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Ketoprofen pharmacokinetics of *R*- and *S*-isomers in juvenile loggerhead sea turtles (*Caretta caretta*) after single intravenous and single- and multidose intramuscular administration

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Ketoprofen is a nonsteroidal anti-inflammatory and analgesic agent that nonselectively inhibits cyclooxygenase, with both COX-1 and COX-2 inhibition. Recent studies on COX receptor expression in reptiles suggest that nonselective COX inhibitors may be more appropriate than more selective inhibitors in some reptiles, but few pharmacokinetic studies are available. The goal of this study was to determine single- and multidose (three consecutive days) pharmacokinetics of racemic ketoprofen administered intravenously and intramuscularly at 2 mg/kg in healthy juvenile loggerhead turtles (*Caretta caretta*). The *S*-isomer is the predominant isomer in loggerhead sea turtles, similar to most mammals, despite administration of a 50:50 racemic mixture. Multidose ketoprofen administration demonstrated no bioaccumulation; therefore, once-daily dosing will not require dose adjustment over time. *S*-isomer pharmacokinetic parameters determined in this study were C_{max} of 10.1 μg/ml by IM injection, C_0 of 13.4 μg/ml by IV injection, AUC of 44.7 or 69.4 μg*hr/ml by IM or IV injection, respectively, and *T*½ of 2.8 or 3.6 hr by IM or IV injection, respectively. Total ketoprofen plasma concentrations were maintained for at least 12 hr above concentrations determined to be effective for rats and humans. A dose of 2 mg/kg either IM or IV every 24 hr is likely appropriate for loggerhead turtles.

1 | INTRODUCTION

Pharmacokinetic studies of analgesics in reptiles are limited despite the widespread and routine use of these medications. A 2004 questionnaire evaluated the use of nonsteroidal anti-inflammatory drugs (NSAIDs) in reptiles by veterinarians and found that 45% of participants used them routinely in patients (Read, 2004). A large portion (40%) were also in support of future pharmacokinetic research in analgesics in different reptile species to increase the knowledge of appropriate doses and dosing intervals (Read, 2004).

Sea turtles commonly present to rehabilitation centers with traumatic injuries, ocular abnormalities, and fishery interactions (fish hooks and entanglements) (Higgins, 2003). These conditions may necessitate medical and/or surgical management, which often includes providing appropriate anti-inflammatory and analgesia treatment. The anti-inflammatory and analgesic effects of NSAIDS are mediated

through the inhibition of the enzyme cyclooxygenase (COX) (Duncan, 2012; Mosley, 2005; Sladky & Mans, 2012; Storms & Klaphake, 2005). Previous pharmacokinetic studies in loggerhead turtles (*Caretta caretta*) evaluating meloxicam, a COX-2 selective NSAID, at 0.1 mg/kg IM and IV and 0.2 mg/kg IV, found low plasma concentrations with more rapid elimination than in mammals; therefore, the authors did not recommend meloxicam at those doses for sea turtles (Claus et al., 2007; Lai et al., 2015). Studies of COX-1 and COX-2 expression in eastern box turtles (*Terrapene carolina carolina*) (Royal, Lascelles, Lewbart, Correa, & Jones, 2012) and ball pythons (*Python regius*) (Sadler et al., 2016) suggest the possibility that a nonselective NSAID with both COX-1 and COX-2 activity, such as ketoprofen, may be more efficacious in controlling pain and inflammation in chelonians than a COX-2 selective drug. Ketoprofen also has the advantage in the United States and some other countries of having a commercially available veterinary formulation for injection at a concentration convenient for larger turtles (Ketofen®, 100 mg/ml solution). Furthermore, ketoprofen administered at 2 mg/kg IM and IV in the green iguana (*Iguana iguana*) exhibited slower elimination than in most mammals (Tuttle et al., 2006), suggesting the possibility of conveniently long dosing intervals in sea turtles.

The goal of this study was to determine the pharmacokinetics of ketoprofen in healthy juvenile loggerhead turtles. The study was divided into three phases. The first phase was an opportunistic pilot study based on sampling two loggerheads. The second consisted of a single dose of ketoprofen administered IM or IV at a dose of 2 mg/kg. The third portion of the study examined plasma drug concentrations with multiple doses of ketoprofen administered IM at 2 mg/kg every 24 hr for three consecutive days.

2 | MATERIALS AND METHODS

2.1 | Pilot study

A pilot study was conducted in two juvenile loggerheads to establish the method of sampling, identify optimal blood sampling times, assess safety of ketoprofen administration, and test the assay to be used in the main study. The two loggerheads were completing rehabilitation at the North Carolina Aquarium on Roanoke Island, Sea Turtle Assistance and Rehabilitation Center. Procedures were approved by the North Carolina Wildlife Resources Commission, the NC State IACUC, and the NC Aquariums IACUC.

2.2 | Main study animals

The animals used in the main study were 18- to 20-month-old juvenile loggerheads housed under conditions previously described (Higgins, 2003) at the National Marine Fisheries Service (NMFS) laboratory in Galveston, Texas, in 2016. All procedures were approved by the Florida Fish and Wildlife Conservation Commission, NC State IACUC; FWCC permit MTP#16-015A; and USFWS permit TE-676379-5; and followed protocols of the US National Marine Fisheries Service (http://www.sefsc.noaa.gov/turtles/TM_579_SEFSC_STRTM.pdf). Prior to enrollment in the study, all turtles were weighed and measured in straight carapace length and straight carapace width. All sea turtles were housed in 11,300-L raceways divided into 14 individual enclosures suspended in the water (Higgins, 2003). Enclosures were circular vinyl-coated wire mesh and measured 76 cm diameter and 45 cm depth. Filtered seawater was pumped into the facility from the Gulf of Mexico. The average daily water temperature was 29.5°C (range 29–30°C), and average salinity was 25 ppt. Turtles were fed 1% of body weight daily, divided into two feedings, of Purina AquaMax Sport Fish 500 4.8-mm (3/16-inch) floating pellets (PMI Nutrition International, LLC, Brentwood, Missouri).

2.3 | Sample collection

Blood collection was performed alternating between the left and right dorsal cervical sinuses (external jugular vein) with a 3-ml syringe and 22-gauge needle that was rinsed with 0.1 ml of 1,000 IU/ml sodium heparin solution. The 1.5 ml of blood collected at each time point was placed into a polyethylene microcentrifuge tube and placed on ice. The blood was centrifuged at 8,000 *g* no longer than 1 hr after collection. The plasma was then collected, placed in a cryovial, stored at −80°C, shipped overnight on dry ice to North Carolina State University, and then stored at −80°C until high-pressure liquid chromatography (HPLC) analysis. All samples were analyzed within 4 months of collection.

2.4 | Single dose

Turtles from a single raceway, comprised of turtles collected from the same nest, were randomly selected using a handheld calculator pseudorandom number generator (Casio *fx-260Solar*, Dover, New Jersey) and assigned to groups for the single-dose portion of the study; six were placed in the IM and six in the IV treatment group. For the six turtles in the IM single-dose study, the means and ranges were as follows: weight: 3.72 kg (3.58–3.89) kg; straight carapace length (SCL): 30.9 (30.3–31.5) cm; and straight carapace width (SCW): 25.12 (24.3–26.0) cm. For the six turtles in the IV single-dose study, the means and ranges were as follows: weight: 3.72 (3.41–4.06) kg; SCL: 30.9 (30.1–32.0) cm; and SCW: 25.2 (24.8–25.9) cm. Turtles were administered ketoprofen 2 mg/kg at time 0, either IM in the pectoral muscles (undiluted 100 mg/ml ketoprofen) or IV via the dorsal cervical sinuses (ketoprofen diluted to 25 mg/ml solution with sterile water), based on their respective group assignment. Diluted ketoprofen was used for IV administration to increase the dose volume and minimize the effect of blood diluting the small volume of drug within the hub of the needle when determining proper IV placement, which could otherwise alter the intended dose. Concentration of the diluted ketoprofen was verified by HPLC analysis. Blood samples were collected at the following times; −24 hr, 0, 25 min, 30 min, and 1, 2, 4, 8, 12, 24, and 48 hr. In each group, all turtles were sampled at −24 hr and time 0, and then, three turtles in each group were sampled at 25 min and 1, 4, 12, and 48 hr and the other three turtles at 30 min and 2, 8, and 24 hr.

2.5 | Multidose

Sea turtles undergoing laparoscopic sex determination for an unrelated study were used for the multidose portion of the study. A single dose of ketoprofen was used for postoperative analgesia for all turtles in that study, but was extended to three doses for the pharmacokinetic multidose study. The first six turtles that received laparoscopies were selected. For the six turtles in the multidose study, the means and ranges were as follows: weight: 3.07 (2.94–3.18) kg; SCL: 29.62 (29.1–30.5) cm; and SCW: 23.88 (22.8–24.3) cm. All turtles were housed in the same raceway and were collected from the same nest, different from the single-dose portion of the study. Laparoscopies and anesthesia were performed as previously described (MacLean, Harms, & Braun-McNeill, 2008; NMFS/SEFSC Sea Turtle Research Techniques Manual http://www.sefsc.noaa.gov/turtles/TM_579_SEFSC_STRTM.pdf, chapter 15), using short-acting general anesthesia with propofol (5 mg/kg IV; Hospira, Inc., Lake Forest, IL) (MacLean et al., 2008) and a lidocaine (Lidocaine 2%, 2 mg/kg intradermal and subcutaneous; Hospira, Inc., Lake Forest IL) local anesthetic block. Additional treatments included preoperative oxytetracycline (Bio-Mycin 25 mg/kg IM; Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) and intraoperative fluids (sterile saline 0.9%, 20 ml/kg ICe; MWI, Boise, ID). Turtles were administered ketoprofen (Ketofen 100 mg/ml, 2 mg/kg IM in pectoral muscles) at time 0 (completion of laparoscopy procedure) and at 24 and 48 hr postoperatively. The blood collection times occurred at 0, 1, 24, 25, 48, 49, and 72 hr after initial injection. Blood samples for times 0, 24, and 48 hr were collected just prior to ketoprofen administration.

2.6 | Ketoprofen analysis

Ketoprofen in turtle plasma was analyzed by reverse-phase HPLC with UV detection using a method developed in the North Carolina State College of Veterinary Medicine Clinical Pharmacology Laboratory. Separation of the *R*- and *S*-isomers of ketoprofen was accomplished with a chiral HPLC column (Ultron ES-OVM, 4.6 × 150 mm, 5 μm, manufactured for Agilent Technologies by Shinwa Chemical Industries Ltd, Japan), kept at a constant temperature of 25°C. This column is specially designed for the separation of chiral isomers (enantiomeric compounds). The mobile phase was 89% potassium monobasic phosphate buffer and 11% acetonitrile, run in isocratic mode at 1 ml/min. The wavelength for detection was 255 nm. Retention times were approximately 16.5 min for the *S*-enantiomer and 17.5 min for the *R*-enantiomer.

Calibration samples and quality control (QC) samples were prepared by fortifying (spiking) blank loggerhead plasma with solution containing reference standards of ketoprofen. A reference standard of pure dexketoprofen tromethamine (S-ketoprofen) was purchased from the United States Pharmacopeia (www.USP.org) (USP, Rockville, MD). Racemic ketoprofen (R and S) was purchased from Sigma Chemical Company (St. Louis, MO). The addition of dexketoprofen was used to verify the elution order of the enantiomers in our chromatograms. Reference solutions were prepared by dissolving a pure (>99% purity) analytical reference standard of ketoprofen into 100% methanol. Further dilutions were performed in 50:50 methanol/water solution. These calibration solutions were used to prepare a range of eight calibration and QC samples ranging from 0.05 to 50 μg/ml. Blank (control) loggerhead plasma samples were also analyzed with each day's run to check for interfering peaks and estimate background noise. All calibration curves were linear with an R^2 value of 0.99 or greater. Limit of quantification (LOQ) for ketoprofen isomers in turtle plasma was 0.05 μg/ml, which was determined from the lowest point on a linear calibration curve that produced an acceptable signal-to-noise ratio and met acceptance criteria of our previously validated assay, and in compliance with the ICH Harmonised Tripartite Guideline (available from https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q2_R1/Step4/Q2_R1__Guideline.pdf.). The accuracy and precision of the assay was determined by analyzing replicate samples at high (10 μg/ml), medium (1 μg/ml), and low (0.1 μg/ml) concentrations. Accuracy was within 8% or less at all concentrations for the R- and S-enantiomer. Precision was less than 7.5% CV at all concentrations for the R- and S-enantiomer.

Calibration plasma samples, QC samples, and all incurred samples were prepared in the same manner. A solid-phase extraction cartridge (Waters Oasis MAX 3 cc cartridge, Milford, MA) was conditioned with water and methanol, followed by addition of a plasma sample of 400 μl. After a wash step of 95:5 water/ammonium hydroxide, the sample was eluted with 98:2 methanol/formic acid. The sample was evaporated to dryness, reconstituted with 200 μl water, vortexed, and injected into the HPLC system. The system consisted of an Agilent 1100 series quaternary solvent delivery pump, an Agilent 1100 series autosampler providing a 30 μl injection, Agilent 1200 series UV detector, and Agilent OpenLAB Software Suite (all from Agilent Technologies, 5301 Stevens Creek Blvd., Santa Clara, CA 95051).

2.7 | Pharmacokinetic analysis

The plasma drug concentrations for each isomer of ketoprofen (*R* and *S*) were analyzed using a population pharmacokinetic method that allows for analysis when sparse sampling is used for collection. Initial estimates of the pharmacokinetic parameters for IM and IV drug administration were determined using the Phoenix pharmacokinetic software (Phoenix WinNonlin/NLME software; Pharsight Corporation, Certara, St. Louis, MO). The concentrations of the *R*- and *S*-isomer of ketoprofen after each administration were plotted to visualize the most appropriate model for these data (Figure 1). Then, initial estimates of parameters were determined using naïve averaged samples (pooled samples) in which a pharmacokinetic model was fit to the average concentration at each time point. This model determines the best initial estimate for primary pharmacokinetic parameters to be used for the population pharmacokinetic method. These animals could not be sampled as frequently as large domestic animals in which traditional standard two-stage (STS) pharmacokinetic methods are used. Instead, a sparse sampling strategy was designed so that each animal was sampled three or four times to cover a wide range of time points from 0 to 48 hr. Population pharmacokinetics (Pop-PK) was

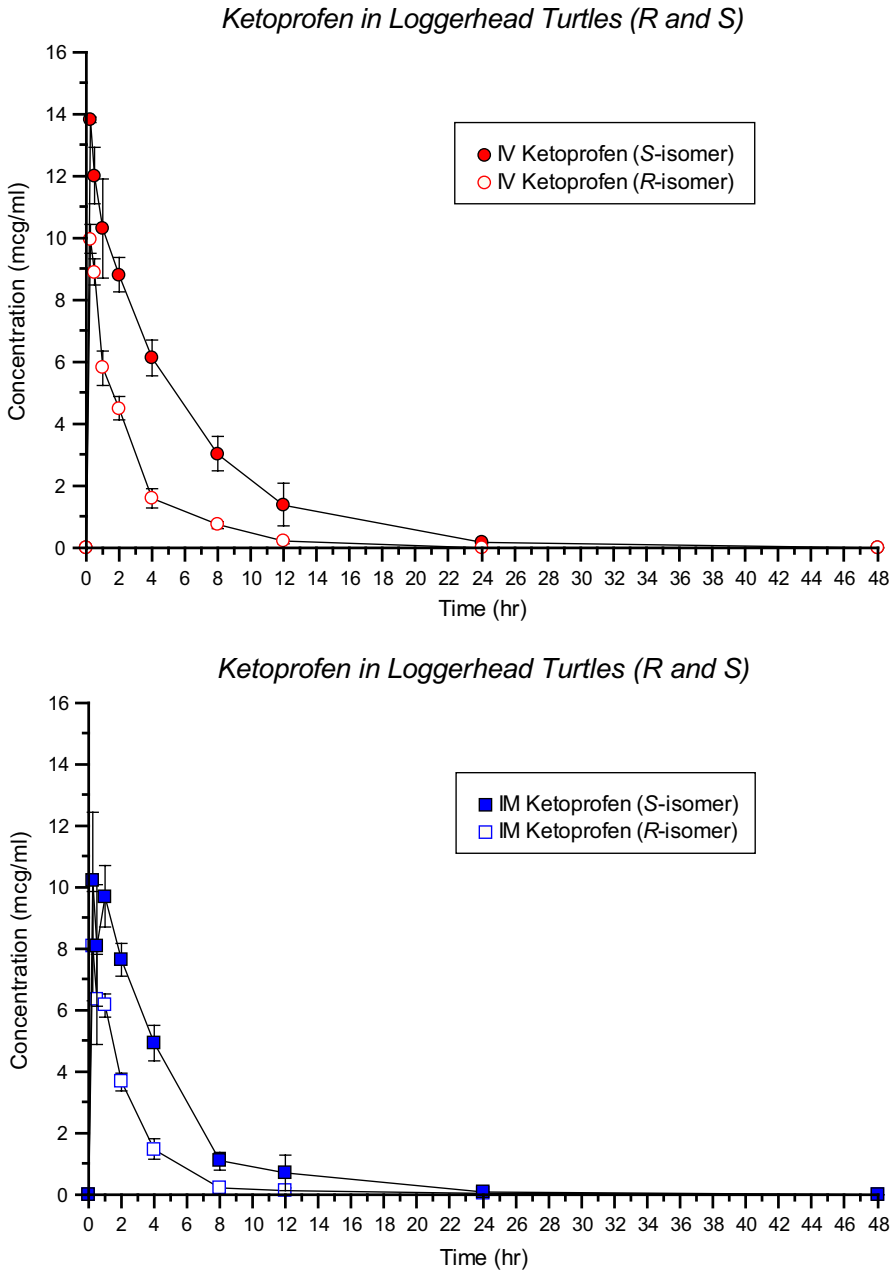

FIGURE 1 Single-dose ketoprofen administered to loggerhead turtles, 2 mg/kg IV and IM. Top panel: IV; bottom panel: IM. Each point represents the mean (±*SD*). Open symbols are the *R*-isomer, and closed symbols are the *S*-isomer [Colour figure can be viewed at wileyonlinelibrary.com]

performed by fitting the concentrations to a model using nonlinear mixed-effects (NLME) modeling and the Phoenix software (Phoenix® NLME™ software, Certara, St. Louis, MO). This analysis allowed for a population-based approach in which the primary pharmacokinetic parameters for the population were considered fixed effects and the interindividual (between-subject) variability was modeled as a random effect. The remaining differences between predicted concentrations and measurements were accounted for by residual errors (within-subject variation).

The IV model was parameterized with compartmental analysis of the data from injection of 2 mg/kg using a one-compartment model with first-order absorption and the following formula (Model 1 in Phoenix):

$$C(T) = Ce^{-KeT} \quad (1)$$

where C is the plasma concentration at time T, e is the base of the natural logarithm, T is the time after injection, and Ke is the elimination phase rate constant (terminal phase). Secondary parameters calculated included the half-life ($T½$), area under the curve (AUC), volume of distribution (VD), mean residence time (MRT), and clearance (CL).

Compartmental analysis of the data from the IM injection was calculated using a one-compartment model with first-order absorption according to the following formula (Model 3 in Phoenix):

$$C(T) = \frac{Ka\,D}{V(Ka - Ke)} \times (e^{-KeT} - e^{-KaT}) \quad (2)$$

where C is the plasma concentration at time T, Ka is the absorption rate, assuming first-order absorption, Ke is the elimination rate constant, V is the apparent volume of distribution, and D is the dose.

Secondary parameters calculated from the model included the peak concentration (C_{max}), time to peak concentration (T_{max}), area under the plasma concentration versus time profile (AUC), and the respective absorption and terminal half-lives (*T*½).

The models were run with the Quasi-Random Parametric Expectation Maximization (QRPEM) engine in Phoenix, which is a member of a general class of NLME estimation procedures. Model selection was based on goodness-of-fit plots, statistical significance between models using -2LL (twice the negative log likelihood), AIC (Akaike information criterion), and coefficient of variation (CV%) of parameter estimates. Interindividual (between-subject) variability (variance of a parameter among different subjects) was expressed using an exponential error model according to the equation:

$$\mathrm{Pi} = \theta P \times \exp(\eta iP), \tag{3}$$

where *P* is the pharmacokinetic parameter of interest for the individual i; θ *P* is *theta*, or the typical value (fixed effect) for the population estimate of the parameter of interest; and ηiP is the η (*eta*) (random effect) for the interindividual (between-subject) differences of the parameter of interest. The η values were assumed to be independent and have a lognormal distribution with a mean of zero and variance of ω^2. A multiplicative model was used to describe the residual random variability (ε) of the data for once-daily dosing, where ε is the residual intrasubject (within-subject) variability with a mean of zero and a variance of σ^2, according to the equation:

$$C_{obs} = C_{pred} \times (1+\varepsilon), \tag{4}$$

where C_{obs} is the observed concentration for the individual and C_{pred} is the model predicted concentration plus the error value (ε).

3 | RESULTS

3.1 | Single dose

For the six turtles in the IM single-dose study, the PCV and TS means and ranges were as follows: PCV: 25.8 (24–27) % and TS: 2.4 (2.2–2.6) g/dl. At the 48-hr sample, there was no anemia present (PCV = 26.0–28.0%; TS = 2.2–2.4 g/dl). The sex of all turtles was determined as female via laparoscopy.

For the six turtles in the IV single-dose study, the PCV and TS means and ranges were as follows: PCV: 28.8 (28–30) %; and TS: 2.2 (2.0–2.3) g/dl. At the 48-hr sample, there was no anemia present (PCV = 26.5–30.5%; TS = 2.2–2.4 g/dl). The sex of five turtles was determined as female via laparoscopy, and the sex of one turtle was undetermined.

Plasma drug concentrations for each isomer of ketoprofen (*R* and *S*) are plotted in Figure 1 for each dose (mean ± *SD*). Figure 2 (IV dose) and Figure 3 (IM dose) represent the model fitted to the data for individuals (panels a and b) and for the population (panels c and d) after individual variation (random effects) was accounted for in the model. Pharmacokinetic parameters are shown in Table 1 for each isomer and each route of administration. Although a racemic mixture was

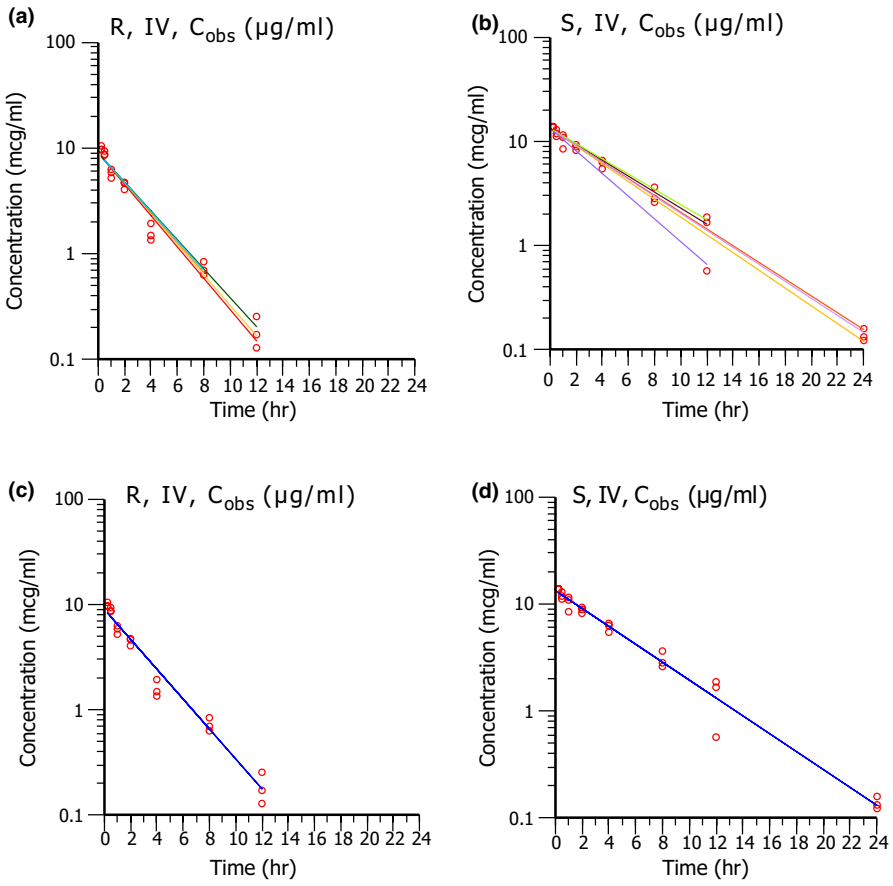

FIGURE 2 Single-dose ketoprofen administered to loggerhead turtles, 2 mg/kg IV, log scale. Panels a and b represent the plots for individual turtles, fitted to the model. (a and b) are the *R*- and *S*-isomer, respectively. Panels c and d represent all the turtles fitted to the model, adjusting for interindividual (between-subject) variation fitted to the model. (c and d) are the *R*- and *S*-isomer, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

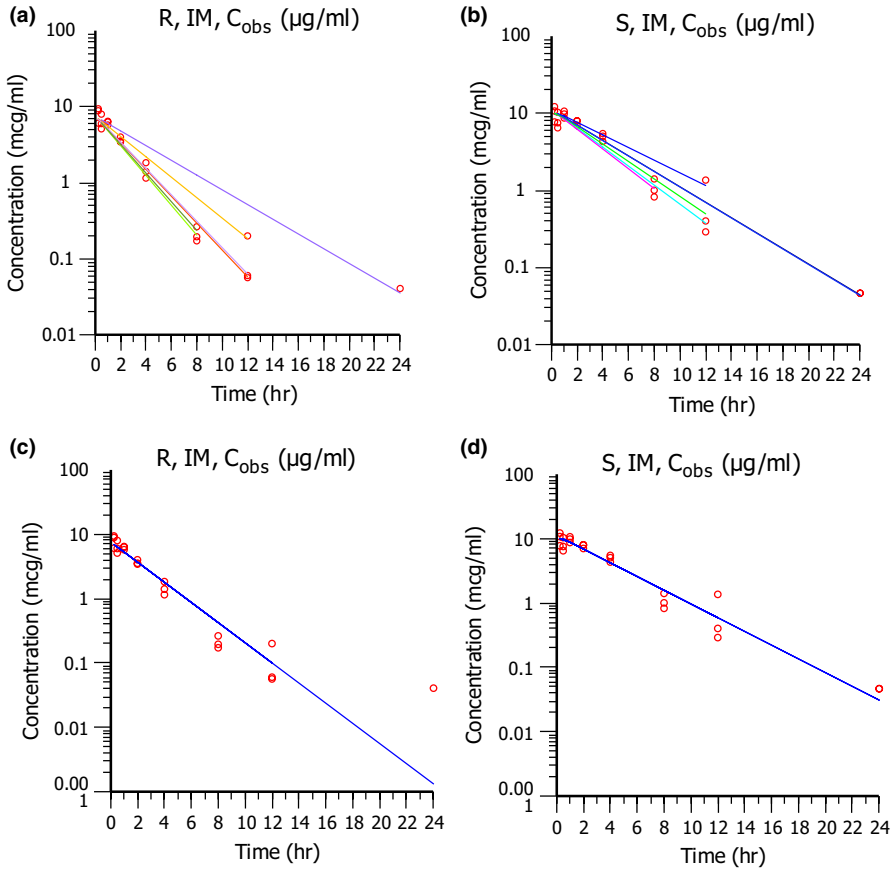

FIGURE 3 Single-dose ketoprofen administered to loggerhead turtles, 2 mg/kg IM, log scale. Panels a and b represent the plots for individual turtles, fitted to the model. (a and b) are the *R*- and *S*-isomer, respectively. Panels c and d represent all the turtles fitted to the model, adjusting for interindividual (between-subject) variation fitted to the model. (c and d) are the *R*- and *S*-isomer, respectively [Colour figure can be viewed at wileyonlinelibrary.com]

administered (equal *R*- and *S*-isomers in the injection formulation), the pharmacokinetics of each isomer were quite different. There was approximately a twofold difference (based on AUC) between isomers with concentrations of the *S*-isomer consistently higher than the *R*-isomer.

3.2 | Multidose

For the six turtles in the multidose study, the PCV and TS means and ranges were as follows: PCV: 28 (26–30) %; and TS: 2.1 (1.8–2.5) g/dl. The sex of the turtles was determined via laparoscopy to be three males, two females, and one undetermined. At the 48-hr sample, it was subjectively noted that one of the turtles, turtle 901, likely had a low PCV based on visual assessment of the spun blood sample, although PCV was not measured. Blood samples collected 6 days later confirmed the presence of anemia, PCV = 14%, in this turtle. All five other turtles at that time had PCV = 24–35%. Resolution of the anemia had occurred by the time of recheck PCV 14 days after the study, PCV = 28%.

Plasma drug concentrations at times 0, 1, 24, 25, 48, 49, and 72 hr after initial injection for each isomer of ketoprofen (*R* and *S*) are plotted in Figure 4 (mean ± *SD*).

4 | DISCUSSION

The pharmacokinetics in this study found substantial differences between the two enantiomers, *R*- and *S*-isomers, of ketoprofen when injected either IM or IV to loggerhead sea turtles (Table 1). The fraction of dose absorbed (*F*) measured from the AUC ratios was 0.75 for the *R*-isomer and 0.64 for the *S*-isomer. Half-lives were slightly longer for the *S*-isomer, and the clearance for the *R*-isomer was consistently faster than for the *S*-isomer. The absorption rate from the IM injection was rapid (Ka *T*½ less than 5 min) but was highly variable. The elimination (terminal) *T*½ was approximately 2 hr for the *R*-isomer from both routes, and 2.8 (IM) or 3.6 (IV) hr for the *S*-isomer. Thus, there is no evidence that route of administration prolongs the *T*½.

The reasons for the differences between isomers are undetermined without further study and an opportunity to administer each isomer separately. In many mammals, there is interconversion, most commonly with ketoprofen converted from the *R*-isomer to the *S*-isomer after injection (Lees, Landoni, Giraudel, & Toutain, 2004). The inversion of *R*- to *S*-isomer varies greatly between species, with humans having 8.9% inversion and horses 48.8% (Lees et al., 2004), while there is no inversion in cattle and llamas, and elephants may convert *S*- to *R*-ketoprofen (Hunter, Isaza, & Koch, 2003). The amount of inversion of *R*- to *S*-isomer in loggerhead sea turtles was not assessed in this study but may account for some of the variation in results.

In mammals, the *S*-isomer is considered more active (eutomer) for biologic activity (COX inhibition) (Plessers et al., 2014). It is unknown which isomer is more biologically active in sea turtles. The concentrations of the *S*-isomer needed for analgesia and suppression of inflammation are also not known in sea turtles. Based on human

TABLE 1 Pharmacokinetic parameters after 2 mg/kg administered either IM or IV to loggerhead turtles

IM Dose 2 mg/kg					IV Dose 2 mg/kg				
Parameter	**Estimate**	**Units**	***SE***	**CV%**	**Parameter**	**Estimate**	**Units**	***SE***	**CV%**
R-isomer					*R*-isomer				
Ka	21.71	1/hr	200.38	922.96	VD	0.11	L/kg	0.03	22.76
VD/*F*	0.13	L/kg	0.02	13.74	Ke	0.33	1/hr	0.03	8.30
Ke	0.36	1/hr	0.07	19.35	AUC	27.55	μg*hr/ml	4.20	15.26
T_{max}	0.19	hr	1.37	712.26	C0	9.01	μg/ml	2.05	22.76
AUC	20.67	μg*hr/ml	6.27	30.33	Cl	0.04	L/kg/hr	0.01	15.26
C_{max}	6.94	μg/ml	3.88	55.93	Ke *T*½	2.12	hr	0.18	8.30
Cl/*F*	0.05	L/kg/hr	0.01	30.33	MRT	3.06	hr	0.25	8.30
Ka *T*½	0.03	hr	0.29	922.96					
Ke *T*½	1.93	hr	0.37	19.35					
S-isomer					*S*-isomer				
Ka	11.35	1/hr	26.62	234.58	VD	0.07	L/kg	0.00	6.02
VD/*F*	0.09	L/kg	0.01	9.34	Ke	0.19	1/hr	0.02	7.86
Ke	0.25	1/hr	0.04	14.67	AUC	69.36	μg*hr/ml	8.89	12.81
T_{max}	0.35	hr	0.61	176.14	C0	13.37	μg/ml	0.80	6.02
AUC	44.68	μg*hr/ml	6.58	14.73	Cl	0.01	L/kg/hr	0.00	12.81
C_{max}	10.07	μg/ml	1.21	11.98	Ke *T*½	3.60	hr	0.28	7.86
Cl/*F*	0.02	l/kg/hr	0.00	14.73	MRT	5.19	hr	0.41	7.86
Ka *T*½	0.06	hr	0.14	234.58					
Ke *T*½	2.83	hr	0.41	14.67					

VD, apparent volume of distribution (corrected for F for IM dose); MRT, mean residence time; Ka, absorption rate constant, and associated half-life (*T*½); Ke, elimination rate, and associated half-life (*T*½); AUC, area under the curve; C_{max}, peak concentration; T_{max}, time to peak concentration; C0, concentration at time zero after IV dose; Cl, clearance (adjusted for *F* for IM dose). Fraction absorbed (*F*) from the IM dose was 0.75 for the *R*-isomer and 0.64 for the *S*-isomer (calculated from AUC ratios).

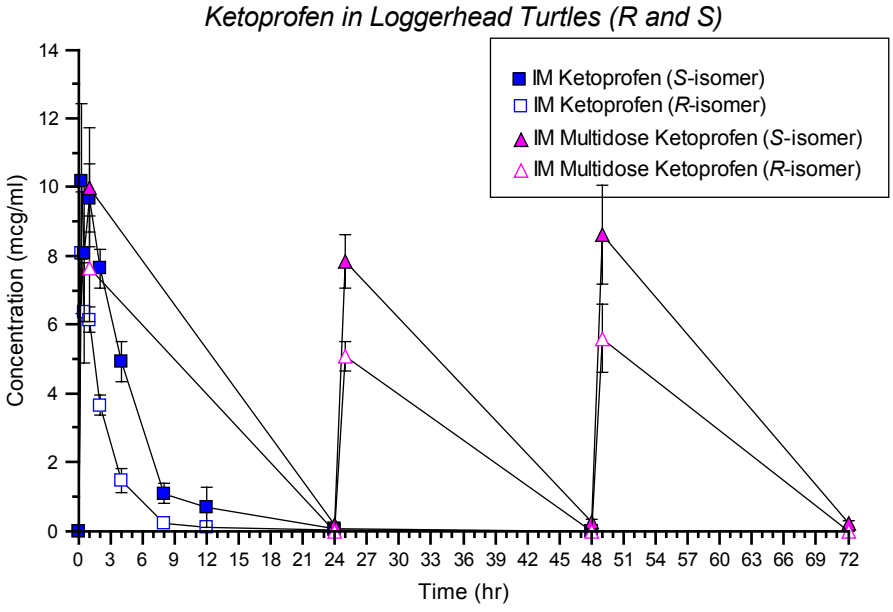

FIGURE 4 Multidose ketoprofen administered to loggerhead turtles, 2 mg/kg IM, with single-dose IM results from Figure 1 superimposed (square markers represent single-dose results, and triangles are multidose results). The three doses of ketoprofen were administered at times 0, 24, and 48 hr. Blood samples were collected at times 0, 1, 24, 25, 48, 49, and 72 hr. Each point represents the mean (±*SD*). Open symbols are the *R*-isomer, and closed symbols are the *S*-isomer [Colour figure can be viewed at wileyonlinelibrary.com]

orthopedic pain models developed in experimental domestic animals, *S*-isomer or total ketoprofen concentrations that were effective were in the range of 0.2–1 μg/ml (Jamali & Brocks, 1990; Kohler, Primbs, Morand, & Rubelt, 1985). Future nociception studies are needed to help establish NSAID doses that are effective at providing analgesia to reptiles.

The administration of ketoprofen in this study showed good absorption from the IM injection with the fraction of dose absorbed estimated to be approximately 75% for the *R*-isomer and 64% for the *S*-isomer. The *T*½ was not prolonged from the IM injection; therefore, there appears to be no flip-flop effect or evidence that the IM injection created a slow-release depot.

The volume of distribution (VD) recorded in this study was low, but typical for highly protein-bound NSAIDs. These NSAIDs are known to concentrate in inflamed sites, thus prolonging their duration of activity (Lees et al., 2004). Therefore, despite a *T*½ in this study of 2.8 or 3.6 hr from the IM or IV injection, respectively, it is anticipated that this dose may provide an effect for at least a 24-hr duration as it has in mammalian species [ketoprofen is commonly administered once daily in other animals, although frequency ranges from q 12 hr in rabbits and some birds (Carpenter, 2013); up to q 48 hr in elephants (Hunter et al., 2003)]. In green iguanas, ketoprofen had a *T*½ of 2.7 hr for IV injection, similar to the values found in this study in loggerhead sea turtles (Tuttle et al., 2006).

During the multidose study, drugs administered in addition to ketoprofen included propofol, oxytetracycline, and lidocaine. There may have been potential interactions between the drugs, or the metabolism, protein binding, or excretion of ketoprofen may have been affected. However, the protocol used in the multidose portion of the study most closely resembles typical application of ketoprofen in sea turtles in a clinical or research setting, such as the laparoscopies being performed here.

The cause of the anemia that occurred in one of 20 of the turtles remains unknown. This highlights the lack of knowledge of the side effects of repeated use of NSAIDS in sea turtles. Review of this animal's data from the laparoscopic surgery noted no significant hemorrhage, but bleeding may have occurred after closure of the incision. Drug-induced immune hemolytic anemia (DIIHA) is a rare condition and occurs in about 1 per million of the human population and has only very rarely been associated with an NSAID (Barbaryan et al., 2013; Johnson, Fueger, & Gottschall, 2007). An intravascular hemolytic cause, such as DIIHA, remains a low differential because the plasma remained clear rather than showing evidence of hemolysis. An extravascular cause of hemolysis may have been possible. Repeated blood collection from the sea turtle may have resulted in subcutaneous hematomas that were not easily visible. This sea turtle maintained a normal appetite and behavior and the anemia resolved quickly within the following weeks without any treatment. The remaining five turtles in the multidose study maintained adequate PCV throughout the study and showed little variation in their PCV on their initial and final blood samples.

In conclusion, after administration of a racemic mixture of ketoprofen, loggerhead sea turtles show enantioselective differences between the *R*- and *S*-isomers, with the *S*-isomer having consistently higher pharmacokinetic values. This study demonstrated favorable pharmacokinetic profiles of ketoprofen, administered via both IM and IV routes. No bioaccumulation of the drug occurs with repeated once-daily IM administration every 24 hr for three consecutive days (Figure 4). Further studies are needed to evaluate the safety and side effects of long-term repeated ketoprofen administration. The study suggests that administration of ketoprofen at 2 mg/kg once-daily dosing, either IM or IV, is an appropriate dose to select for future studies in loggerhead sea turtles.

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CONFLICT OF INTEREST

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