to thank Tori Matta, Meagan Wood, Elizabeth Loos, and Taylor Wright for their assistance with various aspects of this study.

The authors declare that there were no conflicts of interest.

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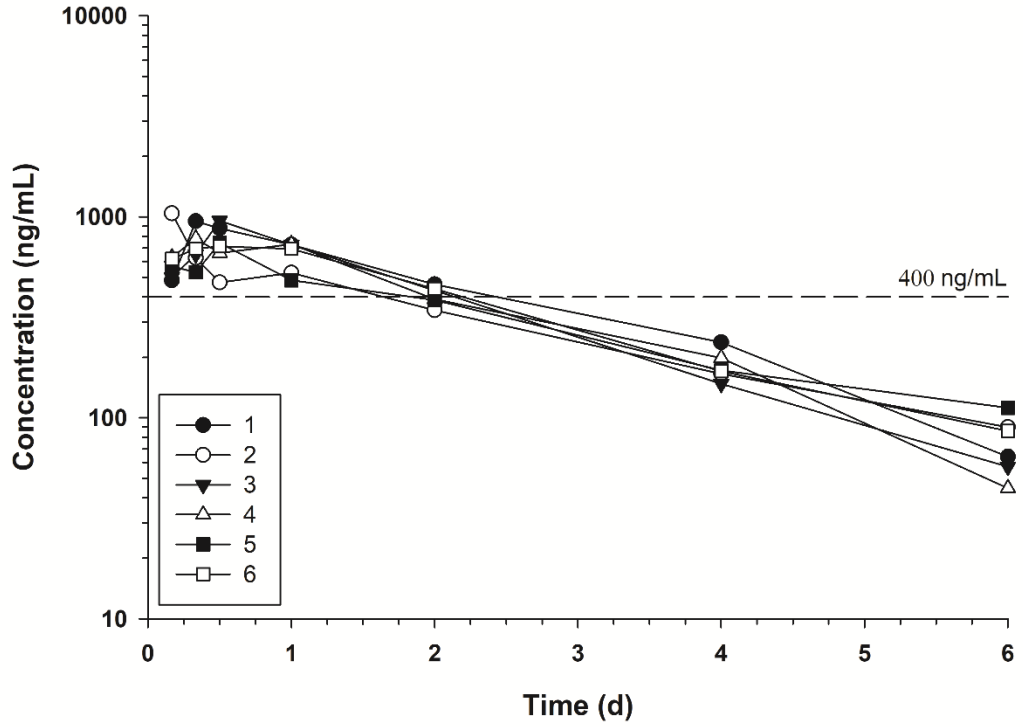
14. Monteiro-Steagall BP, Steagall PVM, Lascelles BDX. Systematic review of nonsteroidal anti-inflammatory drug-induced adverse effects in dogs. *J Vet Intern Med* 2013;27:1011–1019.

Figure Legend

Figure 1 – Individual animal plasma profiles of mavacoxib after administering 6 mg/kg mavacoxib orally to 6 healthy rabbits.

Figures

Figure 1



Tables

Table 1 – Noncompartmental pharmacokinetic parameters after administering 6 mg/kg mavacoxib orally to 6 healthy rabbits.

Animal	C_{MAX} (ng/mL)	T_{MAX} (d)	AUC_{all} (d*ng/mL)	AUC_{inf} (d*ng/mL)	AUC % extrap	$T_{1/2}$ (d)	L_z (/d1)
1	951	0.33	2307	2443	6%	1.47	0.471
2	1041	0.17	1765	2019	14%	1.96	0.353
3	955	0.50	2054	2165	5%	1.36	0.511
4	780	0.33	2029	2113	4%	1.30	0.534
5	741	0.50	1828	2193	20%	2.26	0.306
6	713	0.50	2061	2264	10%	1.64	0.422
Geometric mean	854	0.36	2000	2196	8%	1.63	0.42
Minimum	713	0.17	1765	2019	4%	1.30	0.31
Maximum	1040	0.50	2307	2443	20%	2.26	0.53