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Pharmacokinetics of mavacoxib in New Zealand White rabbits (*Oryctolagus cuniculus*)

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OBJECTIVES
To characterize the pharmacokinetics of a single oral dose (6 mg/kg) of mavacoxib in New Zealand White rabbits (*Oryctolagus cuniculus*) and to characterize any clinicopathologic effects with this medication and dose.

ANIMALS
Six healthy, 4-month-old New Zealand White rabbits (3 male, 3 female).

PROCEDURES
Before drug administration, clinicopathologic samples were collected for baseline data (CBC, serum biochemical analyses, and urinalysis including urine protein-to-creatinine ratio). All 6 rabbits received a single oral dose (6 mg/kg) of mavacoxib. Clinicopathologic samples were collected at set time intervals to compare with the baseline. Plasma mavacoxib concentrations were determined using liquid chromatography with mass spectrometry, and pharmacokinetic analysis was performed using non-compartmental methods.

RESULTS
After a single oral dose, the maximum plasma concentration (C_{max}; mean, range) was 854 (713–1040) ng/mL, the time to C_{max} (t_{max}) was 0.36 (0.17–0.50) days, the area under the curve from 0 to the last measured time point ($AUC_{0\text{-last}}$) was 2000 (1765–2307) days*ng/mL, the terminal half-life ($t_{1/2}$) was 1.63 (1.30–2.26) days, and the terminal rate constant (λ_z) was 0.42 (0.31–0.53) days. All results for CBCs, serum biochemical analyses, urinalyses, and urine protein-to-creatinine ratios remained within published normal reference intervals.

CLINICAL RELEVANCE
This study determined that plasma concentrations reached target levels of 400 ng/mL for 48 hours in 3/6 rabbits at 6 mg/kg PO. In the remaining 3/6 rabbits, the plasma concentrations were 343–389 ng/mL at 48 hours, which is below the target concentration. Further research is needed to make a dosing recommendation, including a pharmacodynamic study and investigating pharmacokinetics at different doses and multiple doses.

Providing proper pain management in non-traditional 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 NSAIDs and opioids; however, these drugs often require frequent dosing to achieve adequate pain control. Due to substantial 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 cyclooxygenase-2 (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 slow elimination and a long duration of action.[2,3] 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 non-compliance. The only pharmacokinetic studies of NSAIDs in rabbits currently are for meloxicam and firicoxib, and both were found to have a short half-life and therefore require a frequent dosing interval.[4–6]

Previous pharmacokinetic studies of mavacoxib in mammalian species have been limited to the domestic dog,[2,3] where one study showed that a single 2 mg/kg PO dose reached therapeutic plasma concentrations for the management of osteoarthritis.[3] Mavacoxib has also been studied in a limited number of avian species, including the cockatiel (*Nymphicus hollandicus*),[7,8] the flamingo (*Phoenicopterus ruber ruber*),[9] and the African grey parrot (*Psittacus erithacus*).[10] 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 half-life of 135 hours, which is prolonged when compared with other NSAIDs.[7] In flamingos dosed at 6 mg/kg PO once, plasma concentrations of the drug reached a concentration higher than canine therapeutic levels for 5–7 days.[9] 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, as well as a half-life of about 116 hours, leading to a less frequent dosing recommendation in this species.[10] 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 was 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). This dose was chosen based on dosing recommendations for other species and the metabolic rates of these species compared with rabbits.[2,3,7–10] 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 New Zealand White rabbits (3 male, 3 female) that were specific-pathogen free *Pasteurella* sp. were used in this study. They were deemed clinically normal after undergoing a physical examination and obtaining a PCV, total plasma protein, and plasma biochemical analysis immediately before the study. The rabbits 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 (Bunny Basics 15/23, Oxbow Pet Products, Murdock, NE) and timothy hay (Western Timothy Hay, Oxbow Pet Products). Each rabbit received a single oral dose (6 mg/kg) of mavacoxib (Trocoxil, Zoetis, Marino del Tronto, Italy) compounded in-house. 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 a sweetened syrup vehicle (ORA-Sweet; Paddock Laboratories, LLC, Minneapolis, MN), 11.6 mL of a syrup vehicle (ORA-Plus; Paddock Laboratories), and 0.23 mL of strawberry flavoring (Strawberry Concentrate Artificial Flavor, Gallipot, St Paul, MN). The resulting concentration was 3 mg/mL of mavacoxib (1:1:0.02 of each syrup vehicle to flavoring ratio). The formulations were made approximately 48 hours before dosing and stored in a refrigerator until administration. No stability testing was done, so there is no stability data for the final compounded product.

Blood samples were collected from the lateral saphenous vein using heparinized syringes and a 23 or 25-gauge needle before 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 at 1,200 X *g* for 10 minutes and the plasma supernatant was 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 PCV, total plasma protein and plasma biochemical analysis, and urinalysis with a urine protein-to-creatinine ratio to monitor for adverse effects that may result from the mavacoxib.

Plasma drug and pharmacokinetic analysis

Plasma drug analysis was performed with liquid chromatography with mass spectrometry (Acquity UPLC, TQD, Waters Corp, Milford, MA) 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 15,000 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 (Acquity UPLC HSS, T3, 1.8 µM, 2.1 X 50 mm, Waters Corp) 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 386 and 381, respectively. The quantifying ions for mavacoxib and celecoxib were m/z 366 and 362, 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, V1.0, Excel; Microsoft Corp) using non-compartmental methods. The $AUC_{0\text{-last}}$ (AUC 0–6 days), C_{max}, and t_{max} were determined directly from the data. The $t_{½}$ and λ_z were determined by log-linear regression of the last 4 time points on the terminal portion of the plasma curve. The area under the curve (AUC) extrapolated to infinity (AUC_{inf}) was determined by extrapolation from the last time point using the λ_z. The percent of the AUC_{inf} extrapolated (AUC $\%_{extrap}$) was determined by dividing the AUC_{inf} by $AUC_{0\text{-last}}$. Summary pharmacokinetic data are presented as geometric mean and range.

Results

The 6 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 were noted throughout the study. The geometric means and ranges of the pharmacokinetics parameters measured for mavacoxib were calculated **(Table 1)**. The mean C_{max} was 854 (range 713–1,040) ng/mL and the mean t_{max} was 0.36 (range 0.17–0.50) days. The mean $AUC_{0\text{-last}}$ for days 1–6 was 2,000 (range 1,765–2,307) days*ng/mL. The AUC_{inf} was 2,196 (range 2,019–2,443) days. The $t_{1/2}$ was 1.63 (range 1.30–2.26) days, and the λ_z was 0.42 (range 0.31–0.53) days. Mavacoxib was present in all plasma samples from days 1 to 6, but none was detected on or after day 7 **(Figure 1)**.

Three of the 6 rabbits achieved the targeted 400 ng/mL plasma concentration of mavacoxib for 48 hours. 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.[11–13]

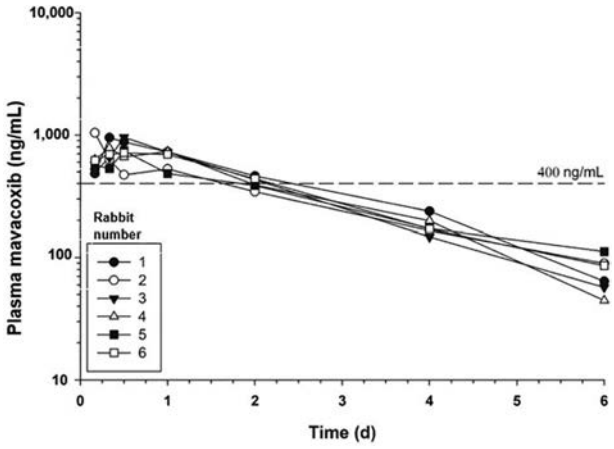

Figure 1—Individual log-transformed plasma mavacoxib concentrations for 6 clinically normal and *Pasteurella* sp specific-pathogen free New Zealand White rabbits (*Oryctolagus cuniculus*) after receiving mavacoxib (6 mg/kg, PO, once at time 0) between March 14 and April 3, 2021. For each time point, each shape represents the result for 1 rabbit (numbered 1 through 6). A solid line connects progressive results for each animal. The dashed horizontal line represents the targeted plasma mavacoxib concentration of 400 ng/mL.

Discussion

The purpose of this study was to determine pharmacokinetic parameters of a single oral dose of mavacoxib at 6 mg/kg. The results demonstrated that 3/6 rabbits reached mavacoxib plasma concentrations associated with efficacy in dogs (400 ng/mL) for 48 hours. The remaining 3/6 rabbits had plasma concentrations of 343–389 ng/mL at 48 hours, which is under the target concentration of 400 ng/mL.

Previous mavacoxib pharmacokinetic studies have been conducted in cockatiels, African grey parrots, and flamingos, as well as in dogs.[2,3,7–10] In cockatiels, a 4 mg/kg oral dose achieved 400 µg/mL for less than 5 days in some birds,[7] whereas in flamingos a 6 mg/kg oral dose exceeded this concentration in all birds for 5–7 days.[9] 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 days, 1.63 days, respectively) than in fasted dogs (1,040 ng/mL, 2.81 days, 19.3 days) at 4 mg/kg orally[2] and flamingos (2,970 ng/mL,

Table 1—Geometric mean and ranges of non-compartmental pharmacokinetic variables for 6 clinically normal and *Pasteurella* sp specific-pathogen free New Zealand White rabbits (*Oryctolagus cuniculus*) after receiving mavacoxib (6 mg/kg, PO, once) between March 14 and April 3, 2021.

Animal	C_{max} (ng/mL)	t_{max} (d)	$AUC_{0\text{-last}}$ (d*ng/mL)	AUC_{inf} (d*ng/mL)	AUC % extrap	$t_{1/2}$ (d)	λ_z (/d1)
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

C_{max} = peak plasma concentration; t_{max} = time to C_{max}; $AUC_{0\text{-last}}$ = 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; $t_{1/2}$ = terminal half-life; λ_z = terminal rate constant.

0.78 days, 3.10 days) at 6 mg/kg orally.[9] 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 days, 5.64 days).[7] 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 and rates, bioavailability, or stability of the compounded formulation when compared with the other pharmacokinetic studies 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] 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.[1,4]

The most common adverse effect associated with mavacoxib in dogs includes 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.[14,15] Because this is a long-acting medication, it is important to consider that an adverse reaction may be more difficult to treat due to being unable to discontinue the medication as part of treatment, resulting in a longer period of supportive care. 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 biochemical panels and urinalyses are relatively insensitive measures of acute kidney injury. Additionally, rabbits cannot vomit, and histologic evaluation of the stomach was not performed in this study to evaluate for gastric ulcers.

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 a determination of the oral bioavailability. Additionally, the medication was compounded from FDA-approved products 48 hours before administration with no stability testing having been done. Veterinarians should adhere to compounding regulations and be aware that pharmacokinetic properties may differ between compounded and FDA-approved products.

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 pharmacodynamic study is needed to determine a therapeutic plasma concentration and efficacious dose. Future studies could include in vitro or ex vivo cyclooxygenase enzyme 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, the volume of distribution, mean absorption time, and clearance of the oral administration.

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The authors declare that there were no conflicts of interest.

References

1. Hawkins MG. The use of analgesics in birds, reptiles, and small exotic mammals. *J Exot Pet Med.* 2006;15:177–192. doi:10.1053/j.jepm.2006.06.004
2. Cox SR, Lesman SP, Boucher JF, et al. The pharmacokinetics of mavacoxib, a long-acting COX-2 inhibitor, in young adult laboratory dogs. *J Vet Pharmacol Ther.* 2010;33:461–470. doi:10.1111/j.1365-2885.2010.01165.x
3. Lees P, Pelligand L, Elliott J, et al. Pharmacokinetics, pharmacodynamics, toxicology and therapeutics of mavacoxib in the dog: a review. *J Vet Pharmacol Ther.* 2015;38:1–14. doi:10.1111/jvp.12185
4. Delk KW, Carpenter JW, KuKanich B, et al. Pharmacokinetics of meloxicam administered orally to rabbits (*Oryctolagus cuniculus*) for 29 days. *Am J Vet Res.* 2014;75:195–199. doi:10.2460/ajvr.75.2.195
5. Turner PV, Chen HC, Taylor WM. Pharmacokinetics of meloxicam in rabbits after single and repeat oral dosing. *Comp Med.* 2006;56:63–67.
6. Gardhouse S, Kleinhenz M, Hocker SE, et al. Pharmacokinetics and ex vivo pharmacodynamics of oral firocoxib in New Zealand White rabbits (*Oryctolagus cuniculus*). *Am J Vet Res.* 2022;83; ajvr.21.11.0177.
7. Dhondt L, Devreese M, Croubels S, et al. Comparative population pharmacokinetics and absolute oral bioavailability of COX-2 selective inhibitors celecoxib, mavacoxib and meloxicam in cockatiels (*Nymphicus hollandicus*). *Sci Rep.* 2017;7:12043. doi:10.1038/s41598-017-12159-z
8. Antonissen G, Martel A, Goessens T, et al. Comparative pharmacokinetics of celecoxib and mavacoxib in cockatiels (*Nymphicus hollandicus*). In: Proceedings Avian Herpetological and Exotic Mammal Medicine, 2nd International Conference, 2015; April;400; Paris, France.
9. Huckins, GL, Carpenter JW, Dias S, et al. Pharmacokinetics of oral mavacoxib in Caribbean flamingos (*Phoenicopterus ruber ruber*). *J Zoo Wildl Med.* 2020;51:53–58. doi:10.1638/2019-0161
10. Montesinos A, Ardiaca M, De Baere S, et al. Population pharmacokinetics of mavacoxib after oral administration of a single dose to African grey parrots (*Psittacus erithacus*). In: Abstracts. 4th Annual Avian Herpetological and Exotic Mammal Medicine; 2019; London. doi:10.2460/ajvr.80.2.201
11. Leineweber C, Müller E, Marschang RE. Blood reference intervals for rabbits (*Oryctolagus cuniculus*) from

routine diagnostic samples. *Tierarztl Prax Ausg K Kleintiere Heimtiere.* 2018;46:393–398. doi:10.1055/s-0038-1677403
12. Melillo A. Rabbit clinical pathology. *J Exot Pet Med.* 2007;16:135–145. doi:10.1053/j.jepm.2007.06.002
13. Fisher P, Graham JE. Rabbits. In: Carpenter JW, Harms CA, eds. *Carpenter's exotic animal formulary.* 6th ed. St Louis: Elsevier, 2023;603–606.
14. Curry SL, Cogar SM, Cook JL. Nonsteroidal anti-inflammatory drugs: a review. *J Am Anim Hosp Assoc.* 2005;41:298–309. doi:10.5326/0410298
15. 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.