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PHARMACOKINETICS OF KETOPROFEN IN HEALTHY DONKEYS IN PAKISTAN

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ABSTRACT

Ketoprofen is a non-steroidal anti-Inflammatory drug. This drug was reported as a safe alternate for diclofenac which was banned for veterinary use in Pakistan. The present study was designed to access pharmacokinetics of ketoprofen after intravenous administration in eight Healthy donkeys. Donkeys were administered through jugular vein, an intravenous dosage of ketoprofen @3.0 mg/kg body weight. Heparinized Vacutainer were used to collect blood samples (3-5 ml) before and then up to 24 hours post – medication. Plasma Ketoprofen concentrations were measured by HPLC. The data obtained the mean (±SEM) pharmacokinetic parameters of ketoprofen were, AUC (Area under the curve) = 17.02±2.61 μg.h/ml, Cl (clearance) = 0.21±0.02l/h/kg, V_{Dss} (Steady state volume of distribution) = 0.47±0.04 l/kg, $t_{1/2}$ (half life) = 2.2± 0.17h, and MRT (Mean Residence Time) = 2.44±0.17 hrs. The short elimination half-life and MRT were observed in donkeys when compared with horses, dogs, rats and human. The small value of AUC of Ketoprofen observed in donkeys. The lower value of Vd may be due to high protein binding of Ketoprofen which limit their ability to reach extra vascular compartments. The low plasma clearance leads to suggestion that use of Ketoprofen in donkey might be beneficial.

Keywords: Ketoprofen, Pharmacokinetics, Intravenous administration, donkeys.

INTRODUCTION

Ketoprofen belongs to class of cyclo-oxygenase inhibitor and a non-steroidal anti-inflammatory drug (NSAID). It has analgesic, antipyretic and anti inflammatory properties. Its structure is *2-(3-benzoyl phenyl) propionic acid or 3-benzoyl alpha-methyl benzene acetic acid*. It is a non selective COX-1 and COX-II inhibitor (Streppa *et al*., 2002). Protein binding ability of Ketoprofen is very high. It is weakly acidic and is less lipid soluble. Absorption of Ketoprofen is rapid in humans, dogs and rats. Ketoprofen is metabolized in the liver by conjugation reactions and half life is 2.5 hours. It is eliminated either as conjugated metabolite through renal route of excretion (Sams *et al*., 2008). Ketoprofen is reported to have good penetration in synovial fluid of cattle. Ketoprofen is mainly approved for osteoarthritis and musculoskeletal pain from soft-tissue injury. For use in animals it reduces fever, alleviate respiratory complications in calf and relive pneumonias in piglets (Landoni *et al*., 2008). It is also effective as a potent analgesic in equine colic and associated pain and inflammation due to various joint disorders of horse and dog. It is also used to mange pain allied with traumatic injuries and postoperative pain in almost every specie (Lees *et al*., 2004). It is also used to alleviate pain associated with gastrointestinal complications and can be administered for a maximum up to five successive days for lessening the severity of inflammation and pain (Allen *et al*., 2010). Drug Interactions may occur when given with other members of anti-inflammatory drugs. Ketoprofen is not extensively studied and established in veterinary practices. Approval for recommendations about Ketoprofen administration in horses was given in 1990. (Mozaffari *et al*., 2010). It is contraindicated with other NSAIDs due to drug interactions. Its use is prohibited in animals which are sensitive to aspirin and metabolites formed from aspirin. Various unwanted effects appear upon the extensive use of Ketoprofen such as gastrointestinal ulceration, hepatopathies, changes in various biochemical parameters, photosensitivity and renal disorders in domestic and laboratory animals (Cabre *et al*. (1998); Collins *et al*. (1998); Narita *et al*.(2005). Pharmacokinetic evaluation of any drug is of great importance for the evaluation therapeutic use of the drug in any species (Mahmood and Ashraf, 2010). A limited data regarding the safety of Ketoprofen in the various species is available. In most cases the genetic composition of native animals and environmental surroundings are unlike from their foreign counterparts and this affects the biodisposition of drugs. So, estimation of pharmacokinetic parameters in native animals is needed. This is therefore the present study was designed and conducted for estimation of pharmacokinetic parameters of Ketoprofen in donkeys when administered through intravenous route.

MATERIALS AND METHODS

Experimental Animals: Eight clinically healthy donkeys were purchased from the market having weight of 64kg, 80kg, 72kg, 60kg, 86kg, 76kg, 65kg and 50kg respectively. All animals were placed in the animal shed

of University of Veterinary and Animal Sciences, Lahore. The health and clinical status of all the animals were checked. (temperature, respiration, and pulse). Feed was provided with *ad libitum* water supply. All the animals were dewormed with oxfendazole. A 14 days wash out period was observed after deworming.

Experimental Drugs / Chemicals: Ketoprofen, reference standard (Sigma-Aldrich USA) was purchased. Ketoprofen (ketoject 100mg/ml) Selmore Pharmaceutical (Pvt) Limited, Lahore, was purchased from market.. HPLC grade acetonitrile, methanol, di-ethyl ether (E. Merck Germany), de-ionized water, di-potassium hydrogen phosphate were purchased from the market.

Experimental design: Each animal received an Ketoprofen @3.0 mg/kg body weight intravenous after Rehman *et al*. (2012). 5ml blood was drawn in heparinized Vacutainer before and then at 0.16, 0.33, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hours post – medication. The blood samples were centrifuged at 4000rpm for 10 minutes. Plasma was collected.

Plasma Sample Extraction: 1ml of plasma was taken and 1ml of 1.0 M hydrochloric acid was added. The mixture was vortexed for 1 minute and then 1ml of analytical grade diethyl ether was added. The mixture was again vortexed and then centrifugation was done at 4000 rpm for 10 min. The clear supernatant was taken and evaporated to dryness and reconstituted with 1ml of mobile phase as described by Rehman *et al*. (2012). Filtered through 0.22 µm syringe filter and 20 µl of the aliquot was injected into HPLC system for analysis.

HPLC Analysis: The analysis was performed on HPLC; Shimadzu LC2000 by using the already established method (Tuttle *et al*,. 2006) with some modifications. The mobile phase comprises of di-potassium hydrogen phosphate buffer and acetonitrile (75:25, v/v). Drug was separated by using a C18 reversed phase column. Column oven temperature was set at 30 °c and pH of buffer was adjusted at 7.0. The maximum absorbance λ_{max} for Ketoprofen was 254nm. 20 µl of the samples were injected into the HPLC column through an auto injector. Flow rate was 1ml/min. Ketoprofen was detected at around 10.0 minutes. The plasma ketoprofen concentration versus time data after I/V administration was analyzed.

Statistical Analysis: The values in the raw data were expressed as range, mean and SEM (standard error of means). Kinetic parameters were calculated by using kinetics software APO, version 3.02. The Pharmacokinetics of Ketoprofen in donkey was best fitted to a two-compartment model.

RESULTS AND DISCUSSION

The concentration of Ketoprofen in plasma was computed to calculate pharmacokinetic parameters. mobile phase was used to prepare stock solution of Ketoprofen (100µg/ml). Standard solutions of different strengths were prepared in plasma that was free from any drug. Then the spiked samples were analyzed on HPLC to get average regression equation as shown in figure 1.

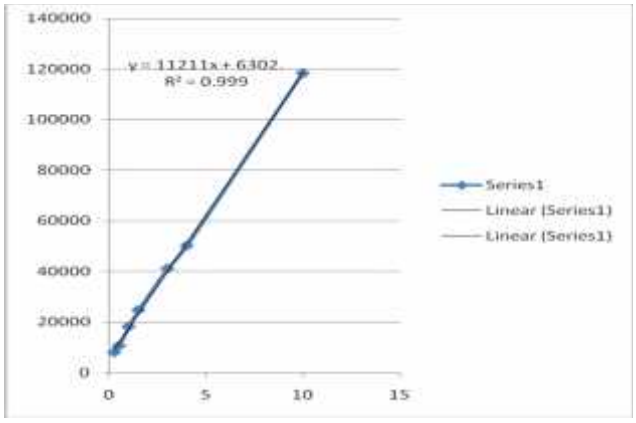

Figure 1. Calibration curve of ketoprofen in plasma at different concentrations (ug/ml)

The graphical presentation of plasma concentrations (µg/ml) of Ketoprofen in donkeys versus time is given in Figure 2.

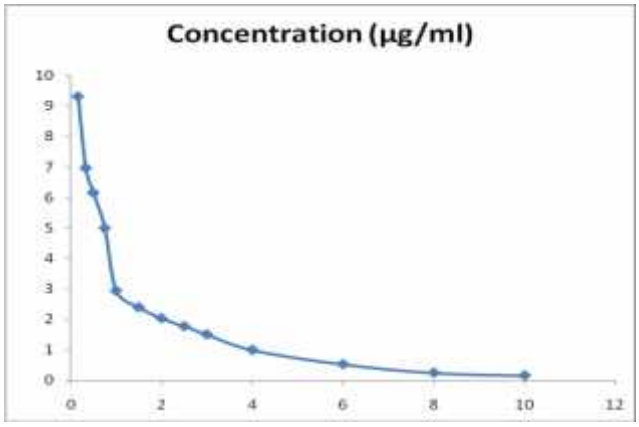

Figure 2. The graphical representation of Plasma concentration (µg/ml) vs time profiles of Ketoprofen in donkeys after intravenous administration at dose of 3.0 mg/kg BWt (n=8).

The pharmacokinetics (PK) of Ketoprofen in donkeys was best fitted to two compartment model. The PK profile is given in Table 1.

Table 1. Pharmacokinetics parameters of Ketoprofen in donkeys administered at dose of 3.0 mg/kg BWt (n=8).

Parameters	Mean±SE	Range
Area Under Curve (AUC)[h.mg/l]	17.02± 7.4	10.46-31.88
AUC poly-exponential (t= 12)	15.83±7.47	8.55-31.82
AUC trapezoidal rule (t= 12)	14.67±6.26	7.48-27.24
Clearance (CL) [l/h]	0.20±0.07	0.094-0.29
Volume of distribution. [l]	0.22±0.11	0.02-0.37
Volume of distribution. steady state [l]	0.47±0.12	0.27-0.62
Volume of distr. [l]	0.61±0.16	0.38-0.89
Half-life phase 1 [h]	0.26±0.13	0.04-0.49
Half-life phase 2 [h]	2.21±0.49	1.70-2.97
k10	1.42±1.54	0.59-5.19
k12	2.33±0.77	0.42-11.59
k21	0.97±0.33	0.65-1.6
MRT	2.44±0.48	2.06-3.31

Ketoprofen is non-steroidal anti-inflammatory drug, reported as a effective NSAID for veterinary and human use. A simple, specific and accurately developed high performance liquid chromatography (HPLC) method for the measurement of Ketoprofen in plasma was applied for the determination of Ketoprofen concentration in different plasma samples collected from healthy donkeys after IV. administration of drug. The dosage of Ketoprofen 3 mg/kg, IV was selected for characterization of pharmacokinetic parameters of Ketoprofen in donkeys. The mean pharmacokinetic parameters of Ketoprofen determined in healthy donkeys were, AUC (Area under the curve) = 17.02±2.61 μg.h/ml, Cl (clearance) = 0.21±0.02l/h/kg, V_{Dss} (Steady state volume of distribution) = 0.47±0.04 l/kg, $t_{1/2}$ (half life) = 2.2± 0.17h, and MRT (Mean Residence Time) = 2.44±0.17 hrs. The short elimination half-life and MRT were observed in donkeys when compared with horses, dogs, rats and human. The small value of AUC of Ketoprofen observed in donkeys. The lower value of volume of distribution may be due to high protein binding of Ketoprofen which limit their ability to reach extra vascular compartments. The low plasma clearance leads to suggestion that use of Ketoprofen in donkey might be beneficial. The main differences between pharmacokinetic parameters of donkeys and other species emphasized the significance in creating pharmacokinetic parameters of a specific drug in individual species instead of using the same data between species, otherwise treatment failures or toxicosis might occur. After completion of study it was observed that inter species variations exist in the pharmacokinetics of Ketoprofen and data of Ketoprofen (e.g. bioavailability and half-life) can't be extrapolated from one specie to specie. Furthermore, Ketoprofen is a safe substitute of diclofenac in veterinary practice. However we need to carry out trials for assessment of minimum effective plasma concentration of Ketoprofen in target animals in order to measure its benefits as an analgesic and anti inflammatory agent. The interaction with antibiotics must be studied as NSAIDs are usually co administered as an adjunct to antimicrobial therapy in veterinary practice. The Government of Pakistan must direct manufacturing of ketoprofen formulations to give species specific dosage regimens instead of a generalized dosage regime.

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