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# PHARMACOKINETICS OF KETOPROFEN IN HEALTHY HORSES IN PAKISTAN

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## ABSTRACT

Ketoprofen (KTP) is a non-steroidal anti-inflammatory drug (NSAID), used to alleviate inflammation and rheumatic problems in humans and animals. A single intravenous dose of ketoprofen was administered in eight healthy horses at dose of 3.0 mg/kg body weight through jugular vein. Blood samples (3-5ml) were drawn pre-medication at zero-hr, and then at 0.08, 0.17, 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10, 12.0, 24, 48, 60, 72, 84 and 96 hrs post medication. Plasma was separated out. The concentration of KTP in plasma was measured by HPLC (high performance liquid Chromatography) method. By using the plasma concentration versus time data, the pharmacokinetic parameters were calculated through computer based pharmacokinetic software APO. Version 3.02, as Mean ±SEM AUC (Area Under the concentration time Curve) ± $\mu g.h.ml^{-1}$, Cl (Clearance) ± $l.hr^{-1}.kg^{-1}$, $t_{½}$ (Half Life) ± $hr^{-1}$, VD (Volume of Distribution) ± $l.kg^{-1}$, $VD_{ss}$ (Volume of distribution at Steady State) ± $l.kg^{-1}$, and $K_{el}$ (Elimination Rate Constant) ± $l.hr^{-1}$ respectively.

**Keywords:** NSAID's**,** Ketoprofen, Pharmacokinetics, HPLC, Intravenous administration, Horses.

## INTRODUCTION

NSAID's are frequently used and commonly prescribed in humans and animals for reduction in pain, fever and inflammation in rheumatic problems (Geof et al., 2008). Chemically, ketoprofen (KTP) is 2-(3-benzoyl phenyl) propionic acid. Molecular formula is C16H14O3. KTP exists in two enantiomeric forms S and R, which have different half lives. KTP is formulated as racemic mixture. The S(+) enantiomer is associated with prostaglandin inhibition and toxicity and the R(-) enantiomer is linked with analgesic action and protection of gastrointestinal tract. The S(+) isomer, due to chiral inversion dominated in cats, dogs and horses. The R(-) isomer, dominated in sheep. Ketoprofen is approved for use in horses and advised in the inflammatory and pain conditions associated with musculo skeletal problems (Huskisson et al. 1996). Ketoprofen has a wide safety margin and low toxicity compared to phenylbutazone and flunixin meglumine in horses (Mozaffari et al., 2010). Ketoprofen has comparatively a better gastro intestinal safety and has less adverse events than aspirin, phenylbutazone and flunixin meglumin in horses (MacAllister et al. 1993). Favourable pharmacokinetic profile of ketoprofen in humans made it a suitable and effective NSAID for veterinary use. The present project was designed to investigate the pharmacokinetic parameters of KTP in local Pakistani horses after intravenous administration at the dose of 3mg/kg body weight so that we could recommend dosage regime in this particular specie.

## MATERIALS AND METHODS

**Animals:** Eight healthy male horses with an average weight of 315kg were used in this study. All the horses were tagged and acclimatized to the experimentation area. Food was provided to animals along with water *ad libitum*. Experimental animals were monitored regularly regarding their health throughout the experiment.

**Drugs and Chemicals:** Ketoprofen standard (Sigma) was purchased for use as external standard from the company. HPLC grade water, di ethyl ether, acetonitrile (E. Merck Germany) was used. Injection (20ml) of Ketoprofen manufactured by Selmore Pharmaceuticals (Pvt) Limited, Lahore, Pakistan were used during experiment. All the chemicals used were of analytical grade.

**Treatment protocol, sampling and drug analysis:** An I/V dose of Ketoprofen @ 3 mg/kg body weight was administered via jugular vein into horses. Blood samples (5 ml) were collected in test tubes prior to medication and then at 0.08, 0.17, 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10, 12, 24, 48, 60, 72, 84 and 96 hours post medication. Separation of plasma was made by centrifugating blood at 4000 rpm for 10 minutes and stored at −80°C till analyzed.

**Extraction of drug:** 1.0 ml of 1.0 M hydrochloric acid was added to 1.0 ml of plasma. Vortexed it for 1 minute and then 1.0 ml of HPLC grade di-ethyl ether was added. Vortexed the material again at high speed vortex (4000 rpm) for 10 minutes. The clear supernatant was taken and evaporated to dryness. The dried portion was

reconstituted with 1.0 ml of mobile phase (buffer: acetonitrile 75: 25). Filtered it through 0.22 µm syringe filter and analysis was made.

**HPLC analysis:** HPLC analysis of ketoprofen in plasma was made with already developed, standardized and validated HPLC method by using Shimadzu LC2000, equipped with a LC-20AT VP pump, a SIL-20AC HT auto-sampler, SPD-M20A, CTO 20 AC and CBM 20A control unit. 20 µl of the sample was run into HPLC system via auto sampler with flow rate of 1 ml/minute. Mixture of phosphate buffer (Di-potassium hydrogen phosphate) and acetonitrile (75:25 v/v) was used as mobile phase. A reversed phase C18 column (Thermo, BDS Hypersil. 5 µm; 4.6 mm × 250 mm) was used as stationary phase. Separation was achieved at 7.0 minutes (retention time) at wavelength of 254nm. Oven temperature was set at 30°C. Ketoprofen in sample was compared with ketoprofen external standard. The discrete peak found in chromatograms of plasma samples of horses was similar to the peak in chromatogram of ketoprofen (external standard) at 7.0 minutes of retention time. The limit of quantification was 0.125µg. The plasma concentration (µg/ml) versus time profile of ketoprofen in horses was prepared semilogarithmically.

**Pharmacokinetic Analysis:** The computer software APO pharmacological analysis MW /PHARM, Version 3.02, (Holland, 1987) was used for calculation of pharmacokinetic parameters through standard two compartmental analysis. Pharmacokinetic parameters were calculated for horses by use of statistical moment theory. The following equations were used for different calculations: Dose= Cl x AUC

Cl = Dose/AUC; AUMC = MRT x AUC;

Where, AU C = Area under the curve, Cl = Clearance, MRT = Mean residence time and AUMC =Area under the first moment curve.

**Statistical analysis:** The software SPSS (Statistical Package for the Social Scientists) 13.0 was used for statistics. The values in the raw data were expressed as individual as well as range, mean, SEM, median and standard deviation.

## RESULTS AND DISCUSSION

The group means for plasma concentrations (µg/ml)-time (h) data and pharmacokinetic parameters of ketoprofen were determined at dose of 3mg.kg-1 body weight in horses after intravenous administration. The I/V dose of 3mg/kg body weight of KTP was selected from literature for administration in buffalo calves to achieve that KTP plasma concentration which was likely to have an anti-inflammatory, analgesic and antipyretic effect (Fosse *et al*., 2010, Neirinckxa *et al.*, 2010, Koshi, 2008, Arifah *et al*., 2003, Boothe, 2001, Landoni *et al*., 1999, Paul *et al*., 1997, Banting *et al*.,2008). The results are given in Table 1and 2. Plasma concentrations (µg/ml)-time profile of ketoprofen was also prepared and is given in Figure 1.

**KTP in horses after I/V administration @ 3mg/kg body weight. (N=8):** The Mean ± SEM pharmacokinetic parameters of KTP determined in our local healthy horses in this study are, Area under conc. time curve (AUC)= 9.62 ± 1.580µg.h/ml, body clearance (Cl) = 0.304 ± 0.054 l/h/kg, Volume of distribution (VD) =1.25±0.476 l/kg, Volume of distribution at steady state (VDss) = 0.693 ± 0.190 l/kg, plasma half life ($t_{1/2}$) =2.665 ±0.658 h, and Elimination constant (Kel) =2.129 ±0.366 l/hrs.

**Table 1: Group means for plasma conc. time data (µg/ml) of KTP in horses after I/V administration @3mg/kg body weight. (N=8).**

| TIME (hours) | RANGE (µg/ml) | MEAN CONCENTRATION (µg/ml) | SEM | SD | CV% | MEDIAN |
|---|---|---|---|---|---|---|
| **0.08** | 8.70-19.80 | 15.52 | 1.41 | 3.98 | 25.664 | 16.26 |
| **0.17** | 5.51-12.52 | 9.46 | 1.01 | 2.855 | 30.18 | 10.45 |
| **0.25** | 4.42-9.81 | 7.40 | 0.83 | 2.357 | 31.85 | 7.48 |
| **0.50** | 2.77-3.93 | 3.21 | 0.19 | 0.525 | 16.35 | 3.075 |
| **0.75** | 1.89-2.35 | 2.09 | 0.06 | 0.178 | 8.13 | 2.155 |
| **1** | 0.96-1.98 | 1.63 | 0.14 | 0.394 | 24.17 | 1.805 |
| **2** | 0.52-1.92 | 1.28 | 0.20 | 0.571 | 44.60 | 1.475 |
| **3** | 0.23-1.44 | 0.88 | 0.18 | 0.503 | 57.15 | 0.855 |
| **4** | 0.93-0.99 | 0.63 | 0.13 | 0.365 | 57.93 | 0.755 |
| **6** | 0.14-0.47 | 0.26 | 0.05 | 0.132 | 50.76 | 0.225 |
| **8** | 0.13-0.30 | 0.17 | 0.02 | 0.057 | 33.52 | 0.15 |

**Table 2: Group means for PK parameters of KTP in horses after I/V administration @ 3 mg/kg body weight. (N=8).**

| Pharmacokinetic Parameters | Unit | Range | Mean | SEM | S.D | CV% | Median |
|---|---|---|---|---|---|---|---|
| AUC (Area under the curve) | µg.h /ml | 5.92-12.92 | 9.62 | 0.965 | 2.728 | 28.35 | 9.825 |
| Cl (Clearance) | l/hr/kg | 0.212-0.466 | 0.304 | 0.033 | 0.095 | 31.25 | 0.265 |
| VD (Volume of distribution ) | l/kg | 0.62-2.784 | 1.25 | 0.291 | 0.825 | 66 | 0.774 |
| VDss(volume of distribution at steady state) | l/kg | 0.428-1.258 | 0.693 | 0.1165 | 0.329 | 47.47 | 0.517 |
| HL( $t_{1/2)}$ Half Life | hr | 1.661-4.487 | 2.665 | 0.4033 | 1.140 | 42.77 | 2.044 |
| Kel(Elimination constant) | l/hr | 1.235-2.80 | 2.129 | 0.224 | 0.634 | 29.77 | 2.287 |

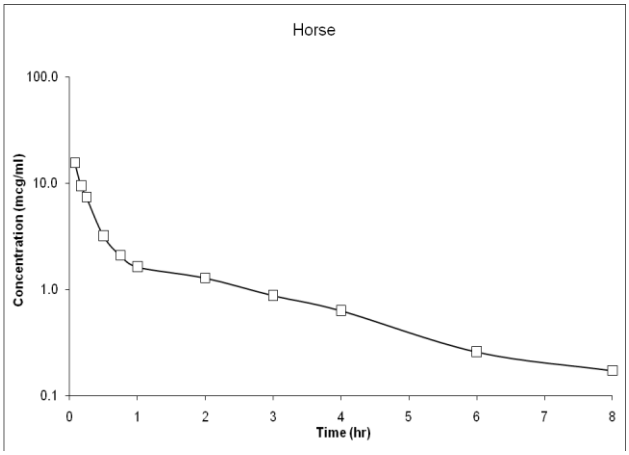


**Figure 1: Semilogarithmic plot of group means for plasma conc. time data (µg/ml) of**

The pharmacokinetic parameters like body clearance (Cl) are comparable with donkeys and mares (Oukessou et al., 1996, Sams et al., 2008). The comparatively large value of Volume of distribution (VD) and Volume of distribution at steady state (VDss) may result in fast elimination of drug in horses. The higher drug metabolizing enzyme activities in the liver and other organs of horses have been linked with fast elimination of KTP like antipyrine, ampicillin (Elsheikh, 1997), sulphadimidine (Nouws *et al.,* 1988), isometamidium (Wesongah *et al.,* 2004) and meloxicam (Mehmood *et al*.,2011) in goats as compared to other species. Heavily protein bound drugs have long plasma half lives (Sindhu and Ram Pal.,2007).The pharmacokinetic parameters observed in horses are different from those reported in literature for horses, dogs, buffalo calves, humans, and laboratory animals.

In this study, dose was also calculated by putting values of AUC and Clearance in formula (Dose= AUC x Cl). In horses it was found to be 2.92mg/kg body weight. The dose was rounded off to 3mg/kg body weight. In the present study, I/V dose of 3mg/kg body weight is suggested in horses. The dose should be repeated after 12 hours in horses. In this study, no toxicity was observed in any animal.

However, it is suggested that clinical trials of ketoprofen should be conducted in horses to assess its MEC (minimum effective concentration) to calculate its activity as an analgesic and anti inflammatory agent. Government of Pakistan should bound pharmaceutical manufacturers to mention specific dosage regimens of ketoprofen for different species on labels of formulations instead of mentioning a generalized dosage regime.

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