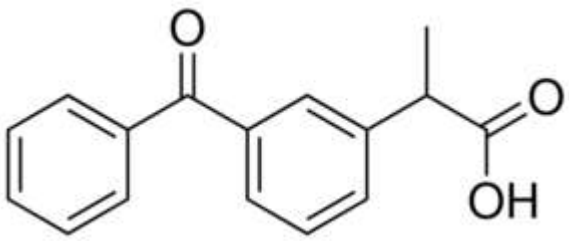
CONTROLLED THERAPEUTIC MEDICATIONS

MONOGRAPH SERIES

Ketoprofen

Background

Ketoprofen is a commonly used non-steroidal anti-inflammatory (NSAID). It is assigned 4/C in the ARCI's Uniform Classification of Foreign Substances. Ketoprofen is administered orally, topically, intravenously or intramuscularly for its anti-inflammatory, anti-pyretic, and analgesic activity.[i] In the horse, ketoprofen is commonly used in the treatment of colic, endotoxemia, and various musculoskeletal disorders including acute injury and more chronic disorders such as osteoarthritis.[ii]



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Ketoprofen is relatively new when compared to other systemically administered NSAIDs. It received FDA approval for use in the horse in 1990. Ketoprofen is a prescription medication and can only be dispensed by, or upon, the request of a licensed veterinarian. It is commercially available in an injectable solution, Ketofen® (Zoetis; Parsippany, NJ). Ketofen® is approved for intravenous administration in equines with a recommended dosage of 1.0 milligram per pound (2.2 mg/kg) of body weight once daily for up to 5 days.[iii] At one time Ketoprofen was available as an over-the-counter oral preparation for human use but has since been withdrawn due to cardiovascular and gastrointestinal complications. Veterinarians have had ketoprofen compounded in paste formulations.

Ketoprofen's primary mechanism of action is the inhibition of the cyclo-oxygenase and lipoxygenase pathways. Ketoprofen is a racemic drug mixture composed of both R(-) and S(+) enantiomers but only the S- enantiomer has pharmacologic activity.[iv] Ketoprofen, like many other NSAIDs, has increased penetration into inflamed joints when compared to healthy joints.[v]

Ketoprofen demonstrates lower toxicity than its counterparts phenylbutazone and flunixin meglumine in equines and thus has fewer adverse effects.[vi] It is also, however, slightly less effective in non-laboratory studies on lameness.[vii] Ketoprofen's relative polarity may cause limited diffusion through most biological membranes, which could also explain why phenylbutazone has been observed to be more effective in treating lameness.[viii] However, those studies also identified that a higher dose may be needed for ketoprofen to be effective on

lameness.[ix] Ketoprofen has been shown to significantly inhibit eicosanoid production in the horse for up to 120 hours. (Knych et al *In Preparation*, 2020)

Administration Studies

In a 2013 study, ketoprofen was administered in a random 3 way-crossover design to eight healthy exercise-conditioned adult Thoroughbred horses intravenously in an aqueous formulation of 100 mg/mL, orally at 2.2 mg/kg (Ketofen®, Zoetis) and an oral paste formulation of 2.2 mg/kg.[x] The paste was formulated by a compounding pharmacy at a label concentration of 30 mg/mL. This dosing scheme was repeated until all eight horses had received all formulations. This study was performed at the University of California, Davis, by personnel in the K.L. Maddy Equine Analytical Chemistry Laboratory under the direction of Dr. Heather K. Kynch.

Blood samples were obtained via IV catheter immediately before dose administration and at the following times after dosing: 5, 10, 15, 30, and 45 minutes and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 18, 24, 30, 36, 48, 72, and 96 hours. Urine samples were collected prior to administration and at 4, 24, 48, and 72 hours post administration.

In a subsequent RMTC study in 2015, ketoprofen was administered as a single intravenous dose of 2.2 mg/kg to 20 exercise-conditioned Thoroughbreds at the University of Florida under the direction of Dr. P. Colahan. Blood samples were obtained by direct venipuncture immediately before dosing and at 20, 24, and 48 hours post-administration. The goal of this study was to investigate the plasma concentrations of ketoprofen to determine if a 48-hour withdrawal interval could be supported by testing.

A third study in 2019 administered ketoprofen by single intravenous dose of 2.2 mg/kg to 16 exercise-conditioned Thoroughbreds at the University of California, Davis, under the direction of Dr. Heather K. Knych. Blood samples were obtained prior to administration and following dosing at: 12, 18, 24, 36, 48, 72, 96, and 120 hours.

Extraction and Analysis Procedures

For the 2013 study, quantification of ketoprofen in plasma and urine samples was performed at the K.L. Maddy Equine Analytical Chemistry Laboratory (Davis, CA) using validated methods similar to those previously described.[xi] Ketoprofen was determined in plasma and urine by tandem liquid chromatography-mass spectrometry (LC-MS/MS) using ketoprofen d-3 (Sigma-Aldrich, St. Louis, MO)as internal standard to improve quantitative accuracy and precision. The lower Limit of Quantification (LOQ) for determination of both ketoprofen enantiomers in plasma was 0.5 ng/mL. The Limit of Detection (LOD) was 0.2 ng/mL.

For the 2015 study, quantification of ketoprofen in plasma was performed at HFL-Sport Science (Lexington, KY) under the direction of Dr. R. Sams. Ketoprofen was determined by LC-MS/MS using an internally validated method. A deuterated standard, ketoprofen d-3 (Sigma-Aldrich, St.

Louis, MO) was used to improve precision and accuracy. The method was characterized by a LOQ of 0.02 ng/mL and an LOD of 0.005 ng/mL.

More recently, quantification of ketoprofen in plasma and urine samples was performed at the K.L. Maddy Equine Analytical Chemistry Laboratory (Davis, CA) (Knych et al *In Preparation*, 2020). using validated methods similar to those previously described.[xii] Ketoprofen was determined in plasma and urine by tandem liquid chromatography-mass spectrometry (LC-MS) using ketoprofen d-3 (Sigma-Aldrich, St. Louis, MO) as internal standard to improve quantitative accuracy and precision. The LOQ for determination of both ketoprofen enantiomers in plasma was improved to 0.25 ng/mL and the LOD to 0.1 ng/mL.

Pharmacokinetic Modelling

Plasma concentrations of ketoprofen are expressed as the mean and range at each collection point (Tables 1.1). Pharmacokinetic analysis was performed on individual plasma concentrations using Phoenix® WinNonlin® pharmacokinetic analysis software (Pharsight Corporation, St. Louis, MO).

The 95/95 tolerance interval was calculated on the natural logarithmic (*i.e.,* ln) transformed plasma concentration data at each collection time for all eight horses.

Results and Discussion

The bioavailability of the intravenous and orally administered injectable formulation was slightly higher than the orally administered compound paste. Mean serum concentrations of (R- and S+) ketoprofen following intravenous and oral administration are shown in Figure 1.1.

Table 1.1. 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. (ND: Not detected.)

Time (h)	Intravenous (Range) ng/mL	Oral-injectable (Range) ng/mL	Oral-paste (Range) ng/mL
24	0.447 (ND–0.944)	1.65 (0.78–4.98)	1.62 (0.66–3.32)
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)

Plasma ketoprofen concentrations increased rapidly, especially for intravenous administration. The mean serum concentration fell below the LOQ in all horses and formulations by 36 hours post administration. Both oral formulations continued to be detectable through the testing period.

Ketoprofen was detectable in urine after a 2.2 mg/kg IV dose at 72 hours.[X] The European Horserace Scientific Liaison Committee published a 96 hours Detection Time for ketoprofen. [xiii]

Scientific Advisory Committee (SAC) Recommendation

This historic threshold of 10 ng/mL associated with a 24-hour restricted administration time was adopted in 2009 and based on the limitations of the testing method's sensitivity at that time. The 2013 RMTC administration study determined that a 10 ng/mL threshold was inadequate for controlling the administration of ketoprofen out to 24 hours prior to a race. Using the 24-hour data from the intravenous administrations, the SAC rounded up the 95/95 TI value of 1.76 to a recommended threshold of 2.0 ng/mL of serum or plasma. The withdrawal guidance recommendation was based upon a single 2.2 mg/kg intravenous administration of ketoprofen. A secondary threshold of 1.0 ng/mL was based on the results of 2015 RMTC administration study and was adopted to support a prohibition on the administration of more than one NSAID within 48 hours of a horse's race.

In 2019, the ARCI adopted a Model Rule with a 48-hour restricted administration for all NSAIDs and a prohibition on stacking (i.e. the detection of two or more NSAIDs in a blood and/or urine sample). The 48-hour data from 2015 and 2019 administration studies were combined and the 95/95 Tolerance Interval calculated for the 36 samples. The SAC rounded up the resulting value of 1.69 ng/mL to a threshold recommendation of 2.0 ng/mL.

Practice Tips

Different formulations of ketoprofen, administration of higher doses, serial administrations, and/or co-administration of ketoprofen with other substances may result in concentrations above the threshold unless an extended withdrawal time is observed. Veterinarians are advised to assess the impact of these potential risk factors on the withdrawal time and to advise their clients accordingly.

Specifically, the withdrawal time recommendations do <u>not</u> apply to ketoprofen administered orally or topically because it is cleared more slowly after these routes of administration and may need a substantially longer withdrawal time.

References

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Equine Plasma by Liquid Chromatography/Tandem Mass Spectrometry, (2005) Rapid Communications in Mass Spectrometry (2005) 19:1245-56.

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