



RESEARCH ARTICLE

Pharmacokinetic Evaluation and Dosage Optimization of Ketoprofen in Healthy Beetal Goats

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ABSTRACT

Ketoprofen (KTP) is a frequently used non-steroidal anti-inflammatory drug (NSAID) in veterinary practice for the treatment of different inflammatory conditions. This work was carried out to evaluate the pharmacokinetic parameters of ketoprofen in healthy beetal goats. Eight goats were administered single intravenous (IV) dose of KTP (3.0mg/kg BW). Blood samples from all the goats were drawn before drug administration and then at different time intervals post administration. The KTP in plasma was estimated by using a high performance liquid Chromatography (HPLC) method. Mobile phase was mixture of phosphate buffer and acetonitrile (75:25 v/v). C18 column was used as stationary phase. The flow rate was 1.0ml/minute and temperature of column oven was adjusted to 30°C. Injection volume was 20µl. Wavelength was adjusted to 254nm. The pharmacokinetic parameters of KTP in beetal goats were calculated from plasma concentration-time data with APO MW/PHARM version 3.02 pharmacokinetic software using two compartmental model. The concentration of drug in plasma at different time intervals was calculated with regression/correlation analysis. All data are reported as mean ± SEM. The pharmacokinetic parameters determined are area under the curve (AUC) 7.711±0.60µg.h/ml, maximum concentration (C_{max}) 13.64±0.98µg/ml, clearance (Cl) 0.325±0.02L/h/kg, volume of distribution (VD) 1.40±0.132L/kg, steady state volume of distribution (VD_{ss}) 0.50±0.09L/kg, half-life (t_{1/2}) 3.10±0.37hrs, and elimination constant (Kel) 2.09±0.29L/hr. Results showed rapid elimination of ketoprofen from goat. Based on pharmacokinetics parameters, KTP at dosage of 2.47mg/kg BW is appropriate in beetal goats and may be repeated after 12 hours.

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INTRODUCTION

The inflammatory reactions are complex processes which are triggered by cell infection or tissue damage and can result in a series of chain reactions (Dong *et al.*, 2018). Inflammation is a sever tissue response to any kind of injury (Kiran *et al.*, 2018). Anti-inflammatory drugs are required to avert it. Non-steroidal anti-inflammatory drugs (NSAIDs) are generally used in animals including, ketoprofen, aspirin, phenylbutazone, flunixin meglumine, piroxicam and naproxen. They act by inhibiting the cyclooxygenases enzyme which is responsible for production of prostanoids. Ketoprofen (KTP) act as analgesic and antipyretic (Sean, 2009). It is non-selective COX-I and COX-II inhibitor (Brunton *et al.*, 2017). It is approved in animals for the treatment of inflammatory

painful illnesses linked with musculo-skeletal complications (Mozaffari *et al.*, 2010). It is administered through parenteral route in animals. It is recommended at the dosage of 2.2mg/kg BW in animals. It exists in two enantiomeric forms i.e. S and R. These forms have different pharmacokinetic parameters. It is formulated as racemic mixture. The S(+) isomer is dominated in horses, dogs and cats. The R(-) isomer is dominated in goat and sheep (Riviere and Papich, 2017). It is comparatively safe and has low toxicity as compared to flunixin meglumine and phenylbutazone in animals (Mozaffari *et al.*, 2010). Beetal is high milk and meat producing indigenous goat breed of Asian countries especially Pakistan and India. They are suitable for intensive farming system with a little or no grazing activities. The effect of biochemical interior environment of animal on the pharmacokinetics of drug is

of utmost value. The difference in the genetic makeup of animals and diverse environmental conditions may affect the bio-disposition of drugs in indigenous animals (Nawaz *et al.*, 1988; Nawaz, 1994). The pharmacokinetic evaluation of a drug in specific specie under local environmental conditions is very much important, so that appropriate dosage regime might be suggested in particular specie. This study was intended to explore the pharmacokinetic parameters of racemic ketoprofen subsequent to single IV dosage of 3mg/kg BW in beetal goats under local environmental conditions, so that specie specific dosage regimen may be suggested.

MATERIALS AND METHODS

Experimental animals: For this study eight healthy beetal goats of 6 to 9 months of age with an average weight of 35kg were purchased and placed them in animal shed of Pharmacology Department of UVAS, Lahore. Goats were dewormed and acclimatized for 2 weeks. Feed and water were provided *ad libitum*. The health and clinical status of goats were monitored through physical and hematological evaluation before medication to ensure the health of goats.

Chemicals and instruments: The pure standard of KTP was purchased from Sigma-Aldrich, USA. Injections of KTP (Ketoject®, Lot. No. KT014) were procured from Selmore pharmaceuticals (Pvt) Limited, Lahore. Acetonitrile, di-ethyl ether, methanol, di-potassium hydrogen phosphate, de-ionized water and ortho-phosphoric acid were procured from market. All were of analytical grade. Shimadzu LC2000 series HPLC equipped with a LC-20AT pump, SPD-M20A, CTO 20 AC, SIL-20AC HT auto-sampler, CBM 20A control unit, a computer with software was used. The other instruments used were centrifuge machine, rotifox 32, vortex mixer and ultrasonic bath.

Preparation of mobile phase, working and calibration standards: Di-potassium hydrogen phosphate (0.1M) buffer was prepared. Ortho-phosphoric acid was used to adjust the pH at 7.0. Phosphate buffer and acetonitrile mixture (75:25 v/v) was prepared for mobile phase. The 1mg/ml stock solution of KTP was prepared by adding 100mg of KTP standard in 100ml of mobile phase. Sequential dilution of KTP stock solution was done in mobile phase to form working standards having KTP concentrations of 100, 10, 1.0, 0.1, 0.01 and 0.001µg/ml. Plasma standards were prepared by spiking the known amount of KTP in blank plasma to get the concentrations of 10, 4, 3, 2, 1.5, 1.0, 0.5, 0.25 and 0.125 µg/ml of KTP.

Drug administration and sample collection: The 3mg/kg BW dosage of KTP administered in goat was selected based on literature described by Arifah *et al.* (2003); Banting *et al.* (2008); Koshi, (2008); Fosse *et al.* (2011); Neirinckxa *et al.* (2011). For this study single IV dosage of 3mg/kg BW of KTP was administered in goats through jugular vein. The 3-5 ml of blood from all goats were taken before KTP administration 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, 10.0, 12.0, 24.0, 48.0, 60.0, 72.0, 84.0 and 96.0hrs post administration. Plasma was separated and stored at -40°C.

Extraction of KTP from plasma: 1.0ml of plasma was added into 1.0ml of (1M) hydrochloric acid. The mixture was vortexed for one minute and then 1.0ml of di-ethyl ether was added. Again the mixture was vortexed and centrifuged for 10 minutes at 4000 rpm. The supernatant was collected and air dried till dryness. The dried extract was reconstituted with 10ml of mobile phase. Vortexed it for one minute and filtered through 0.22µm syringe filter and run on HPLC for analysis.

HPLC method/conditions: HPLC method used by Baeyens *et al.* (1998) was followed minor alterations. The method was standardized and validated. The wavelength was set at 254nm. The mobile phase was phosphate buffer and acetonitrile (75:25 v/v) having pH 7.0. A reverse phase C₁₈ column (Thermo, BDS Hypersil 5µm; 4.6 mm × 250 mm) was used. The temperature of the column oven was set at 30°C. The 20µl of the sample was injected with flow rate of 1 ml/minute.

Pharmacokinetic analysis: Different pharmacokinetic parameters were calculated from plasma concentration-time data by using APO software MW/PHARM 3.02 version. Standard two compartmental model approach was used. Following equation was used for dosage optimization.

$$\text{Dosage} = (\text{AUC} \times \text{Cl})$$

Statistical analysis: The concentrations of the drug in the plasma at different time intervals were determined with regression/correlation analysis. The results in the data were expressed as range, mean, SEM, standard deviation and coefficient of variation.

RESULTS

The Chromatogram of blank plasma spiked with KTP (3µg/ml) at 7 minutes of retention time is given in Fig. 1.

Standardization of KTP in plasma: The calibration curve of spiked plasma sample with standard KTP was drawn to evaluate the linearity of KTP in serial dilution. Different concentrations of KTP (100, 10, 1.0, 0.1, 0.01 and 0.001µg/ml) were used to calculate the average regression equation. All peaks of KTP concentrations showed specificity and linearity within the concentration of 0.125 to 10µg/ml. The calibration curve displayed a good linearity over the range of concentrations evaluated: $Y = 49.659X - 1.5001$, $R^2 = 0.997$. Regression curve is also shown in Fig. 2. The recovery of KTP from the spiked plasma was in the range of 85-90%. The intraday and inter day assays were performed by testing three different concentration of 0.125, 1.25 and 3 µg/ml of KTP. Five reading were taken for intraday assay. Inter day assays were carried out for five days. The recovery was found to be more than 80%. The Limit of Detection (LOD) of KTP was 0.01µg/ml and Limit of Quantification (LOQ) was 0.125µg/ml in the plasma. The stock solutions of KTP in plasma were stable for 3 months at 4°C.

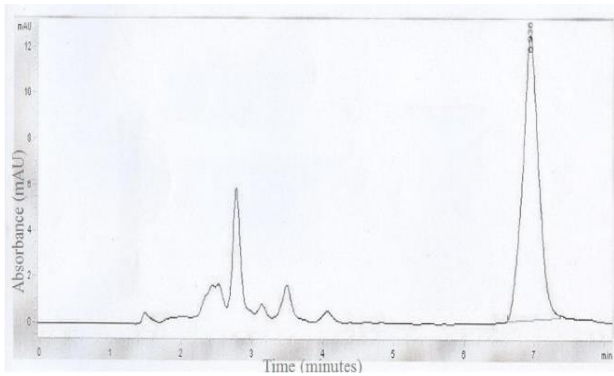


Fig. 1: Chromatogram of blank plasma spiked with KTP (3µg/ml) at 7 minutes. Time (minutes) on x-axis versus Absorbance (mAU) on y-axis.

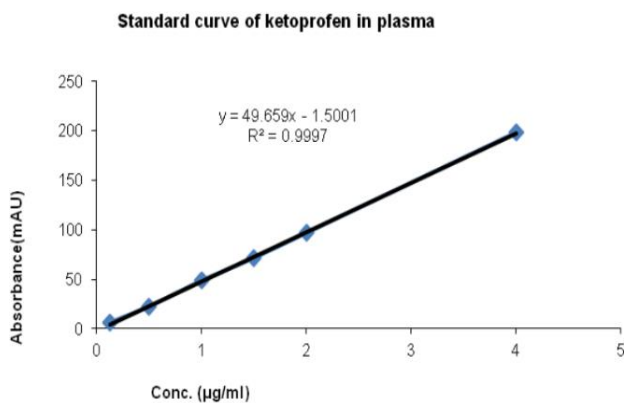


Fig. 2: KTP standard calibration curve at different concentrations.

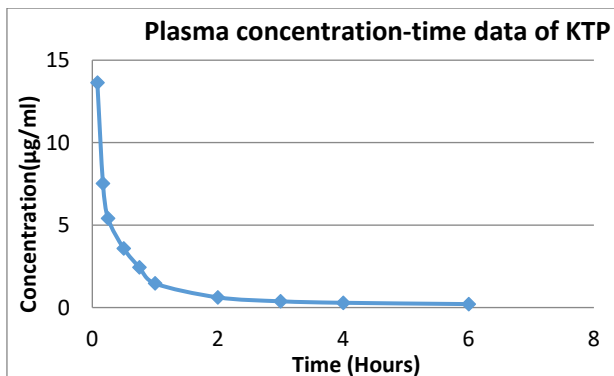


Fig. 3: Semilogarithmic representation of group means of plasma concentration-time data of KTP in beetal goats after IV administration at dosage of 3mg/kg BW (n=8).

Table 1: Group means for plasma concentration-time data of KTP in beetal goats after single IV administration at a dosage of 3 mg/kg BW (n=8).

Time (hours)	Range (µg/ml)	Mean concentration (µg/ml)	SEM	Std. Dev	CV%
0.08	10.95-16.39	13.64	0.65	1.845	13.52
0.17	6.64-9.86	7.52	0.38	1.069	14.21
0.25	3.46-6.63	5.41	0.36	1.024	18.92
0.51	2.21-5.57	3.59	0.47	1.342	37.38
0.75	1.31-3.97	2.44	0.38	1.075	44.05
1	0.80-2.65	1.46	0.22	0.612	41.91
2	0.21-1.41	0.62	0.13	0.373	60.16
3	0.19-0.53	0.38	0.05	0.145	23.38
4	0.16-0.42	0.29	0.04	0.115	39.65
6	0.10-0.33	0.21	0.03	0.076	36.19
12	ND	ND	ND	ND	ND

SEM= Standard Error Means, Std. Dev= Standard Deviation, CV%=Coefficient of Variance percentage, ND=Not Detected.

Pharmacokinetics parameters: The groups mean plasma concentrations (µg/ml)-time data of KTP following intravenous administration of 3mg/kg BW in goats was calculated and results are described in Table 1. The groups mean plasma concentrations-time profile was prepared semi-logarithmically and is given in Fig. 3. The (mean±SEM) KTP pharmacokinetic parameters determined in beetal goats were area under curve (AUC) µg.h/ml, maximum concentration (C_{max}), clearance (Cl) L/h/kg, volume of distribution (VD) L/kg, steady state volume of distribution (VD_{ss}) L/kg, half-life ($t_{1/2}$) hrs and Elimination constant (Kel) L/hr. The results are demonstrated in Table 2.

KTP dosage optimization: The dosage of KTP in beetal goats was optimized by multiplying the values of AUC with Clearance (Dosage = AUC x Cl). An IV dosage of 2.47mg/kg body weight after every 12 hours interval is optimized in beetal goats under local environmental conditions.

DISCUSSION

The pharmacokinetics of KTP in beetal goats under local environmental conditions has never been studied and reported earlier. The Mean ± SEM AUC (area under the curve) determined in this study was 7.711±0.60µg.h/ml. The results are comparable with Landoni (1999) who evaluated the AUC of ketoprofen 8.728±0.608µg.h/ml and Ali (2012) who stated the AUC of ketoprofen in sheep as 5.47±2.72h.mg/l. The pharmacokinetic parameters calculated in this study were maximum concentration (C_{max}) 13.64±0.98µg/ml, clearance (Cl) 0.325±0.02 L/h/kg, Volume of distribution (VD) 1.40±0.132L/kg, Steady state volume of distribution (VD_{ss}) 0.50±0.09L/kg, half-life ($t_{1/2}$) 3.10±0.37hrs and Elimination constant (Kel) 2.09±0.292L/hr. These results are similar and comparable with Musser *et al.* (1998) who determined the pharmacokinetic parameters of Ketoprofen after single IV administration at the dosage of 2.2 mg/kg of BW in Toggenburg goats.

Our findings of pharmacokinetic parameters in beetal goats are comparable with pharmacokinetics values obtained in local horses (Rehman *et al.*, 2012). These values are diverse when matched with other species (buffalo calf, and dog) and also dissimilar from mares, donkeys and camels. The larger volume of distribution may result in fast elimination of KTP in goats. KTP is excreted mostly as metabolites formed after biotransformation. The enhanced metabolizing activity in the liver and other organs of sheep and goat may be attributed to fast elimination of KTP (Ali *et al.*, 2012), (Wesongah *et al.*, 2004) isometamidium chloride (ElSheikh, 1997), meloxicam (Tahir *et al.*, 2011) as compared to other species. In this study, dosage of KTP in beetal goats was also optimized and it was found to be 2.47mg/kg BW. The KTP in beetal goats can be repeated after 12 hours because the drug was not detected in plasma at 12th hour. The results of this study are in line with previous findings, that 2.47mg/kg BW dosage is appropriate in goats (Banting *et al.*, 2008; Singh *et al.*, 2009; Riviere and Papich, 2017) and can be repeated after 12 hours (Musser *et al.*, 1998; Landoni *et al.*, 1999; Arifah *et al.*, 2003).

Table 2: Group means of KTP Pharmacokinetics parameters in beetal goats after IV administration at dosage of 3mg/kg BW (n=8)

Pharmacokinetic Parameters	Range	Mean	SEM	S.D	CV%
Area under Curve (AUC) $\mu\text{g}\cdot\text{h}/\text{ml}$	5.46-9.23	7.71	0.60	1.72	22.31
Maximum Concentration (C_{max}) $\mu\text{g}/\text{ml}$	11.67-15.79	13.64	0.98	2.54	27.56
Clearance (Cl) $\text{l}/\text{hr}/\text{kg}$	0.26-0.45	0.32	0.02	0.07	23.07
Volume of distribution (VD) l/kg	0.84-1.96	1.40	0.13	0.37	26.45
Volume of distribution at steady state (VD _{ss}) l/kg	0.26-1.00	0.50	0.09	0.24	49.50
Half Life($t_{1/2}$) hr	1.94-5.17	3.10	0.37	1.06	34.38
Elimination constant (Kel) l/hr	1.02-2.97	2.09	0.29	0.82	39.52

SEM= Standard Error Means, Std. Dev= Standard Deviation, CV%=Co-efficient of Variance percentage.

Conclusions: In this study pharmacokinetics of ketoprofen was carried out using sensitive HPLC method. Pharmacokinetic parameters were calculated. KTP showed rapid elimination from goat. The dosage of KTP was optimized based on pharmacokinetic parameters. KTP at the dosage of 2.47mg/kg body weight (BW) is appropriate in beetal goats and may be repeated after 12 hours.

Authors contribution: ZUR planned this research work. MA supervised this research. ZUR, MA, MAR developed and standardized the laboratory procedures to conduct this research work. All the authors wrote, revised and approved the manuscript.

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