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Disposition of ketoprofen in Camels

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ABSTRACT

The disposition of ketoprofen was studied in camels following intravenous administration of 2 mg/kg body weight. Blood samples were collected at 0.00, 0.08, 0.17, 0.25, 0.33, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 8.0, 10.0, 12.0, 13.0, 14.0, 15.0 and 16.0 hours. Urine was collected in fractions during the entire blood, sample collection period. A ketoprofen metabolite, a hydroxylated product, was identified in camel serum and urine by gas chromatography-mass spectrometry (GC/MS) in electron impact and chemical ionization scan modes after it was purified by thin layer chromatography. The serum concentrations of hydroxy ketoprofen were measured by high performance liquid chromatography. Its elimination half-life was 5.94 – 11.99 hours and area under the curve was 28.83 – 46.45 μg.h.ml

Key Words:, Ketoprofen, Camel, United Arab Emirates.

INTRODUCTION

Ketoprofen (KP), a 2-arylpropinoic acid, belongs to the family of nonsteroid anti-inflammatory drugs (NSAIDS). It has similar pharmacological actions to other drugs in this class such as ibuprofen, fenoprofen and naproxen. Ketoprofen has been used extensively in treatment of chronic rheumatoid arthritis and various painful conditions in various species. The principal mechanism of action is considered to be via inhibition of cyclo-oxygenase mediated generation of prostanoids (Vane, 1971). Ketoprofen pharmacokinetics has been studied in mares (Sams *et al.,* 1995), dairy cattle (DeGraves *et al.,* 1996), horses (Owens *et al.,* 1995), donkeys (Oukessou *et al.,* 1996), camels (Oukessou *et al.,* 1995), monkeys (Mauleon *et al.,* 1994), calves (Landoni *et al.,* 1995), and humans (Lewellen and Templeton, 1976).

The metabolic fate of ketoprofen has been studied in the horse (Kurosawa *et al.,* 1994; Ryan *et al.,* 1994). Urinary excretion of free and conjugated ketoprofen have been reported in horses (Ryan *et al.,* 1994; Sams *et al.,* 1995) and humans (Leis *et al.,* 1996). Our preliminary work has indicated that the renal clearance of free ketoprofen accounted for 0.273 – 3.277% of total clearance, which suggests that extensive metabolism must occur for ketoprofen.

The objective of this study was to investigate the metabolic fate of ketoprofen in the camel by analyzing its metabolites in serum and urine.The latter piece of information would be helpful for veterinarians, as this would enable them to know when to discontinue ketoprofen use before racing, in order to avoid penalties imposed by the camel-racing commissioner if a camel tests positive for a foreign substance and/or its metabolite(s).

MATERIALS AND METHODS

Chemicals

Ketoprofen (ketoprofen, 100 mg/ml, Nature Vet. Pty, Limited, Richmond, Australia) was obtained locally. Sulindac as internal standard (ISD) was purchased from Sigma. Hydroxylate ketoprofen was a kind gift from Dr. M. Kurosawa (Tokyo, Japan). HPLC grade acetonitrile, acetic acid, ethyl acetate and potassium phosphate (monobasic) was purchased from Aldrich. Methelute was purchased from Pierce, (Rockford, IL, USA).

Animals

Five clinically healthy camels (3 males, 2 females) 4-7 years old and ranging in body weight from 225 to 500 kg were used. They were kept in open pens. None had received any drug for at least 6 months. Good quality hay and lucerne were fed once daily and water was provided *ad libitum*.

Drug administration and sample collection

Ketoprofen was administered as a bolus i.v. dose of 2 mg/kg of body weight. Blood samples (10 ml) were collected from the

opposite jugular vein at 0 (pre-dose), 5, 10, 15, 20, 30, 45 and 60 min and at 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8, 10, 12, 13, 14, 15 and 16 hours after dosage. The blood samples were allowed to clot, serum was separated by centrifugation (2,000 x g for 10 min) and was stored at –20 °C pending analysis. Voided urine samples were collected in fractions up to 12 hours, as reported previously (Wasfi *et al.,* 1997). Its pH was immediately adjusted to 3.0 with glacial acetic acid to stabilize the glucuronide conjugates and was stored at -20 °C.

Sample preparation and analysis of metabolites

To each serum sample (1 ml) in screw capped test tubes, was added 1 ml phosphate buffer (1 M, pH 3) containing 2 µg/ml ISD; then 6 ml of ethyl acetate was added and the tubes were agitated for 15 min. The tubes were then centrifuged (2,000 x g for 5 min) and 5 ml of organic layer was removed and evaporated to dryness under nitrogen. The residue was dissolved in 250 µl of mobile phase and 150 µl was injected in the HPLC system. A calibration curve was prepared by spiking drug free serum with HKP at concentration of 0.25, 0.5, 1, 2.5, 5 and 10 µg/ml. Extraction was performed as described above.

The serum and urine extracts were analyzed using High-Performance Liquid Chromatographic (HPLC 1090, Hewlett Packard, Palo Alto, CA) with a diode array detector, and an injector (HP 1090 M, Hewlett Packard, Palo Alto, CA) with sample tray. Injections were made onto a Suplcosol ABZ + plus HPLC column (15 cm x 4.6 mm id, 5 µm). Analysis was performed isocratically with a mixture of 65% of solvent A (0.2% acetic acid pH 3.6) and 35% of solvent B (acetonitrile) (Kurosawa *et al.,* 1994).

The flow rate was 1.5 ml/min and the detector was monitoring 220 nm for HKP. The limit of quantitation, defined as the concentration at which all acceptance chromatographic criteria are met and the quantitative value, which was 50 ng/ml, is within ±20% of the target concentration. The percent recovery of HKP in serum for a concentration of 0.625 to 10 µg/ml ranged (88-93%). The inter and intra assay coefficients of variation of HKP in serum for 1 and 5 µg/ml was 7.56, 2.36 and 6.38, 2.09%, respectively.

Identification of ketoprofen metabolites in urine was done by GC/MS as reported by (Jaussaud *et al.,* 1987) and modified elsewhere (Wasfi *et al.,* 1998). GC/MS was carried out using a Hewlett Packard 5972 Mass Selective Detector interfaced to a HP 5890 gas chromatograph with HP 7673 auto injector and sample tray. Injections were made in the split less mode onto a 15 m x 0.25 mm I.d. DP-5MS column (Fison). The initial column temperature was 70 °C and was programmed to 290 °C at 25 °C/min. Injection and interface temperatures were 250 °C and 280 °C, respectively. Helium was used as a carrier gas at a flow rate of 20 cm/s. In electron impact (EI) scan mode, spectra were obtained at 70 eV and scanned from 50 to 500 a.m.u. The source temperature was 150 °C. In positive chemical ionization (CI) scan mode, spectra were obtained at 30 eV and methane was used as a reagent gas at a source pressure of 1.0 x 10^{-4} mBar. The source temperature was 150 °C.

RESULTS

Identification of metabolites

The preparatory thin layer chromatography of the urine samples showed two spots. The spectrum of the spot with the higher Rf value compared well with the spectrum of methylated ketoprofen standard.

The mass spectrum of the methylation of the second spot with lower Rf value had a suspected molecular ion of m/z 270, a base peak ion of m/z 105, and other major ions m/z 211, 191 and 165. The molecular was confirmed by CI scan mode which showed pseudo molecular ion of 271. Ion m/z 299 is an adduct (M^+ + 28). The spectrum of this spot agreed very well with methylated HKP standard which is due to reduction of the ketone group of ketoprofen(Fig. 1).

Serum

The HPLC method for HKP was linear from 0.25 to 10 µg/ml. The method gave good separation between ketoprofen, HKP and ISD. Retention times for HKP, ISD and KP were 7.16, 10.45 and 14.29, respectively. The total time needed to analyze one sample was less than 18 min.

The disposition of HKP was best fitted by a first order two compartment model (Fig. 2). The elimination half-life of HKP calculated from the terminal portion of the concentration vs. time graph ranged from 5.77 to 11.99 hours and the area under the curve was 28.83 to 46.45 μg.h.ml.

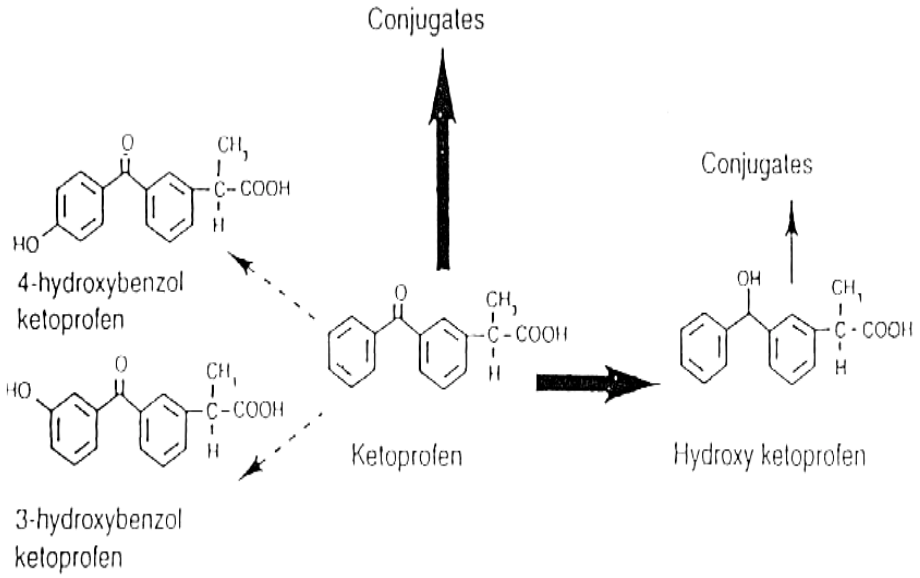

Fig. 1: Metabolite pathways of ketoprofen in camel after intravenous administration of mg/kg of ketoprofen.

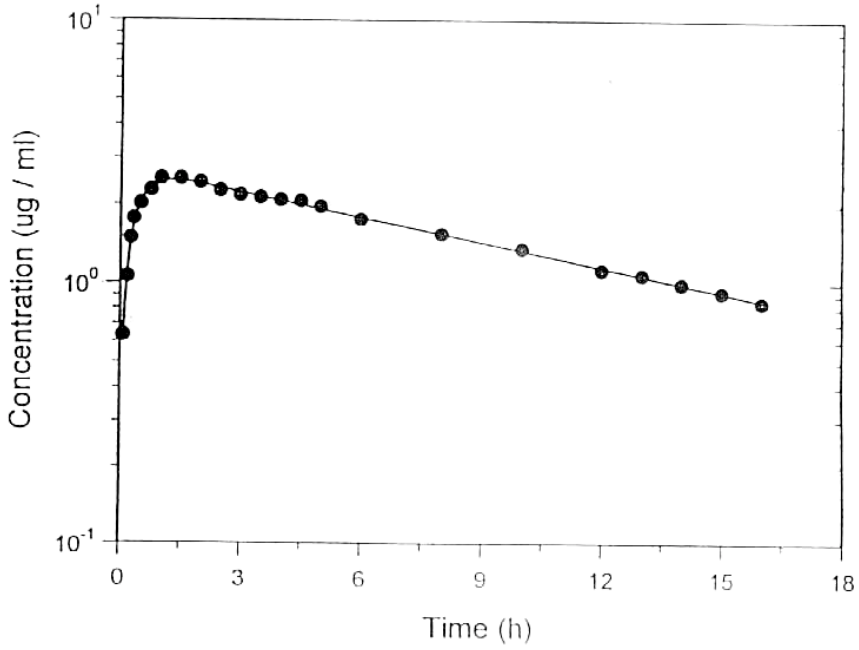

Fig. 2: Serum concentration (mean±SEM) of Ketoprofen and hydroxy Ketoprofen vs time following administration of 2 mg/kg of Ketoprofen intravenously. Each point is an average of 3 camels.

DISCUSSION

To the best of our knowledge this is first report which describes metabolism of ketoprofen in the dromedary camel. We have found that one way to the metabolic pathways of ketoprofen is by reduction of its ketone group resulting in a hydroxylated product. This pathway has also been previously reported for the horse (Kurosawa *et al.,* 1994). We can not ascertain from this study if this route of elimination of ketoprofen is a major one or not because we have not calculated the absolute values of HKP in the urine of different

camels. However, in the horse, this route of elimination constitutes about 20% in twelve hours of administrated dose (Kurosawa *et al.,* 1994).

The renal clearance of ketoprofen itself represented a small percent of the administered dose (1.18%) (Al Katheeri *et al.,* 1998). Clearly, other routes of ketoprofen elimination must exist. In this regard it has been shown that, in humans, dogs, rats and rabbits, ring hydroxylation occurs at position 3 or 4 resulting in 3 or 4 hydroxy benzoyl ketoprofen (Populaire *et al.,* 1973). We are currently evaluating this pathway in camels.

The elimination half-life of hydroxy ketoprofen, calculated from the terminal portion of the concentration vs. time curve, ranged from 5.94 to 11.99 hours. The long half-life could be due to the low glomerular filtration rate of camels. For example, under normal conditions the glomerular filtration rate of camels is half that of cows (Wilson, 1984). Other factors like enterohepatic recycling or a large volume of distribution can not be ruled out. The half-life of hydroxy ketoprofen in camels is longer than that reported in horses (Kurosawa *et al.,* 1994).

The camel racing authority in UAE prohibits the presence of any foreign substance and/or its metabolite(s) in biological samples obtained after racing. Detection of a prohibited substance in those samples usually results in loss of any prize and severe penalties. For this reason, guide lines are needed by owners, trainers and veterinarians to help them determine when to discontinue the use of legitimate, therapeutic drugs in order to avoid their accidental presence post racing. Based on the half-life of hydroxy ketoprofen obtained from the present study, we therefore, propose withholding ketoprofen administration before racing for an minimum period of 4 days. This however, needs to be verified experimentally following single and multiple administration of ketoprofen and using the routine screening method.

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