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PHARMACOKINETICS OF KETOPROFEN IN THE GREEN IGUANA (*IGUANA IGUANA*) FOLLOWING SINGLE INTRAVENOUS AND INTRAMUSCULAR INJECTIONS

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Abstract: The nonsteroidal antiinflammatory drug ketoprofen (KTP) is a commonly used antiinflammatory and analgesic agent in reptile medicine, but no studies documenting its pharmacokinetics in this species have been published. Ketoprofen was administered as a racemic mixture to green iguanas (*Iguana iguana*) intravenously (i.v.) and intramuscularly (i.m.) at 2 mg/kg. Pharmacokinetic analyses were performed and indicated that ketoprofen in iguanas administered by the intravenous route has a classical two-compartmental distribution pattern, a slow clearance (67 ml/kg/hr) and a long terminal half-life (31 hr) compared to ketoprofen studies reported in mammals. When delivered by the intramuscular route, bioavailability was 78%. These data indicate the daily dosing that is generally recommended for reptile patients, as an extrapolation from mammalian data, may be more frequent than necessary.

Key words: Analgesia, green iguana (*Iguana iguana*), ketoprofen, pharmacokinetics, NSAID, reptile.

Ketoprofen [(±)2-(3-benzoylphenyl) propionic acid] is a nonsteroidal antiinflammatory drug (NSAID) that belongs in the 2-arylpropionic acid class. Its method of action is to block access of arachidonic acid to its binding site on the cyclooxygenase enzyme. Ketoprofen is a chiral compound of two enantiomers, R(−) and S(+). The product that is available for veterinary use is a racemic mixture (50:50) of both enantiomers.[8] Reports describing pharmacokinetics and pharmacodynamics of ketoprofen in domestic and nondomestic species have resulted in widespread use of ketoprofen as an analgesic and antiinflammatory agent in veterinary medicine.[1,6,7,9] Despite common use in reptiles for treatment of musculoskeletal pain and to reduce inflammation, no published ketoprofen pharmacokinetic data in any reptile species are available.

Ketoprofen is a nonselective inhibitor of cyclooxygenase (COX), which has isoenzymes COX-1, COX-2, and a newly discovered COX-3, which may be a subset of COX-1.[2,12] Reptiles undergoing an inflammatory response have been reported to respond by upregulation of the COX-1 receptors over the COX-2 receptors (Lillian Royal, pers. comm.). Therefore, drugs with a higher COX-2 specificity may be less effective in reptiles for control of inflammation than nonselective COX inhibitors. In reptiles, ketoprofen is used off-label at 2 mg/kg i.m. once daily, based on extrapolation from mammalian dosages.[3] However, anatomic and metabolic differences in reptiles and mammals could make extrapolations ineffective or dangerous, resulting in some adverse effects that have been reported in mammals, including surgical site bleeding, gastric ulceration, acute renal failure, and pruritus.[8,10,11]

The purpose of this pilot study was to determine the pharmacokinetics of ketoprofen following intramuscular and intravenous administration at 2 mg/kg in green iguanas (*Iguana iguana*) undergoing gonadectomy. Because it was a pilot study, a chiral assay to differentiate the R(−) and S(+) isomer was not performed. In addition, as a pilot study, we used a pooled sampling strategy whereby time points were pooled to obtain a complete time–concentration profile.

Twelve green iguanas—six males and six females—weighing from 0.68 to 6.5 kg, were subjects. The iguanas were owned by a local iguana rescue league. They were maintained in individual cages at 25.5°C, and provided a basking light for behavioral thermoregulation. Animals were fed a fruit and vegetable salad daily with water access ad libitum. All iguanas were judged to be healthy based on a physical examination, complete blood count, and plasma chemistry profile.

The iguanas were randomly split into two groups of six. A baseline blood sample of 0.6 ml was collected from each iguana from the lateral tail vein.

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Following induction of anesthesia with isoflurane gas (Attane; Minrad, Inc., Buffalo, New York 14202, USA), one group received ketoprofen (Ketofen; Aveco Company, Fort Dodge, Iowa 50501, USA) at 2 mg/kg i.v. via a catheter in the lateral tail vein and the other group received the same dose i.m. in the left thigh. Blood samples (0.6 ml) were collected at 2, 4, 6, 8, 12, 16, 24, 48, 60, and 72 hr following the dose administration. Samples were collected from three of six animals from each group at each time point and animals were alternated such that each one was sampled at every other time point. Blood samples could not be collected prior to 2-hr postdose because of concurrent gonadectomy during this period. Samples were immediately centrifuged at 13,000 *g* for 6 min and the plasma was removed and frozen at −70°C until analysis.

Plasma samples were analyzed by reverse-phase high-performance liquid chromatography (HPLC) with the use of a method developed in our laboratory. There was insufficient plasma to develop an assay for the chiral isomers of ketoprofen. Therefore, the total (racemic) concentrations are reported.

A reference standard of racemic ketoprofen was obtained from the United States Pharmacopeia (USP, Rockville, MD 20852, USA). Ketoprofen was dissolved in 100% methanol to compose a 1 mg/ml stock solution, from which further dilutions were made in distilled water:methanol (1:1 v/v) as fortifying solutions for plasma. Blank (control) plasma ketoprofen solutions were added to make up nine calibration standards (including zero and ranging from 0.05 μg/ml to 10 μg/ml).

The mobile phase for HPLC analysis consisted of 0.02 M potassium dihydrogen phosphate buffer (75%) and acetonitrile (25%). One milliliter of triethylamine (TEA) was added to each liter of mobile phase to improve peak shape. The pH of the buffer was adjusted to 3.5 with 85% phosphoric acid. Fresh mobile phase was prepared daily.

The HPLC system consisted of a quaternary solvent delivery system (Agilent Technologies, Wilmington, DE 19805, USA) at a flow rate of 1 ml/min, an autosampler (1100 Series Autosampler, Agilent Technologies, Wilmington, DE 19805, USA), and an UV detector set at a wavelength of 255 nm (1100 Series Autosampler, Agilent Technologies). The column was a reverse-phase, 4.6 mm × 15 cm C8 column (Zorbax) and kept at a constant temperature of 40°C.

All plasma samples, calibration samples, and blank (control) plasma samples were prepared identically. Solid-phase HLB extraction cartridges (Oasis HLB solid-phase extraction cartridges, Waters Corporation, Milford, MA, 01757, USA) were conditioned with 1 ml methanol followed by a wash with 1 ml distilled water. Each plasma sample was added to a conditioned cartridge, followed by a wash with 1 ml distilled water:methanol (80:20). The drug was eluted with 1 ml 100% methanol and collected in clean glass tubes. The tubes were evaporated under a stream of air at 40°C for 15 min. Each tube was then reconstituted with 200 μl of mobile phase and vortexed. Fifty microliters of each tube was injected into the HPLC system. Retention time for peak of interest was 4.5–5.5 min. All calibration curves were linear with $R^2 \geq 0.99$. Limit of quantification for ketoprofen in iguana plasma was 0.05 μg/ml. Assay precision and accuracy were within 15% CV for precision and ±15% of the true value for accuracy.

We could not sample each individual often enough to perform a standard two-stage pharmacokinetic analysis. Therefore, we used the naïve average sampling method in which three samples collected in each group at each time point were used to derive the plasma concentration. The intravenous plasma ketoprofen concentration vs. time data were analyzed by nonlinear regression analysis with a two-compartment model with the use of computer software (WinNonlin Version 4.0, Pharsight Corporation, Mountain View, CA 94043, USA). The data were weighted for heavier emphasis of lower points. The compartmental parameters calculated for the intravenous route included A and B intercepts, alpha and beta (distribution and terminal rate constants, respectively), area under the plasma concentration vs. time curve from zero to infinity (AUC), area under the moment curve (AUMC), mean residence time (MRT), steady-state volume of distribution (V_{SS}), systemic clearance, and half-life ($T_{1/2}$) of the distribution and terminal phases. Equations for primary and secondary parameters used were obtained from published references.[5]

The intramuscular plasma ketoprofen concentration versus time data were analyzed with a noncompartmental model because there were insufficient data during the absorption phase for nonlinear regression analysis using compartmental methods. For noncompartmental analysis, the same computer program (WinNonlin Version 4.0) was used. Noncompartmental parameters calculated include AUC, AUMC, MRT, and the terminal $T_{1/2}$. From the plasma concentration vs. time curve, the maximum concentration (C_{MAX}) and time to reach C_{MAX} (T_{MAX}) were determined. The systemic availability (F) for intramuscular dosing was calculated from:

$$F = \text{AUC i.m.}/\text{AUC i.v.}$$

The time-concentration profile of ketoprofen

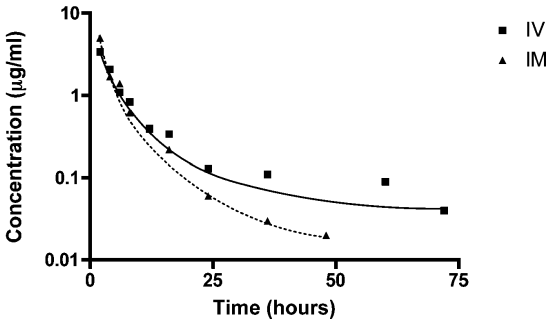

Figure 1. Plasma concentration profile (μg/ml) of ketoprofen in the green iguana after intravenous (■) and intramuscular (▲) administration of a single dose (2 mg/kg). The symbols represent the observed values and represent naïve averaged data from all animals sampled at a given time point. Each data point represents data from three animals. The lines indicate the curve predicted from calculated parameters.

Table 1. Pharmacokinetic parameters of ketoprofen in the green iguana.

Pharmacokinetic parameter	IV dosing (compartmental analysis)	IM dosing (noncompartmental analysis)
A (μg/ml)	5.0	NA
α (1/hr)	0.3	NA
B (μg/ml)	0.2	NA
β (1/hr)	0.02	NA
$T_{1/2\alpha}$ (hr)	2.7	NA
$T_{1/2\beta}$ (hr)	31	8.3
AUC (hr* μg/ml)	30	23
AUMC (hr* hr*μg/ml)	531	190
MRT (hr)	18	8.2
V_{ss} (ml/kg)	1,195	NA
F (%)	100	78
Clearance (ml/kg/hr)	67	NA

plasma concentrations for i.v. and i.m. dosing is shown in Figure 1. The calculated pharmacokinetic parameters for ketoprofen administered by the i.v and i.m. route are presented in Table 1.

Several factors affect interpretation of the data, and we are therefore hesitant to extrapolate unduly from the results. Anesthesia and surgery make this a realistic indication for ketoprofen use, but results may not apply well to other situations. The lack of a time point prior to 2 hr postadministration may affect the pharmacokinetic analysis for the i.v. study. Likewise, earlier time points on the i.m. curve could have identified a higher C_{MAX} value and allowed a compartmental analysis that could have identified an absorption curve. In addition, the results reported in this article represent both forms in a racemic mixture. In most mammals, it is the S(+) enantiomer that is more active, and differences in clearance between enantiomers and chiral inversion may occur.[9] It is not known which isomer is more active in reptiles.

Despite the limitations of our study, we observed that ketoprofen, after i.v. administration, showed a classical two-compartmental distribution pattern (Fig. 1) with a slow clearance and a long terminal half-life compared to ketoprofen studies in mammals and birds.[4,6,7] The apparent volume of distribution (VD) was higher than 1 L/kg, which is common for the highly lipophilic NSAIDs. Some NSAIDs have lower VD values because they are highly protein bound in plasma and distribution is limited; however, we lacked sufficient blank plasma to perform protein binding studies.

After i.m. administration, we observed a peak that was as high as the highest point from i.v. administration and systemic absorption of 78%. Although the terminal half-life after i.m. injection was shorter than the i.v. study, the noncompartmental analysis and sampling strategy of the i.m. data limited conclusions. Although predicting a dosage regimen is beyond the scope of this study, it is reasonable to conclude that an ideal dose frequency may be less in iguanas than mammals for a given dose.

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