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Short Communication

Pharmacokinetics of ketoprofen enantiomers following intravenous and oral administration to exercised Thoroughbred horses

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ABSTRACT

Ketoprofen (KTP) is currently only available as an injectable formulation for intravenous administration to horses. The primary goal of the study reported here was to characterize the pharmacokinetics of KTP, including determination of bioavailability following oral administration of the currently available injectable formulation as well as a paste formulation. KTP was administered intravenously and orally, and blood and urine samples were collected at various time points up to 96 h. KTP enantiomer concentrations were determined using LC–MS/MS, and pharmacokinetic analyses were performed.

Mean ± standard error values for systemic clearance, steady state volume of distribution and terminal elimination half-life were 0.345 ± 0.033 [R(−) KTP] and 0.167 ± 0.016 [S(+) KTP] L/kg/h, 0.344 ± 0.044 [R(−) KTP] and 0.298 ± 0.025 [S(+) KTP] L/kg, and 2.49 ± 0.077 [R(−) KTP] and 2.86 ± 0.102 [S(+) KTP] h, respectively. Oral bioavailability was calculated as 69.5 ± 10.3% and 88.2 ± 15.9% for R(−) KTP and S(+) KTP, respectively, following administration of the injectable formulation and 53.0 ± 6.0 and 53.0 ± 16.0% for the R(−) KTP and S(+) KTP, respectively, following administration of KTP paste.

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In veterinary medicine, oral administration of drugs may be a more convenient and less expensive means by which to administer drugs. To the authors' knowledge there is only one previous report describing oral administration of ketoprofen (KTP) to horses (Landoni and Lees, 1995). The investigators concluded that oral dosing of KTP might be a practical route of administration provided the drug is formulated appropriately to allow for drug dissolution (Landoni and Lees, 1995).

In the current study we investigated the enantiomer specific pharmacokinetics, including bioavailability, of two KTP formulations following oral administration. Additionally, because its use in performance horses is common and because of anecdotal reports of oral administration in this group, a secondary goal was to provide concentration data upon which an appropriate withdrawal time or detection time recommendation could be made.

Eight University owned healthy exercised adult Thoroughbred horses (four geldings, four mares; 4–7 years of age; weight: 512 ± 31 kg) were used. In a randomized crossover design, all horses received the US Food and Drug Administration (FDA) approved aqueous formulation (100 mg/mL) intravenously (IV) or orally (2.2 mg/kg Ketofen, Zoetis) and an oral paste formulation (2.2 mg/kg KTP). The paste was manufactured by a compounding pharmacy and was formulated in polyglycol 1450 and 350 at a label concentration of 30 mg/mL. This dosing scheme was repeated until all eight horses had received all formulations, with a minimum washout period of 2 weeks between administrations.

Prior to and throughout the course of the study, horses were exercised 5 days a week (Knych et al., 2014). No medications were administered for at least 4 weeks prior to the start of the investigation. Food was withheld for 12 h before and 4 h following drug administration, while water was available ad libitum. The study was approved by the Institutional Animal Care and Use Committee of the University of California, Davis (18572; approval date 12 January 2014).

Blood samples were collected via IV catheter and transferred to serum separator tubes at time 0 and at 5, 10 (IV), 15, 30 and 45 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 18, 24, 30, 36, 48, 72 and 96 h post drug administration as described previously (Knych et al., 2014). Urine samples were collected at time 0 and at 4, 24, 48 and 72 h post drug administration by free catch. Samples were stored at –20 °C until analysis (96 h from final collection).

Samples were prepared as described previously (Knych et al., 2015) and concentrations were quantitated using tandem liquid chromatography–mass spectrometry (LC–MS/MS) and d3-KTP was used as the internal standard. Plasma calibrators and quality control samples were prepared by dilution of working standard solutions with drug free equine serum. The response was quadratic and gave

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correlation coefficients (R^2) of 0.99 or better. Accuracy and precision for both assays were acceptable based on the FDA guidelines for bioanalytical method validation (Appendix: Supplementary Table S1). The limit of quantitation (LOQ) was 0.5 ng/mL and the limit of detection (LOD) was 0.2 ng/mL for both enantiomers. Recovery in serum was 90% and 85% for R(−)KTP and S(+)KTP, respectively. Recovery in urine was 85% for both enantiomers. KTP compounded oral paste was serially diluted with a mixture of methanol and water and analyzed by LC/MS/MS.

Determination of pharmacokinetic parameters for KTP enantiomers was conducted using commercially available software (Phoenix WinNonlin Version 6.3, Pharsight). To improve the model fit and coefficient of variation values (CV), IV and oral serum concentrations for each enantiomer were modeled simultaneously using compartmental analysis. This analysis was performed separately for each oral formulation.

The compounded KTP paste was found to contain 27 mg/mL, which corresponds to 90% of the labeled 30.0 mg/mL concentration. The mean serum concentration–time curve for ketoprofen enantiomers following IV and oral administration of 2.2 mg/kg is depicted in Fig. 1. Total KTP concentrations (used for regulation of KTP in drug testing programs) are reported in Table 1. Based on CV, Akaike information criterion (Yamaoka et al., 1978) and visual inspection of the residual plots, a two-compartment model with a multiplicative error gave the best fit to concentration data. Selected pharmacokinetic parameters for KTP enantiomers are listed in Table 2. The average urine concentrations of each enantiomer at each time point sampled are listed in Table 3.

The clearance of both enantiomers was slower and the elimination half-life was prolonged relative to previous reports (Landoni and Lees, 1995; Verde et al., 2001) and is most likely due to the more sensitive analytical method in the current study allowing for detection of drug concentrations for a longer period of time. Similar to previous reports (Landoni and Lees, 1995; Verde et al., 2001), S(+) KTP concentrations were higher than R(−) KTP at all time points studied and the AUC was greater for S(+) KTP.

Although there is currently no commercially available FDA approved oral KTP formulation, this route may offer a more convenient means for administration. In the current study, the bioavailability following oral administration of the injectable was slightly higher than that of the compounded paste formulation. Furthermore, the bioavailability of the injectable formulation was slightly higher and that of the compounded paste was comparable to that reported previously by Landoni and Lees (1995) for KTP powder formulated in gelatin capsules. Conversely, the bioavailability for both formulations in the current study and the powder formulation in the Landoni and Lees (1995) study were significantly greater than that of KTP formulated in an oral oil based paste (Landoni and Lees, 1995). Since one of the rate limiting steps in absorption is dissolution, the authors attributed the poor oral bioavailability following administration of the oil-based paste to a low degree of dissolution in the gastrointestinal tract, limiting the release and subsequent absorption of the active moiety (Landoni and Lees, 1995).

KTP is commonly used in performance horses for treatment of pain and inflammation associated with musculoskeletal disorders, and as such, drug withdrawal is necessary prior to competition. Since it is labeled for IV use, recommended withdrawal and restricted administration times are often times based on this route of administration. The results of our study suggest that, depending on the threshold concentration or detection limit, the withdrawal or restricted administration time should be adjusted to account for the longer terminal elimination half-life and detection time following oral administration.

Table 1
Mean (range) total serum ketoprofen concentrations at various time post intravenous and oral (injectable and paste formulations) of 2.2 mg/kg to eight exercised Thoroughbred horses.

Time (h)	Intravenous (ng/mL)	Oral-injectable (ng/mL)	Oral-paste (ng/mL)
0	ND	ND	ND
0.08	21,299 (18,594–25,613)	ND	ND
0.16	17,037 (12,563–29,447)	ND	ND
0.25	10,006 (8,846–11,333)	975 (137–2218)	1,126 (318–2581)
0.50	5,427 (4,592–6,672)	2,560 (1003–4447)	2,639 (1485–4730)
0.75	3,385 (2,666–4,527)	2,713 (1538–3891)	2,586 (1920–4088)
1.0	2,240 (1,626–2,899)	2,379 (1430–3145)	2,200 (1556–3168)
1.5	1,032 (758–1,565)	1,473 (1025–2297)	1,446 (960–2574)
2.0	579 (449–1005)	1,139 (732–1641)	1,147 (817–2411)
2.5	360 (277–631)	822 (333–1345)	909 (471–1651)
3.0	231 (186–405)	613 (229–973)	587 (363–935)
4.0	125 (94.2–244)	320 (182–510)	310 (160–454)
5.0	65.8 (51.2–130)	167 (97.9–243)	166 (74.5–254)
6.0	39.2 (30.2–73.8)	101 (55.0–132)	108 (49.1–184)
8.0	18.4 (13.9–35.2)	46.5 (21.7–79.6)	71.2 (13.8–267)
12	5.47 (4.14–8.57)	11.0 (4.53–23.7)	17.1 (2.02–61.3)
18	1.62 (0.805–2.40)	6.0 (1.26–22.1)	7.95 (1.09–22.5)
24	0.447 (ND–0.944)	1.65 (0.78–4.98)	1.62 (0.66–3.32)
30	ND	0.84 (0.35–1.99)	0.76 (0.37–1.70)
36	ND	0.48 (0.09–1.13)	0.32 (0.14–0.49)
48	ND	0.24 (0.07–0.40)	0.14 (ND–0.17)
72	ND	0.12 (ND–0.16)	0.13 (ND–0.10)

ND, not determined.

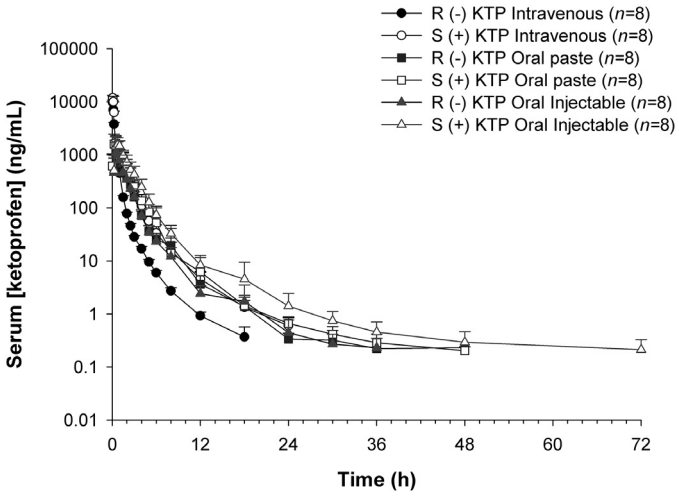

Fig. 1. Mean ± SD serum concentrations of R(−) KTP and S(+) KTP following intravenous and oral administration of 2.2 mg/kg of racemic ketoprofen to eight exercised Thoroughbred horses.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.tvjl.2015.09.018.

Table 2
Pharmacokinetic parameters for ketoprofen following intravenous administration of 2.2 mg/kg of ketoprofen (KTP) to eight exercised Thoroughbred horses.

Parameter	Intravenous		Oral injectable		Oral paste	
	R(−)KTP	S(+)KTP	R(−)KTP	S(+)KTP	R(−)KTP	S(+)KTP
C_{max} (ng/mL)	–	–	1226 ± 535	1814 ± 558	1113 ± 384	1802 ± 688
T_{max} (h)	–	–	0.339 ± 0.047	0.553 ± 0.064	0.520 ± 0.040	0.840 ± 0.200
T_{last} (h)			32.25 ± 2.27	52.5 ± 12.7	31.5 ± 7.69	51.0 ± 17.9
α (1/h)	2.74 ± 0.105	1.48 ± 0.076	7.27 ± 1.36	3.97 ± 0.641	5.01 ± 0.580	2.58 ± 0.65
β (1/h)	0.279 ± 0.009	0.243 ± 0.009	0.122 ± 0.009	0.092 ± 0.005	0.070 ± 0.010	0.10 ± 0.01
$T_{1/2\alpha}$ (h)[a]	0.253 ± 0.010	0.470 ± 0.024	0.095 ± 0.018	0.174 ± 0.028	0.140 ± 0.020	0.270 ± 0.070
$T_{1/2\beta}$ (h)[a]	2.49 ± 0.077	2.86 ± 0.102	5.67 ± 0.401	7.52 ± 0.372	10.4 ± 1.59	6.82 ± 0.910
AUC (h·μg/L)	3191 ± 153	6597 ± 311	1776 ± 192	3035 ± 321.6	1846 ± 169.3	3655 ± 473
K_{12} (1/h)	0.109 ± 0.014	0.095 ± 0.018	–	–	–	–
K_{21} (1/h)	0.292 ± 0.010	0.263 ± 0.012	–	–	–	–
V_c (L/kg)	0.264 ± 0.016	0.245 ± 0.015	–	–	–	–
V_t (L/kg)	0.029 ± 0.004	0.088 ± 0.013	–	–	–	–
Vd_{ss} (L/kg)	0.344 ± 0.044	0.298 ± 0.025	–	–	–	–
Cl_B (L/kg/h)	0.345 ± 0.033	0.167 ± 0.016	–	–	–	–
F (%)	–	–	69.5 ± 10.3	88.2 ± 15.9	53.0 ± 6.0	53.0 ± 16.0
F, adjusted (%)	–	–			65.9 ± 12.9	65.2 ± 13.4

All values in this table were generated using compartmental analysis. Data are presented as means ± SEM.
[a] Harmonic mean.
C_{max}, maximal plasma concentration; $T_{max,}$ time to maximal plasma concentration; T_{last}, time of last detected plasma concentration; α, distribution and elimination rate constants; β, exponential term for elimination phase; $T_{1/2\alpha}$, distribution half-life; $T_{1/2\ \beta}$, elimination half-life; AUC, area under the plasma concentration time curve; K_{12}, K_{21}, transfer constants between compartments 1 and 2; V_c, volume of the central compartment; V_t, volume of the peripheral compartment; $Vd_{ss,}$ volume of distribution at steady state; Cl_B, clearance from the central compartment; F, bioavailability; F, adjusted, bioavailability calculated based on actual paste dose (2.0 mg/kg) and using non-compartmental analysis to calculate AUC.

Table 3
Urine ketoprofen (KTP) enantiomer concentrations (means ± SD) following intravenous and oral administration of 2.2 mg/kg to eight exercised Thoroughbred horses.

	Intravenous		Oral-injectable		Oral-paste	
	R-KTP (ng/mL)	S-KTP (ng/mL)	R-KTP (ng/mL)	S-KTP (ng/mL)	R-KTP (ng/mL)	S-KTP (ng/mL)
Baseline	ND	ND	ND	ND	ND	ND
4	3,396 ± 2,694	36,512 ± 24,399	30,877 ± 24,889	131,183 ± 72,411	51,706 ± 29,249	167,142 ± 73,862
24	11.0 ± 9.30	215 ± 86.7	138 ± 175	861 ± 923	303 ± 672	772 ± 599
48	0.592 ± 0.381	19.6 ± 5.26	9.41 ± 9.36	71.8 ± 46.6	254 ± 704	282 ± 674
72	ND	9.20 ± 3.94	5.00 ± 4.77	28.0 ± 17.5	4.80 ± 7.40	30.2 ± 30.3

ND, not determined.

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