

PHARMACOKINETICS OF KETOPROFEN IN HEALTHY BUFFALO CALVES IN PAKISTAN

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ABSTRACT

The present research project was designed to study the pharmacokinetics of Ketoprofen (KTP), a non-steroidal anti-inflammatory drug (NSAID) in local healthy buffalo calves. For this purpose, eight healthy buffalo calves were administered, a single intravenous bolus of KTP at the dose of 3.0 mg/kg body weight through jugular vein. Blood samples (3-5ml) were drawn in heparinized vacutainers, 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, 10.0, 12.0, 24.0, 48.0, 60.0, 72.0, 84.0, 96.0 hrs post medication. The plasma was separated by centrifugation at 4000 rpm for 10 minutes and stored at -80°C till analyzed. The concentration of KTP in plasma was determined through a standardized HPLC (high performance liquid Chromatography) method. The concentration -time profile of KTP of each animal was prepared semi-logarithmically. The plasma concentration-time data was analyzed by a computer based pharmacokinetic software, APO, Version 3.02, and the pharmacokinetic parameters of ketoprofen in buffalo calves were calculated as Mean \pm SEM, AUC (Area Under Curve) $14.42 \pm 1.97 \mu\text{g}\cdot\text{h/ml}$, Cl (Clearance) $0.19 \pm 0.025 \text{ l/hr/kg}$, $t_{1/2}$ (Half Life) $3.58 \pm 0.418 \text{ hr}$, VD (Volume of Distribution) $0.985 \pm 0.175 \text{ l/kg}$, VD_{ss} (Volume of distribution at Steady State) $0.551 \pm 0.0895 \text{ l/kg}$, and K_{el} (Elimination Rate Constant) $1.46 \pm 0.196 \text{ l/hr}$, respectively.

Keywords: HPLC, Ketoprofen, NSAID, Pharmacokinetics, Intravenous administration.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used in humans and animals for mild to moderate pain disorders like arthritis, osteoarthritis, ankylosing spondylitis and acute musculo-skeletal disorders (Brooks and Day 1991, Abransomet *al.*, 1989). It is reported in literature that drug ketoprofen (KTP) is effective and safe NSAID in buffalo calves (Boothe, 2001, Barhate *et al.*, 2009). In Pakistan, KTP is a registered drug. It is frequently prescribed in bovine, equine, canine and humans as an anti pyretic, analgesic and anti inflammatory drug. It is marketed/sold for use in cattle without indigenous pharmacokinetic study. KTP is a NSAID. Chemically it is 2-(3-benzoyl phenyl) propionic acid or 3- benzoyl alpha-methyl benzene acetic acid. KTP exists in two enantiomeric forms, S and R which have different half lives. It is also available as racemic mixture. Favorable pharmacokinetic profile of KTP 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 buffalo calves under local conditions of Pakistan after intravenous administration at the dose of 3mg/kg body weight. So that appropriate dosage recommendations could be made.

MATERIALS AND METHOD

Experimental Animals: Eight healthy buffalo calves with an average weight of 105kg were taken and used in this research project. Buffalo calves were tagged for easy identification and were acclimatized to experimental environment. Food was offered to buffalo calves with water supply *ad libitum*. Buffalo calves were checked daily by a qualified veterinarian to know their health status throughout the experiment.

Drugs/Chemicals: Ketoprofen external standard (Sigma) was procured from the company. HPLC grade water, diethyl ether, acetonitrile (E. Merck Germany) were used. Injections of ketoprofen (20ml) manufactured by Selmore Pharmaceuticals (Pvt) Limited, Lahore, Pakistan were used during experiment. All the chemicals used were of analytical grade.

Drug Treatment and Sampling Protocol: Ketoprofen at the dose of 3 mg/kg body weight was administered intravenously via jugular vein into the buffalo calves. Blood samples of each buffalo calve (3-5 ml) were drawn and collected in heparinized vacutainers 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, 10.0, 12.0, 24.0, 48.0, 60.0, 72.0, 84.0 and 96.0 hours post medication. Plasma was

separated by centrifugation of blood samples at 4000 rpm for 10 minutes and then plasma was stored at -80°C till analyzed.

Ketoprofen Extraction from plasma: KTP was extracted through acidified extraction. To 1ml of plasma, 1ml of 1.0 M hydrochloric acid was added. Vortexed this material to high speed for 1 min. 1.0 ml of analytical grade diethyl ether was added. The mixture was vortexed again and centrifuged at 4000 xg for 10 min. Took the clear supernatant and evaporated it to dryness. Reconstituted with 1ml of mobile phase. Filtered it with 0.22 μm syringe filter and 20 μl , was injected into HPLC system for analysis.

HPLC analysis: Analysis was done through HPLC method (Baeyens *et al.*, 1998), which was modified, standardized and validated. Shimadzu LC2000 instrument, equipped with a LC-20AT VP pump, a SIL-20AC HT auto-sampler, SPD-M20A, CTO 20 AC and CBM 20A control unit was used for analysis. 20 μl of the sample was run into HPLC system via auto sampler with flow rate of 1 ml/minute. Mixture of 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 \times 250 mm) was used as stationary phase. Separation was achieved and ketoprofen spiked at 7.0 minutes (retention time) at a wavelength of 254nm. Oven temperature was set at 30°C . The limit of quantification was 0.125 μg . The plasma concentration ($\mu\text{g/ml}$) versus time profile of KTP in buffalo calves was prepared semi-logarithmically. Standard curve was prepared by plotting concentration ($\mu\text{g/ml}$) versus area (mAU).

Pharmacokinetic Analysis: The pharmacokinetic parameters of ketoprofen in buffalo calves were analyzed by computer based pharmacokinetic software, APO pharmacological analysis MW /PHARM version 3.02 (Holland, 1987). Two compartmental model technique was used for the analysis. The following pharmacokinetic equations were used for different calculations: Dose=Cl x AUC

$\text{Cl} = \text{Dose}/\text{AUC}$; $\text{AUMC} = \text{MRT} \times \text{AUC}$;

$\text{Dose} = \text{VD}_{\text{ss}} \times \text{AUC}$; $\text{AUMC} = \text{VD} \times \text{Dose}/\text{Kel} \times \text{AUC}$

$\text{VD} = \text{Dose}/\text{concentration measured in plasma}$; $\text{VD}_{\text{ss}} = \text{Cl} \times \text{MRT}$

$\text{HL}(t_{1/2}) = 0.693/\text{Kel}$

Statistical Analysis: The Microsoft Excel 2007 was used for computation and analysis of the drug concentrations in plasma versus time data and the graphics. The individual and group means data for plasma concentration-time and pharmacokinetic parameters were determined statistically and presented as individual mean, group mean, mean \pm SEM, standard deviation, coefficient of variation and medians.

RESULTS AND DISCUSSION

The group means plasma concentration-time data and pharmacokinetics of KTP at different time intervals following I/V administration in buffalo calves @ 3mg/kg body weight were determined and are presented in Table 1 and 2. The semi logarithmic graph of group mean plasma conc. time data was plotted and is given in fig 1.

The mean \pm SEM pharmacokinetic parameters of KTP determined in healthy buffalo calves in the present study are: AUC (Area under the curve) = $14.42 \pm 1.97 \mu\text{g.h/ml}$, Cl (clearance) = $0.190 \pm 0.025 \text{ l/h/kg}$, VD (Volume of distribution) = $0.985 \pm 0.175 \text{ l/kg}$, VD_{ss} (Steady state volume of distribution) = $0.551 \pm 0.0895 \text{ l/kg}$, $t_{1/2}$ (half life) = $3.58 \pm 0.418 \text{ h}$ and Kel (Elimination constant) = $1.46 \pm 0.196 \text{ l/hr}$.

These pharmacokinetic values are comparable with reported values in Kankrej cow calves regarding area under conc. time curve (AUC) and body clearance (Cl) (Barhate *et al.*, 2009). The pharmacokinetic values of KTP determined in Kankrej cow calves after I/V administration of 3mg/kg body weight are, VD (volume of distribution) = $0.48 \pm 0.03 \text{ L/kg}$, (AUC) area under plasma drug concentration = $14.91 \pm 0.69 \mu\text{g.h/ml}$, ($t_{1/2}$) elimination half-life = $1.58 \pm 0.05 \text{ h}$, and (Cl) total body clearance, is $3.29 \pm 0.14 \text{ ml/h/kg} = 0.329 \text{ L/h/kg}$.

The pharmacokinetic parameters of KTP in buffalo calves are not comparable to the reported values in cattle, goats, sheep, horses, donkeys, rat and humans. These values are different even among dairy cattle, sheep and goat despite the fact that all species are ruminants. It is reported in many studies that interspecies / inter ethnic variations in clinical response to ketoprofen exist among different animal species and pharmacokinetic data cannot be extrapolated from one species to another (Lees *et al.*, 1991, Xu and Dong, 2001, Mose and Bertone, 2002, Dasandiet *et al.*, 2002, Rani *et al.*, 2004). The genetic factors and the environment have also noticeable impact on drug disposition (Nawaz *et al.*, 1988, Vesell, 1997).

The pharmacokinetic parameters of KTP in buffalo calves are somewhat comparable to the values in dogs (Montoya *et al.*, 2004). The comparatively short values of VD and VD_{ss} may be due to high protein binding of KTP in buffalo calves (92.8%). Heavily protein bound drugs have long plasma half lives (Sindhu and Ram Pal., 2007). However protein binding was not measured in this study.

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 (Koshi *et al.*, 2008, Arifahet *et al.*, 2003, Arifahet *et al.*, 2001, Boothe, 2001, Landoniet *et al.*, 1995, Landoniet *et al.*, 1999, Bantinget *et al.*, 2008). In this study, dose was also calculated by putting values of AUC

and Clearance in formula (Dose= AUC x Cl). In buffalo calves the dose was found to be 2.7mg/kg body weight. The dose was rounded off to 3mg/kg body weight. In the present study an I/V dose of 3mg/kg body weight is suggested in buffalo calves. The dose could be same in different animals but different dosing intervals are required for different species. The dose should be repeated after 24 hours in buffalo calves. In this study, no

toxicity was observed in any animal. Thus, it is in line with previous findings, the above dose in target animals is safe (Boothe, 2001, Banting *et al.*, 2008, Singh *et al.*, 2009, Mozaffari *et al.*, 2010, Kantor, 1986). The pharmacokinetics of KTP in calves may be different from adult due to difference in age and physiological status like lactation and gestation (Igarza *et al.*, 2004, Julia *et al.*, 2006, Fosse *et al.*, 2011).

Table 1. Group means for plasma conc. time data ($\mu\text{g/ml}$) of KTP after intravenous administration @ 3mg/kg body weight in buffalo calves. (N=8).

TIME (hours)	RANGE ($\mu\text{g/ml}$)	MEAN conc. ($\mu\text{g/ml}$)	SEM	SD	CV%	MEDIAN
0.08	10.02-38.12	19.87	4.09	11.56	58.178	14.6
0.17	5.66-16.89	11.06	1.30	3.664	33.12	10.275
0.25	3.53-12.78	8.00	1.17	3.322	41.52	8.035
0.5	2.57-9.29	5.46	0.88	2.495	45.69	5.2
0.75	1.97-4.91	3.53	0.36	1.031	29.20	3.705
1	0.83-4.40	2.88	0.44	1.244	43.19	2.98
2	0.82-2.42	1.53	0.21	0.607	39.67	1.51
3	0.66-0.42	1.11	0.21	0.596	53.69	0.95
4	0.45-1.59	0.84	0.14	0.396	47.14	0.825
6	0.25-0.94	0.51	0.08	0.221	43.33	0.455
8	0.18-0.78	0.38	0.08	3.664	64.21	0.285

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

Pharmacokinetic Parameters	Unit	Range	Mean	SEM	S.D	CV%	Median
AUC (Area under the curve)	$\mu\text{g} \cdot \text{h} / \text{ml}$	8.78-23.32	14.42	1.97	5.56	38.55	13.6
Cl (Clearance)	l/hr/kg	0.097-0.314	0.190	0.025	0.0697	36.68	0.192
VD (Volume of distribution)	l/kg	0.436-1.854	0.985	0.175	0.494	50.15	0.889
VD _{ss} (volume of distribution at steady state)	l/kg	0.223-1.85	0.551	0.0895	0.2531	45.93	0.493
HL($t_{1/2}$) Half Life	hr	2.31-5.39	3.58	0.418	1.1843	33.08	3.204
Kel (Elimination constant)	l/hr	0.744-2.118	1.46	0.196	0.5534	37.90	1.477

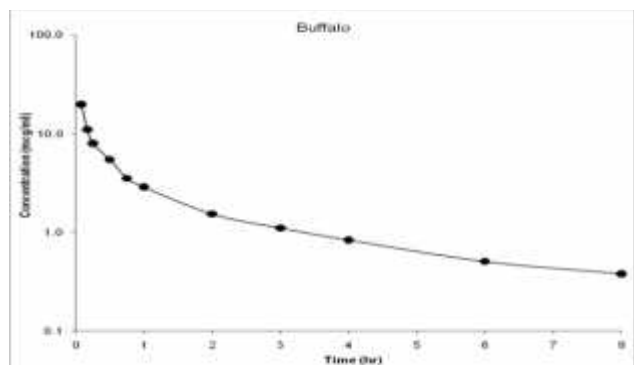


Figure 1. Graph of group means for plasma conc. time data ($\mu\text{g/ml}$) of KTP in buffalo calves after IV administration @ 3mg/kg body weight (N=8).

However there is a need to conduct efficacy trials for the assessment of minimum effective plasma concentration of KTP in buffalo calves in order to get its benefits as an analgesic and anti-inflammatory agent. The Government of Pakistan must direct manufacturers of

drugs to provide indigenous pharmacokinetic data of drug at the time of registration of drug and must mention species specific dosage regimens on product inserts and labels of drug instead of giving a generalized dosage regime.

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