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Possibility of enterohepatic recycling of ketoprofen in dogs

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Abstract

Ketoprofen is mainly cleared by glucuronidation. The rate of glucuronidation of this compound has been demonstrated to be greater in dog than in human liver microsomes. Dog is the most common secondary nonprimate species used in drug metabolism studies in the pharmaceutical industry. Therefore, this study was undertaken to provide valuable information to pharmaceutical companies using dog as a model species for pharmacokinetic analyses when differences in glucuronidation occur across species for therapeutic drugs known to be extensively glucuronidated. The pharmacokinetics of ketoprofen was investigated after intravenous (0.27, 0.57 and 1.10 mg/kg) and oral administration of ketoprofen (∼10 mg/100 ml) of the racemate in dogs. Serial blood samples were collected at timed intervals for 7 and 24 h following intravenous and oral administration of the dose, respectively, and concentrations in plasma were determined by a sensitive and specific HPLC method. By comparing the $AUC_{0-\infty}$ following oral and intravenous administrations, ketoprofen bioavailability was ∼100%. A possibility of enterohepatic cycling of ketoprofen in dogs was proposed because of multiple peak phenomenon in the concentration–time profiles after intravenous and oral dosing was observed.
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Keywords: Ketoprofen; Enterohepatic circulation; Pharmacokinetics; Absorption

1. Introduction

Ketoprofen (2-(3-benzoylphenyl)-propionic acid), KET, is an effective inhibitor of cyclooxygenases and inhibits the synthesis of prostaglandins. Ketoprofen is clinically used in its racemic form in doses from 50 to 200 mg to treat rheumatic disorders and various non-rheumatic musculoskeletal joint diseases, and in lower doses from 12.5 to 25 mg, for mild to moderate pain and fever (Jamali and Brocks, 1990; Veys, 1991; Cashman, 1996; Brady et al., 1997). (*R*)-enantiomer was found to possess analgesic properties independent of prostaglandin synthesis inhibition (Wechter, 1998). In contrast to other arylpropionate derivatives, ketoprofen enantiomers undergo only limited chiral inversion (9–12%) in healthy subjects (Bannwarth et al., 1999).

In humans, ketoprofen follows a simple metabolic pathway (primarily glucuronidation) leading to the formation of an unstable glucuronic ester that is excreted in urine (Debruyne et al., 1987).

Drugs undergoing direct glucuronidation may be cleared more rapidly by dog liver than by human liver, possibly due to a greater efficiency/capacity of glucuronidation. Ketoprofen exhibits greater *in-vitro* CL_{int} (V_{max}/K_m) value in DLM (dog liver microsomes) (2.4 μl/min/mg) compared with HLM (human liver microsomes) (0.2 μl/min/mg). The greater CL_{int} value in DLM suggests that ketoprofen may be cleared more rapidly by dog liver than by human liver (Soars et al., 2001).

Traditionally, rodents such as the rat and nonprimate species such as the dog have been used as animal models in studies aimed at evaluating the pharmacodynamics, metabolism, pharmacokinetics and safety of new chemical entities.

Work carried out by other researches has shown that both ketoprofen enantiomers are extensively eliminated into the rat bile after their glucuronidation and thus, it is very probably also their intestinal reabsorption, suggesting the existence of enterohepatic circulation (EHC) of ketoprofen. Glucuronide of ketoprofen is hardly absorbed from the gastrointestinal tract, and the hydrolysis of the conjugate is needed before the reabsorption (as intact form) (Yasui et al., 1996).

The present study was undertaken to provide valuable information to pharmaceutical companies using dog as a model species for pharmacokinetic analyses when differences in glucuronidation occur across species for therapeutic drugs known to be extensively glucuronidated. Ketoprofen was administrated intravenously and orally to dogs at different doses, and the

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pharmacokinetics was investigated. A possibility of enterohepatic cycling of ketoprofen in dogs was proposed because of multiple peak phenomenon in the concentration–time profiles after intravenous and oral dosing was observed.

2. Materials and methods

2.1. Chemicals

Racemic ketoprofen was purchased from Sigma Chemical Co. (St. Louis, MO). HPLC-grade solvents used for HPLC analysis were obtained from Fisher Scientific Co. (St. Louis, MO). All other chemicals were of analytical grade and were used as received.

2.2. Animal preparation

Four adult Mongrel dogs, ranging in weight from 18 to 36 kg body weights and in age from 2 to 7 years, of both sexes, were used for the study, which was approved by the University of Michigan Committee on Use and Care of Animals. A randomized crossover study design was implemented. Before each administration of ketoprofen, the animals were fasted for 20 h but had free access to water. On each occasion, the appropriate dose was administered and the washout period between applications was at least 1 week for each dog. During the study, the animals were restrained in a dog sling and the back and foreleg shaved.

2.3. Dosage forms

For intravenous applications, the dosing solution was prepared by dissolving the compound in a solvent mixture of polyethylene glycol-400 and bacteriostatic 0.9% sodium chloride inj., USP, at a ratio of 50:50 (v/v). The dosing volume was 2 ml per dog. To insure sterility, all intravenous solutions were filtered through a 0.45 μm filter (Acrodisc® 13 GHP, Gelman Laboratory). For oral administration, the dosing solution of ketoprofen was prepared mixing ∼10-mg of ketoprofen with 100 ml of HPLC-grade water. The accurate concentration of the aqueous solution of ketoprofen was determined, after filtering the suspension through a 0.45 μm filter (Acrodisc® 13 GHP, Gelman Laboratory), by HPLC.

2.4. Pharmacokinetic studies

Pharmacokinetic parameters were calculated from plasma concentration–time data using Kinetica™ 1.1 software. For intravenous applications, three-way crossover experimental design in which four dogs received a single intravenous dose of ketoprofen via the femoral vein, at 0.27, 0.57, or 1.10 mg/kg dose levels, was performed. There was at least a 1-week washout period between successive treatment periods. Blood samples (approximately 2 ml) were collected via an indwelling catheter into heparinized vaccutainer tubes (contralateral to the iv administration) at predose, and 0.02, 0.05, 0.08, 0.17, 0.25, 0.50, 0.75, 1.00, 1.25, 1.75, 2.00, 2.75, 3.00, 4.00, 5.00, 6.00 and 7.00 h postdosing. The catheter was flushed with heparinized saline after each blood draw. For oral applications, 100 ml of aqueous solution containing ∼10 mg of ketoprofen was given by esophageal tube; after the administration, the tube was rinsed with 20 ml of water. Blood samples (2 ml) were collected into heparinized vaccutainer tubes before and at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 4.00, 5.00, 6.00, 7.00 and 24 h post-dose. Blood samples were taken from the femoral vein by means of an indwelling catheter. Twenty-four-hour samples were collected by individual venipuncture. Blood volume was replaced with normal saline. Plasma samples were separated by immediate centrifugation at 5000 rpm for 10 min at −4 °C and stored at −20 °C until the time of analysis.

2.5. Analytical method

2.5.1. Sample processing

Plasma samples were thawed at room temperature. To plasma aliquots (200-μl) were added 200 μl of acetonitrile to precipitate the proteins. The samples were then vortexed, centrifuged (16,000 rpm for 15 min at 4 °C); filtered through a 0.45 μm filter (Acrodisc® 13 GHP, Gelman Laboratory) and 30 or 50 μl of supernatant was injected and analyzed by HPLC.

2.5.2. Sample analysis

Ketoprofen was analyzed by an HPLC method. The chromatographic system consisted of a pump (model 501, Waters Associates, Milford, MA) operated at 1 ml/min. A sample processor (WISP Model 712, Waters Associates, Milford, MA), a variable wavelength UV detector (Spectroflow 783 Absorbance detector, Kratos analytical Instruments, Ramsy, NJ) set at 258 nm, connected to an integrator (HP 3396 Series II, HP Company, Avondale, PA). The mobile phase consisted of a mixture of acetonitrile and water, adjusted at pH 3.02 (35:65, v/v). The analytical column used was a LiChrospher® 100 RP-18 endcapped, 5 μm particle, size column (250 mm × 4.6 mm I.D.), preceded by a LiChroCART® guard column (4 mm × 4 mm) of the same packing material. The retention time of ketoprofen under these conditions was ∼17 min. The standard reference curves were obtained by adding known amounts of diluted stock standard to drug-free plasma. The injection volumes were 30 or 50 μl. The method limit of detection (LOD) was calculated from the calibration curves, area versus concentration, according to $LOD = 3.3\,\delta/S$; with δ being the standard deviation of intercepts of regression line and S being the slope of the calibration curve. The limits of quantification, defined here as $LOQ = 10\,\delta/S$, were determined on the basis of standard deviation of the response and the slope. The LOD were 0.43 and 0.23 μg/ml for calibration curves with 30 and 50 μl injection volumes, respectively. The lower limits of quantification were 1.20 and 0.70 μg/ml for calibration curves with 30 and 50 μl injection volumes, respectively. Detector linearity was determined by linear regression analyses (model $y = ax + b$) of eight level calibration curves. Ranges of concentrations were 0.71–24 μg/ml ($r^2 = 0.999$; 30 μl injection volume) and 0.71–6.00 μg/ml ($r^2 = 0.998$; 50 μl injection volume). During the assay of the study samples the intrabatch precision and

accuracy of the analytical procedure were evaluated after replicate analysis ($n = 3$) of control plasma samples spiked at three concentrations levels: 0.70, 6.00 and 24.00 μg/ml. The individual recovery of each quality control sample was calculated and the mean recovery was 107%.

2.6. Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using non-compartment methods. The maximum concentration (C_{max}) and time to reach C_{max} (T_{max}) were taken directly from the data. The area under the concentration versus time curve (AUC_{0-t}) was calculated using the linear trapezoidal method up to the last measurable time (C_{last}) and extrapolated to infinity ($AUC_{0-\infty}$) according to:

$$AUC_{0-\infty} = AUC_{0-t} + \frac{C_{last}}{\lambda_Z},$$

where λ_Z is the elimination rate constant calculated using least-squares regression of the logarithm of data points, which yielded a minimum mean square error. The area under the first moment–time curve ($AUMC_{0-\infty}$) was assessed by the trapezoidal approximation up to the last experimental measurement, with extrapolation to infinity using standard procedures. Mean residence time (MRT) was given by the $AUMC_{0-\infty}$ to $AUC_{0-\infty}$ ratio.

The elimination half-life ($t_{1/2}$) was calculated as: $t_{1/2} = 0.693/\lambda_Z$. The plasma clearance ($Cl_p$) was calculated as: $Cl_p = dose_{iv}/AUC_{0-\infty,iv}$. After intravenous administration, the steady-state volume of distribution (V_{ss}) was estimated from the MRT–Cl_p ratio. The absolute bioavailability (F) was calculated as: $F(\%) = (dose_{iv}/dose_{po}) \times (AUC_{0-\infty,po}/AUC_{0-\infty,iv}) \times 100$. Dose proportionality was assessed using a power analysis of $AUC_{0-\infty}$ and C_{max} versus dose, i.e. fitting the equation: $P = a \times dose^b$, where P presents the parameter ($AUC_{0-\infty}$ and C_{max}) and a and b are constants. If the system is linear, i.e. dose proportional, then $b \approx 1$.

2.7. Statistical analysis

Data were expressed as individual values or mean ± SD. Comparisons between groups were performed by one-way ANOVA. A p-value of 0.05 or less was considered significant.

3. Results

3.1. Intravenous administration

The mean plasma concentration–time profiles of ketoprofen at various doses after iv bolus injection of *rac*-ketoprofen are shown in Fig. 1. Pharmacokinetic parameters for iv administrations are listed in Table 1. Using one-way ANOVA, statistical comparisons of individual pharmacokinetic parameters such as λ_z, clearance and volume of distribution at steady state demonstrated no significant ($p > 0.05$) differences when each parameter was compared for the three test doses. Also, not significant differences were found for the AUC normalized to a ketoprofen dose of 1 mg/kg for the four dogs used in the study ($p > 0.05$).

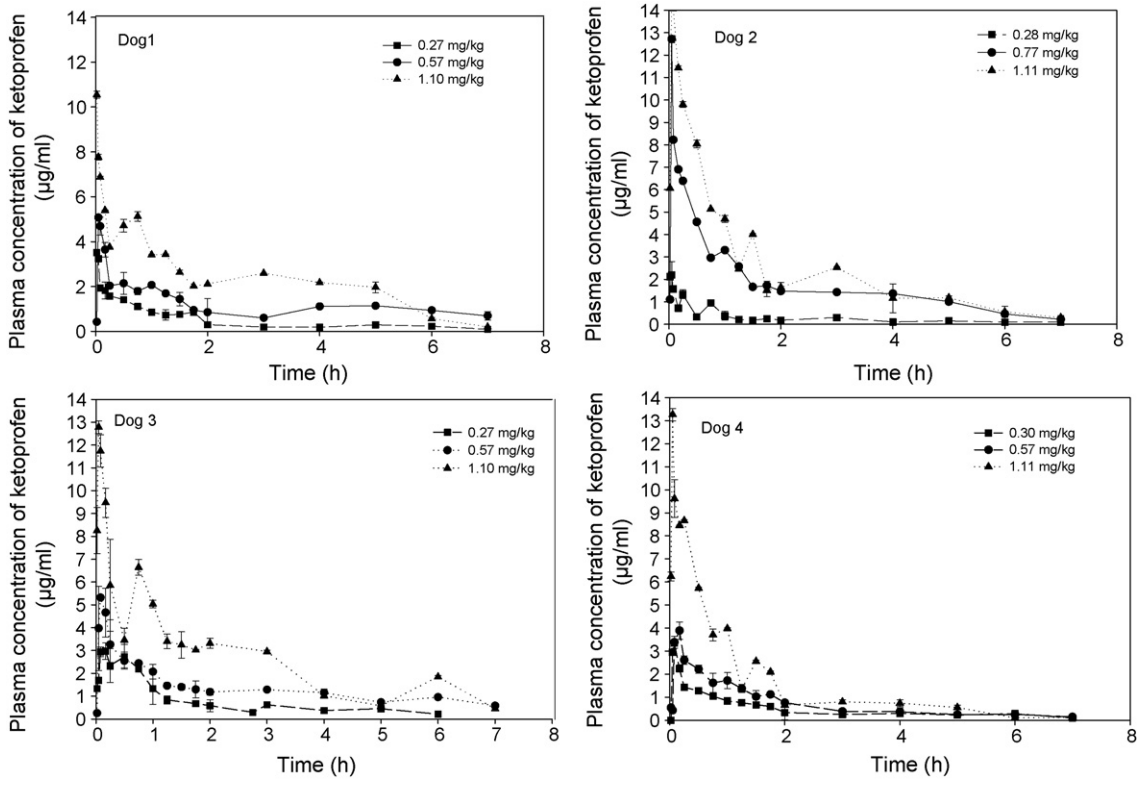

Fig. 1. Mean plasma concentration vs. time plots of ketoprofen racemate obtained after single intravenous administration of 0.27, 0.57 and 1.10 mg/kg doses to four dogs.

Table 1
Individual pharmacokinetic parameters from four dogs of ketoprofen racemate after intravenous administration

Parameter		Dose (mg/kg)				p-Value[a]
		0.27	0.57	1.10	1.0[b]	
C_{max} (μg/ml)	Mean ± S.D. (CV)	3.13 ± 0.22 (7.18)	5.15 ± 0.21 (4.07)	13.78 ± 1.21 (8.77)	10.45 ± 10.32 (3.05)	0.7499
$AUC_{0-\infty}$ (μg h/ml)	Mean ± S.D. (CV)	3.23 ± 0.15 (4.73)	10.87 ± 0.92 (8.46)	17.31 ± 1.16 (6.72)	15.06 ± 1.41 (9.34)	0.556
T_{max} (h)	Mean ± S.D. (CV)	0.08 ± 0.06 (81.01)	0.09 ± 0.06 (64.90)	0.04 ± 0.02 (35.29)		0.4359
λ_Z (h^{-1})	Mean ± S.D. (CV)	0.3257 ± 0.0671 (20.59)	0.3389 ± 0.1589 (46.89)	0.6145 ± 0.3674 (59.79)		0.1996
MRT (h)	Mean ± S.D. (CV)	2.77 ± 0.45 (16.17)	3.62 ± 1.49 (41.19)	2.20 ± 0.45 (20.36)		0.1539
V_{ss} (ml/kg)	Mean ± S.D. (CV)	250.22 ± 139.86 (55.90)	220.93 ± 55.40 (25.08)	154.46 ± 16.06 (10.39)		0.3287
Cl_p (ml/h/kg)	Mean ± S.D. (CV)	86.89 ± 33.65 (38.73)	66.93 ± 26.39 (39.44)	154.46 ± 16.06 (10.39)		0.3287
$t_{1/2}$ (h)	Mean ± S.D. (CV)	2.11 ± 0.50 (23.54)	2.29 ± 1.10 (47.89)	1.36 ± 0.52 (38.53)		0.2372

[a] Significant difference at $p < 0.05$.
[b] Parameter normalised to a ketoprofen dose of 1 mg/kg.

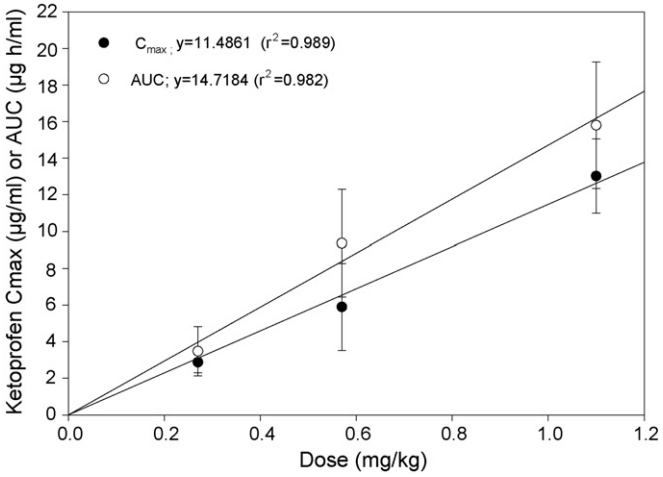

Fig. 2. Relationship between C_{max} and AUC vs. dose in dogs receiving single 0.27, 0.57 and 1.10 mg/kg intravenous doses of racemic ketoprofen.

Fig. 2 depicts the plot of C_{max} and AUC as a function of dose. The peak plasma concentrations, as well as the AUC, increased in near proportion with increasing doses of KET. The power model analysis indicated that the parameter b did not differ significantly from 1. Proportionality was also tested after normalization of C_{max} and AUC to a 1 mg/kg dose. Pairwise comparisons of C_{max} and AUC normalized to 1 mg/kg dose did not result in any significant differences among the three doses studied. Consequently, the linear elimination of ketoprofen was observed at doses used. Also, MRT was not changed over the dose range studied. Moreover, multiple peaks were observed in the plasma concentration versus time profiles, regardless of the dose. The dispersion of the data is high for some parameters. If EHC is accepted in this treatment, then drug reabsorption processes will take place following the discharge of bile into the gastrointestinal tract. The times at which these events occur (gap times) have a considerable effect on parameters such as λ_z and MRT, and in addition on those calculated form them. Inter-individual variations in the time of gallbladder emptying bring about large variation coefficients (CV). The volume of distribution was smaller than the total water (60–70% body-weight) (Davis and Morris, 1992), suggesting that ketoprofen did not appear to be widely distributed throughout the body.

3.2. Oral administration

Ketoprofen concentration–time profiles in the four dogs after a single oral administration are depicted in Fig. 3. The plasma ketoprofen concentrations increased rapidly to peak level between 0.50 and 0.75 h after oral dose and started to decline as a function of time, although minor secondary peaks after dosing were observed. These results indicate the rapid absorption of this drug in dogs. Pharmacokinetic parameters for the oral administration are shown in Table 2. By comparing the $AUC_{0-\infty}$ following oral and intravenous administrations, ketoprofen bioavailability was ~100% (no statistical differences between $AUC_{0-\infty}$ obtained after both administration routes was found; $p = 0.7685$). As shown in Fig. 3, a similar pharmacokinetic profile was observed after intravenous administration.

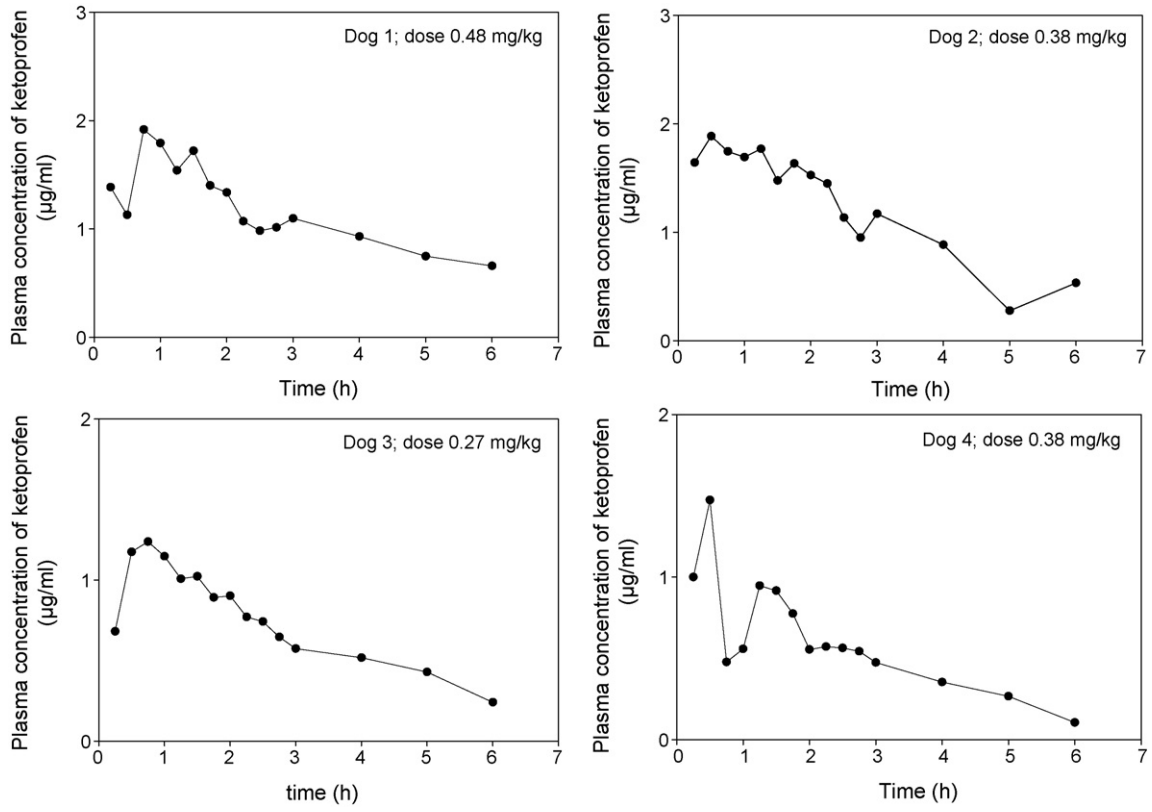

Fig. 3. Mean plasma concentration vs. time plots after a single oral administration of ketoprofen racemate to four dogs.

4. Discussion

The present study describes the pharmacokinetics of ketoprofen after single oral and intravenous administration of the racemate to dogs. The concentration–time profiles of ketoprofen exhibited multiple peaks regardless of administration route. Similar multiple peak phenomena have been described for other structurally diverse drugs such as phenolphthalein (Colburn et al., 1979), *R* and *S* flurbiprofen (Eeckhoudt et al., 1997), carprofen (Priymenko et al., 1998), acebutolol (Piquette-Miller and Jamali, 1997), alprazolam (Wang et al., 1999), danazol (Charman et al., 1993) and valproic acid (Davis and Morris, 1992). Several mechanisms have been proposed to explain this phenomenon, such as enterohepatic recycling, presence of absorption windows along the gastrointestinal tract, variations in the condition of the intestinal lumen pH and time-related fluctuations in gastric emptying. All these reasons, except enterohepatic cycling, are related to the absorption process and hence multiple peaking should be observed only when ketoprofen is given orally. The presence of peaks after both oral and intravenous administration suggests enterohepatic recycling as a likely explanation for multiple peaking in ketoprofen plasma concentrations. However, further studies should be carried out to confirm this hypothesis. The *in vivo* metabolism of ketoprofen in humans and dog is dominated by glucuronidation reactions. Significant differences in the glucuronidation of xenobiotics by microsomes from the livers and kidneys of human and dog were found by Soars et al., indicating that many drugs undergoing direct glucuronidation may be cleared more rapidly by dog liver than human liver, possibly due to a greater efficiency/capacity of glucuronidation. Among the drugs studied, the rate of glucuronidation of ketoprofen was 8-fold greater in dog liver microsomes than in human liver microsomes. In addition, no glucuronidation of ketoprofen was detectable in dog kidney. This species difference in hepatic glucuronidation capacity suggests that ketoprofen is cleared more effectively by liver than kidney in dogs; therefore this drug may be secreted by the dog liver into the bile, and then reabsorbed from intestine after reconversion of conjugates to the free drug. The differences in glucuronidation rates between human and dog taken together

Table 2
Pharmacokinetic parameters of ketoprofen racemate after a single oral administration to four dogs

Dog	Dose (mg/kg)	C_{max} normalised (μg/ml)[a]	T_{max} (h)	AUC normalised (μg h/ml)[a]	MRT (h)	$t_{1/2}$ (h)	λ_Z (h^{-1})
Mean	0.38	4.36	0.63	18.73	3.07	1.85	0.388
S.D.	0.09	0.51	0.14	0.66	0.45	0.41	0.077
CV	22.72	11.7	23.09	3.52	14.52	22.19	19.94

[a] Parameter normalised to a ketoprofen dose of 1 mg/kg.

with the pharmacokinetic data obtained in the current study may lend support the hypothesis that the enterohepatic recycling process may be used to explain the complex profiles of ketoprofen after oral and intravenous administrations in dogs.

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