

PHARMACOKINETIC STUDY OF KETOPROFEN IN HEALTHY SHEEP UNDER LOCAL CONDITIONS OF PAKISTAN

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ABSTRACT

Pharmacokinetic of ketoprofen was studied in sheep after a single intravenous dose of 3mg/kg body mass. Blood samples were collected before and within 24 hours after drug administration. Plasma was separated immediately and concentration of ketoprofen at different time intervals were determined by validated HPLC method by using phosphate buffer pH:7.0 and acetonitrile (75:25) as mobile phase at wavelength of 254nm. Pharmacokinetic parameters were determined from the plasma concentration-time curves of by using two-compartment model. On average with ketoprofen, the area under curve (AUC) was 5.47 ± 2.72 h.mg/L, distribution half life ($t_{1/2\alpha}$) was 0.12 ± 0.10 h, elimination half life ($t_{1/2\beta}$) was 1.91 ± 0.95 h, total body clearance (cl) was 0.65 ± 0.25 L/kg/h, steady state volume of distribution (vdss) was 0.82 ± 0.46 L/kg, maximum plasma concentration (C_{max}) was 6.68 ± 2.28 mg/L and time to reach maximum concentration (T_{max}) was 0.12 ± 0.02 h. Pharmacokinetic parameters showed that drug is rapidly eliminated from Pakistani sheep so dosage adjustment is required and dose should be higher than the recommended dose.

Key words: Pharmacokinetics, Ketoprofen, High Performance liquid, Area under curve, Peak plasma concentration, time to reach maximum plasma concentration.

INTRODUCTION

Ketoprofen (KP) is a non steroid anti inflammatory drugs (NSAIDS) and also acts as analgesic and antipyretic drug (Sean, 2009). It produces comparable pharmacological actions as other drugs of this family such as fenoprofen, naproxen, and ibuprofen and is used in various painful conditions in different species. The principal mechanism of action is that it acts by inhibition of cyclo-oxygenase that is responsible for production of prostanoids (Vane, 1971) and it is thought to be non selective COX-1 and COX-II inhibitor. (Hardman and Limbird, 1996; Charles and Robert, 2003). Chemical name of ketoprofen is (RS)-2-(3-Benzoylphenyl) propionic acid. (Ph. Eur.) as given in fig 1

Absorption and bioavailability of ketoprofen is rapid in humans, dogs and rats (Meunier and Verbeeck 1999 ;Granero and Amidon, 2008; Allegrini *et al.* 2009). Ketoprofen is known to enter synovial fluid in cattle. (Brink *et al.* 1998; Verde *et al.* 2001). It is eliminated via the kidneys as a conjugated metabolite and also as unchanged drug. (Laurence *et al.* 2008). Excretion of a drug is of great importance as some drugs are excreted by bile, saliva, urine and sweat. In dairy animals drugs can also be excreted by milk. (De graves *et al.* 1996,). Drug Interactions involve avoid combination of ketoprofen with other anti inflammatory drugs as may produce synergistic effect. (Radwa, 2000; Qiu *et al.* 2007 .) Avoid using in animals allergic to aspirin (Barbra, 2010).

Pharmacokinetic studies provide necessary data for the calculation of dosage regimen of the drug. Due to influence of biochemical interior milieu of organism on the disposition and fate of drug, there has been increasing application of pharmacokinetic studies to describe the kinetic in specific environment and individualize the dose. In most cases the genetic makeup of indigenous animals and environmental conditions are different from their foreign counterparts and this affects the biodisposition of drugs. So, evaluation of kinetic parameters in indigenous animal species is necessary (Nawaz *et al.* 1988 and Nawaz, 1994). Although on sheep chiral pharmacokinetic studies of R (-) and S (+) enantiomer of ketoprofen by Landoni *et al.* 1999 and Arifah *et al.* 2003 has been published but the present study was designed to investigate the pharmacokinetics of Racemic ketoprofen following single intravenous administration at the dose rate of 3mg/Kg body weight in Sheep of Pakistan. However the chiral analysis to differentiate between R and S enantiomer was not performed.

MATERIALS AND METHODS

Study subjects: The study was conducted in 8 healthy sheep. On the basis of clinical examination and routine laboratory tests, healthy sheep were selected for the undertaking study. For acclimatization to the experimentation environment, all animals were kept for one week in the animal shed of the Pattoki campus, University of Veterinary and Animal Sciences, Lahore.

Feed / Food was provided as per standard requirement with water supply *ad libitum*. The sheep were fasted over-night before the experiment.

Inclusion criteria: Healthy and adult sheep with age between 1 to 3 years and weight less than 50kg were selected. The health and clinical status was monitored on the basis of physical parameters (body temperature, color of conjunctiva, respiration and pulse rate) and blood parameters (complete blood counts (CBC) serum chemistry (LFT) before medication to ensure sheep is healthy. All the animals were dewormed with Oxfendazole (Oxifax liquid) manufactured by Glaxo Wellcome Ltd, Pakistan. Demographic data is given in figure 2.

Drug administration and blood sample collection: A single dose of 3mg/kg body weight (IV) was used for sheep after overnight fasting. Blood samples were collected up to 24 hours at regular time intervals of 0, 0.16, 0.33, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24 hrs. 4-5ml blood was taken through jugular vein to vacutainer containing anti-coagulant. Blood samples were centrifuged immediately to separate plasma at 4000 rpm for 10 minutes and stored at -20°C till analyzed.

Chemicals and reagents: Ketoprofen reference standard was obtained through a local Pharmaceutical company. Acetonitrile, phosphate buffer, methanol and phosphoric acid were purchased from Merck, Germany.

Instruments: The High performance liquid chromatography with autosampler (LC-20A, Shimadzu, Japan), having a reverse phase column (Lichrospher 5 µm RP-18 column (250x4.6 mm), Merck, Germany) and a computer (Pentium IV) with software (LC-20 A for data handling). The other instruments used were: Centrifuge machine, Vortex mixer and ultrasonic bath.

Chromatographic conditions: A standard method was followed with small modifications. (Baeyens *et al.* 1998) All the chromatographic analyses were carried out at 30 °C. The compound was separated isocratically with a mobile phase consisting of acetonitrile and 0.05M dipotassium hydrogen phosphate (25: 75). The pH was a very sensitive factor in this separation, which was adjusted at 7.0 ± 0.1 with the help of ortho-phosphoric acid. Before use, the mobile phase was filtered by passing through a 0.45 µm membrane filter and was sonicated through sonicator for 10 minutes. A constant flow rate of 1.0 mL/min was maintained. The analyte was monitored at 254 nm by using UV-Vis detector. Injection size was 20µl.

Preparation of mobile phase: 8.7 g of dipotassium hydrogen phosphate was weighed and dissolved in 500ml of water. Buffer was sonicated in ultrasonic bath and pH was adjusted with orthophosphoric acid to 7. Buffer and

acetonitrile was filtered through 0.45µm filter paper and run on HPLC with acetonitrile at ratio of 75: 25.

Standard solution: Standard stock solution (1000 µg/ml) of ketoprofen was prepared by dissolving 10mg of ketoprofen in 10 ml of mobile phase. A series of standard working solutions containing drug are also prepared in mobile phase. The stock and working solutions were stored in the dark under refrigeration. Further dilutions were prepared in plasma ranging from 10µg/ml to 0.125µg/ml.

Extraction of plasma samples: Drug was extracted from plasma by taking 1 ml plasma and adding 0.1 M HCl to it in glass tube and was vortexed. 2 ml of di-ethyl ether was added to the tube and vortexed again. The tubes were centrifuged at 4000 rpm for 10 minutes. The transparent supernatant layer of di-ethyl ether was separated by micropipette to 25ml glass beaker and evaporated till dryness. After evaporation, drug residue was reconstituted with 1 ml of mobile phase and vortexed. The sample was filtered through 0.2µm filter and transferred to HPLC vials and run on HPLC. The sample volume was 20µl.

Pharmacokinetic analysis: Different pharmacokinetic parameters were calculated on the basis of plasma concentration of the ketoprofen obtained from each sheep at different time intervals. For this purpose PC-Computer Program, APO, MWPHARM version 3.02, a MEDIWARE product, Holland, was used for two-compartment analysis.

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 plasma concentrations of the drug were calculated with regression/correlation analysis. All data are reported as the mean \pm SD.

RESULTS AND DISCUSSION

As illustrated in Figure 3, the blank plasma had no interfering peak at the retention time of interest. Peak in spiked plasma is given in figure 4.

Calibration of ketoprofen concentration: Concentration of ketoprofen was first calibrated in plasma by using HPLC. Calibration was done to find out **linearity** of ketoprofen in serial dilutions. Stock solution of ketoprofen (1000µg/ml) was prepared in mobile phase. Standard dilutions were prepared in blank plasma at concentration range of 0.125 µg/ml - 10µg/ml and run in HPLC to obtain average regression equation. The standard curve showed a good linearity over the range of concentrations examined: $Y = 75482X + 8429$, $R^2 = 0.999$. Regression curve is also shown in fig 5.

Pharmacokinetic parameters: The mean \pm SD plasma concentration of ketoprofen vs time obtained following intravenous administration of 8 sheep are plotted in Figure 6. Pharmacokinetic parameters were evaluated by using two compartmental model approach. All the parameters obtained by 2 compartment model are given in Table 1.

The purpose of this study was to evaluate the ketoprofen pharmacokinetic. Area under curve shows the presence of drug in the body and for ketoprofen AUC_{0-t} was 5.47 ± 2.72 h.mg/l after intravenous administration. CLEARANCE with 2 compartmental analysis was 0.65 ± 0.25 L/kg/h, so it was seen that ketoprofen start rapidly clearing from the body. The mean VOLUME OF DISTRIBUTION after 3mg dose volume of distribution was more (0.82 ± 0.46 L/kg)

The factors that affect volume of distribution may be due to the difference in one or combination of the following.

- I. The volume of tissues in which drug distributes.
- II. Partition coefficient of the drug between tissues and circulatory blood.
- III. The blood flow to the tissues.
- IV. Binding of the drug to the plasma or tissue proteins. The volume of distribution more than one indicates tissue localization of the drug more than that in the blood.

Distribution half life according to two compartmental model was 0.12 ± 0.10 hours and **Elimination half life** was 1.91 ± 0.95 hrs. (0.96-2.86 hrs). However, according to literature data (Hardman and Limbird, 1996 ; Charles and Robert, 2003) the elimination half life 1 to 3 hours. So the half life obtained in our research work lies within range.

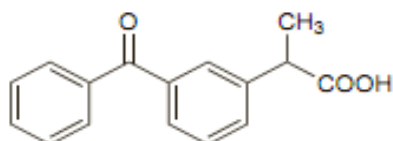


Figure.1 Structure of ketoprofen

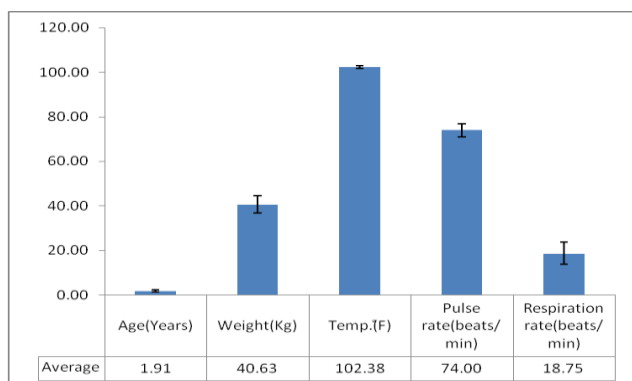


Figure.2 Mean and S.D demographic data of sheep

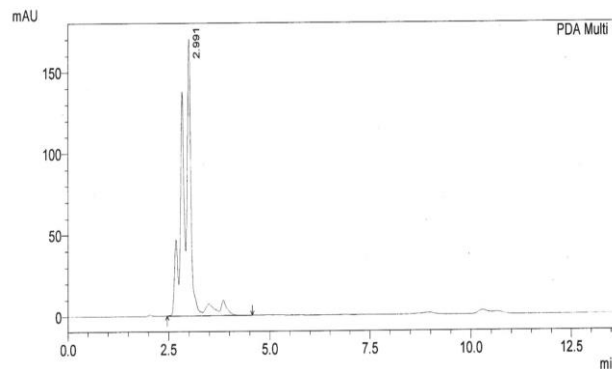


Figure 3: Chromatogram in blank plasma

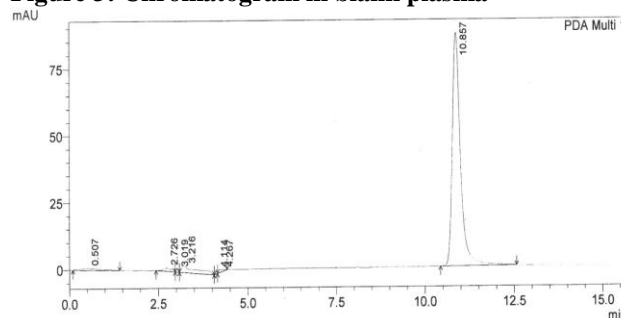


Figure 4: Chromatogram of ketoprofen (10ug/ml) in stock solution.

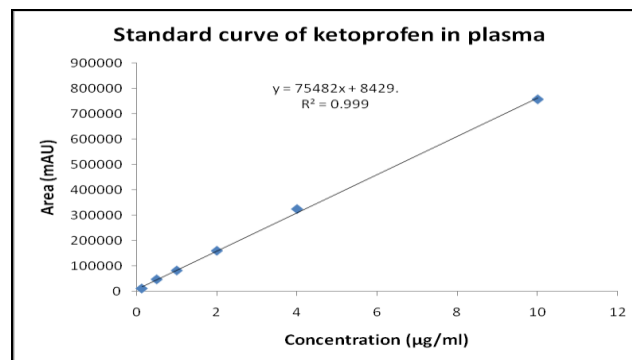


Fig 5: Calibration curve of ketoprofen in plasma at different concentrations (ug/ml).

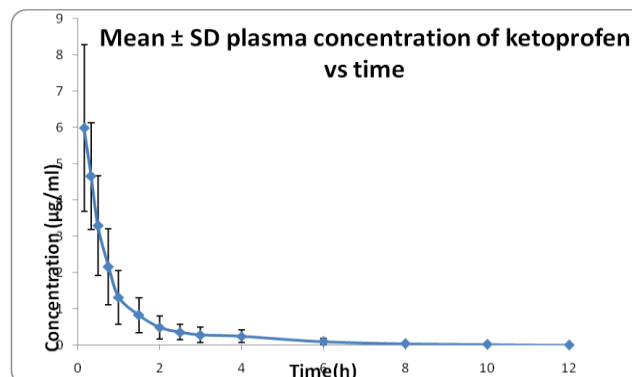


Figure 6: Graphical representation of average plasma concentration, time interval and standard deviation of ketoprofen in healthy sheep.

Table 1. Two compartment pharmacokinetic parameters of ketoprofen 3mg following i. v administration in sheep

Parameterse	2 compartment parameters	
	Mean	± SD
Total AUC (ug.h/ml)	5.47	2.72
Tmax (h)	0.12	0.06
Cmax (ug/ml)	6.68	2.84
Half life (h)	1.91	0.95
Clearance	31.22	18.12
MRT (h)	1.52	0.58
Vd (L)	84	75.45
Vss (L)	40.74	33.06
Elimination rate	0.64	0.37

Peak plasma concentration was 6.68 ± 2.28 mg/L and mean plasma drug concentration data showed that after 6 hours the plasma concentration of ketoprofen started decrease and was not detectable after 8 – 10 hours. According to previous study, Arifah *et al.* 2001, the mean concentration in exudate was constant between one and six hours with concentrations then decreasing to undetectable levels by nine hours

Conclusion: The aim of this study was to evaluate the pharmacokinetic parameters of ketoprofen in sheep after giving 3mg/kg intravenous dose. According to literature, ketoprofen concentration in plasma is maximum at 12 hours reside for 24 hours. (Laurence et al, 2008) However, according to this study, the concentration of ketoprofen starts decline at six hours and drug was eliminated from body at 12 hours. It means dosage adjustment is required. Difference with the literature could be due to decrease volume of tissue to which the drug distribute, decrease blood flow, the plasma protein binding, environment, fast metabolism, genetics etc. However, more studies are required to evaluate the genetic effect on pharmacokinetic of ketoprofen in sheep, to verify the factors that are cause of difference. Also studies of ketoprofen are required in established pain model for further modification in dose.

REFERENCES

- Allegrini, A. L. Nuzzo, M. Zucchelli, A. T. Scaringi, S. Felaco, D. Giangreco, D. Pavone, E. Toniato, A. Mezzetti, S. Martinotti, S. Comuzio and M. Di Grigoli (2009). Fast HPLC method for the determination of ketoprofen in human plasma using a monolithic column and its application to a comparative bioavailability study in man. *Arzneimittelforschung*. 59(3):135-140.
- Arifah, A. K., M. F. Landoni and P. Lees (2001). Pharmacodynamics and pharmacokinetics of ketoprofen enantiomers in sheep. *Am. J. Vet. Res.* 62(1):77-86.
- Arifah, A. K., M. F. Landoni and O. Lees (2003). Pharmacodynamics, chiral pharmacokinetics and PK-PD modelling of ketoprofen in the goat. *J. Vet. Pharmacol. Ther.* 26(2):139-150.
- Baeyens, W. R. G., G. Van der Weken, J. Haustraete, H. Y. Aboul-Enein, S. Corveleyn and J. P. Remon (1998). Direct HPLC analysis of ketoprofen in horse plasma by applying an ADS-restricted access-phase. *Br. J. Clin. Pharmacol* 45 (6): 158-161.
- Barbra Forney (2010). Ketoprofen for Veterinary Use. Available from URL: <http://www.wedgewoodpetrx.com/learning-center/professional-monographs/ketoprofen-for-veterinary-use.html>
- Brink, P., F. DeGraves, W. R. Ravis, D. Johansen, J. D. Campbell and S. H. Duran (1998). Stereospecific pharmacokinetics of free and protein-bound ketoprofen in serum and synovial fluid of horses after intravenous and intramuscular administration. *Am. J. Vet. Res.* 59(6):739-743.
- Charles, R. C., and E. S. Robert (2003). *Modern Pharmacology with clinical applications*. 6th Ed. Lippincott Williams and Wilkins Publisher. U.S.A. 508 p
- De Graves, F. J., M. G. Riddell and J. Schumacher (1996). Ketoprofen concentrations in plasma and milk after intravenous administration in dairy cattle. *Am. J. Vet. Res.* 57(7):1031-1033.
- Granero, G. E. and G. L. Amidon (2008). Possibility of enterohepatic recycling of ketoprofen in dogs. *Int J Pharm.* 349(1-2):166-171.
- Hardman, G. and L. E. Limbird (1996). *Goodman's The pharmacological basis of therapeutic*. 9th edn, McGraw-Hill companies, New York. p:633-634.
- Landoni, M. F., W. Comas, N. Mucci, G. Anglarilli, D. Bidal and P. Lees (1999). Enantiospecific pharmacokinetics and pharmacodynamics of ketoprofen in sheep. *J. Vet. Pharmacol. Ther.* 22(6):349-359.
- Laurence L. B., K. L. Parker, D. K. Blumenthal and I. L. O. Buxton (2008). *Goodman and Gilman's Manual of Pharmacology and Therapeutics*. The McGraw-Hill Companies. U.S.A. 434 p
- Meunier, C. J. and R. K. Verbeeck (1999). Glucuronidation kinetics of R,S-ketoprofen in adjuvant-induced arthritic rats. *Pharm. Res.* 16(7):1081-1086.
- Nawaz, M. (1994). Genetical factors effecting biodisposition of drug. *Can. J. Phy. Pharm.* 72 (Supp.1), 301.
- Nawaz, M., T. Iqbal and R. Nawaz (1988). Genetical consideration in disposition kinetic evaluation of

- chemotherapeutic agents. Cong. Europ. Assoc. Vet. Pharmacol. Thera. 2, Proc.4th Cong.Europe Assoc. Budapest. p: 260.
- Qiu, H. X., J. Liu, H. Kong, Y. Liu and X. G. Mei (2007). Isobolographic analysis of the antinociceptive interactions between ketoprofen and paracetamol. Eur. J. Pharmacol. 557(2-3):141-146.
- Radwan, M. A. (2000). Zidovudine, diclofenac and ketoprofen pharmacokinetic interactions in rats. J. Pharm. Pharmacol. 52(6):665-669.
- Sean, C. S. (2009). Montelukast Sodium. In: Martindale, The Complete Drug Reference, 36th ed. An imprint of RPS Publishing, UK.2009. 1126 p
- Vane, J. R. (1971). Inhibition of postaglandin synthesis as a mechanism of action for aspirin ñ like drugs. Nature. New. Biology. 231: 232-235.
- Verde, C. R., M. I. Simpson, A. Frigoli, and M. F. Landoni (2001). Enantiospecific pharmacokinetics of ketoprofen in plasma and synovial fluid of horses with acute synovitis. J. Vet. Pharmacol. Ther. 24(3):179-185.