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Pharmacokinetics of Meloxicam in Healthy Donkeys

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Abstract.- Meloxicam, a non-steroidal anti-Inflammatory drug (NSAID), has been reported as a safe substitute for diclofenac which was banned for veterinary use during 2005-06, due to its relay toxicity associated with the catastrophic decline in vulture populations in Indian subcontinent. It is a preferential cyclooxygenase-2 (COX-2) inhibiter with higher therapeutic index as compared to diclofenac, indomethacin and piroxicam. The pharmacokinetics of meloxicam was studied in donkeys. Eight donkeys used in the experiment were administered 0.6 $mg.kg^{-1}$ body weight as an intravenous bolus of meloxicam through jugular vein. Blood samples (5ml) were drawn pre medication and then up to 96 h post-medication. Plasma concentrations of meloxicam were measured in triplicate by HPLC. The plasma concentration versus time profile was prepared. Mean (±SEM) values of pharmacokinetic parameters *viz*., area under curve, steady state volume of distribution, half-life, mean residence time and clearance were 6.017±0.009 µg.h/ml, 0.136±0.002 L/kg, 1.002±0.008 h, 1.404±0.053 h and 0.094±0.002 L/h/kg, respectively. These pharmacokinetic parameters of meloxicam in donkeys were comparable to the reported values in donkeys but different from those of other species like sheep, goats, horses, chicken, rabbits and rats. A fast elimination with short half life and higher clearance are suggestive that current dosage regimens of meloxicam may not be clinically effective in donkeys and further research is recommended.

Keywords: NSAIDs, diclofenac toxicity, meloxicam, pharmacokinetics, donkeys.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are frequently prescribed and commonly used in humans, as well as in animals, to reduce pain, fever and inflammation for the treatment of different clinical conditions such as rheumatic disorders (Huskisson *et al*., 1996). It has been established scientifically that relay toxicity of diclofenac was responsible for dramatic fall in vulture population within Indian subcontinent. Vultures were exposed to diclofenac when they had consumed carcasses of livestock that were treated with this drug, before death. Residual diclofenac in the dead livestock animal had caused death as it had elevated uric acid concentrations in serum causing visceral gout leading to kidney failure of vulture

This commonly available NSAID was extensively used in veterinary practice in south asia as an analgesic and anti inflammatory agent (Prakash *et al.,* 2003; Green *et al*., 2004). However, diclofenac was banned for veterinary use in Pakistan, India and Nepal during 2005-06, following evidence of its role in the decline of vulture populations. Another NSAID, meloxicam has been reported as a safe substitute of diclofenac sodium (Swan *et al.,* 2006; Swarup *et al*., 2007).

Meloxicam is chemically designated as 4-hydroxy-2-methyl- N -(5-methyl-2-thiazalyl)-2 H -1,2-benzothiazine-3-carboxamide-1,1-dioxide and belongs to oxicam class of NSAIDs. It has molecular formula $C_{14}H_{13}N_3O_4S_2$ and the molecular weight of 351.4 Dalton (BNF, 2003). It preferentially inhibits cyclooxygenase-2 which is responsible for pathophysiological conditions rather than cyclooxygenase-1 responsible for physiological processes (Churchill *et al.,* 1996). It has a half-life of 20-24 hours in human and once-daily administration is considered appropriate. It is strongly bound to plasma proteins (99.5%) (Davies and Skjodt, 1999).

Most of meloxicam is eliminated after biotransformation. The metabolites of meloxicam do not alter the renal blood flow and consequently the drug is not capable for nephrotoxicity (Engelhardt and Trummlitz, 1990). The therapeutic index of meloxicam is higher when compared with other NSAIDs like piroxicam, diclofenac and indomethacin (Engclhardt *et al.,* 1995).

All the NSAID drugs have been shown to

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have almost similar efficacy. But, meloxicam was shown to be superior as far as GIT tolerability was concerned. It was probably due to preferential and selective inhibition of cyclo-oxygenase-2 as compared to cyclo-oxygenase-1 (Barner, 1996). Its gastrointestinal tolerability was superior to that of nonselective NSAIDs (Schoenfeld, 1999).

The study of pharmacokinetics is of great significance for evaluating therapeutic use of the drug in any species. The pharmacokinetic profile of meloxicam has not been studied / reported for donkeys in Pakistan although it has been reported in USA (Sinclair *et al.*, 2006).

The basic aim of the present research work was to characterize pharmacokinetic parameters of meloxicam in donkeys under local conditions of Pakistan, to explore interspecies variations and to make some recommendation regarding its use in donkeys.

MATERIALS AND METHODS

Experimental animals

Eight healthy and clinically normal male adult donkeys with average weight of 275kg were used in the study.

All the donkeys were tagged, dewormed and acclimatized to the experimental environment at the animal sheds of Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Lahore, Pakistan, for a period of 15 days. Seasonal green fodder was provided as food with water supply *ad libitum*. The health status of these experimental animals was regularly monitored throughout the experiment by project veterinarians.

Experimental chemicals and drugs

The standard of meloxicam (Sigma), HPLC grade water, phosphoric acid and acetonitrile (E. Merck, Germany), and chemicals of analytical grade were purchased and used in this experiment. The injection meloxicam 5mg/ml, manufactured by INTAS Pharmaceutical Limited Ahmadabad, India were received as gift from India for administration to donkey.

Drug treatment, sampling and analysis

It was reported that IV dose 0.6 mg.kg-1, of meloxicam had produced anti-inflammatory effects in carrageenan-sponge model of acute inflammation in horses (Lees *et al.*, 1991). So eight experimental donkeys were administered an intravenous bolus of injection meloxicam 0.6 mg/kg body weight, into the jugular veins in order to achieve plasma concentrations of meloxicam that were likely to have an effect against inflammation.

Blood samples (5 ml) were collected from all the eight donkeys in heparinized vacutainer test tubes before medication and then at 0.12, 0.65, 0.5, 1, 1.5, 2, 3, 4, 6, 7, 8, 9, 12, 18, 24, 36, 48, 60, 72 and 96 h post medication. A saline solution (0.9% NaCl) was used to wash IV cannula pre and post sampling. Plasma was separated from blood samples by centrifugation at 3000 rpm for 10 min and stored at −20°C till analyzed.

HPLC analysis

Meloxicam in plasma was measured in triplicate by HPLC method developed and validated previously (Mahmood and Ashraf, 2008). In brief, HPLC grade acetonitrile (1 ml) was added to 1ml plasma for extraction of meloxicam. The mixture was subjected to high speed vortex mixing at 1500 rpm for 3 min, followed by ultracentrifugation at 8000 x g for 15 min. The clear supernatant (1 ml) was mixed well with 1 ml of HPLC grade water filtered through 0.22 µm filter and 10 µl injected into HPLC system for the analysis through an injector valve with a 10 µl sample loop. The mobile phase comprising phosphate buffer and acetonitrile (38:62, v/v) was pumped into Water 1525 Binary HPLC Pump 1525 at the rate 0.5ml/min. Separation was achieved by using a reversed phase C18 column (Phenomenex, particle size 5 µm; 4.6×150 mm) at retention time of 7.4 min. Oven temperature was set at 25°C. The meloxicam was detected at 352 by using a Water 2487 dual absorbance detectors. Meloxicam (Sigma) was used as external standard. The distinct peak observed in chromatograms of meloxicam extracted from plasma of donkeys was similar to the peak in chromatogram of external standard at retention time of 7.4 min.

The plasma concentration (µg/ml) versus time profile of meloxicam in donkeys was prepared.

Pharmacokinetics

The computer software APO PC-Program,

MWPHARM Version. 3.02, a MEDIWARE product, Holland. was used for calculation of pharmacokinetic parameters. This software calculate parameters for compartmental and non compartmental models.

Statistical analysis

The software SPSS (Statistical Package for the Social Sciences) 13.0 was used for statistical analysis. The values in the raw data were expressed as range, mean and SEM (standard error of means).

RESULTS AND DISCUSSION

Plasma concentrations (µg/ml) of meloxicam at the various time intervals after intravenous administration are given in Table I. Meloxicam was not detected 5 h after injection

Table I.- The plasma concentration (µg/ml) versus time profiles of meloxicam in donkeys after intravenous administration at dose of 0.6 mg.kg^{-1} BWt (n=8).

Time (hours)	Range (µg/ml)	Mean± SEM (µg/ml)	CV(%)
0.12	3.98-4.23	4.09±0.04	2.8
0.65	3.56-3.87	3.70±0.04	3.3
0.5	3.00-3.26	3.12±0.03	3.3
0.75	2.53-2.74	2.63±0.03	3.4
1	2.10-2.32	2.21±0.03	4.0
1.5	1.50-1.99	1.63±0.05	9.6
2	1.02-1.42	1.15±0.04	10.6
3	0.51-0.60	0.56±0.01	5.7
4	0.25-0.31	0.284±0.004	8.1
5	0.12-0.15	0.13±0.00	3.3
6-96	0	0	0

The graphical representation of plasma concentrations (µg/ml) of meloxicam in donkeys versus time is given in Figure 1. The pharmacokinetics (PK) of meloxicam in donkeys was best fitted to a one compartment model. The PK profile is given in Table II.

The result of the present study in the donkey had shown that mean (± SEM) values of pharmacokinetic parameters *viz*., area under curve (AUC), steady state volume of distribution (V_{Dss}), half-life ($t_{1/2}$), mean residence time (MRT) and clearance (Cl) were 6.017±0.009 µg.h/ml,

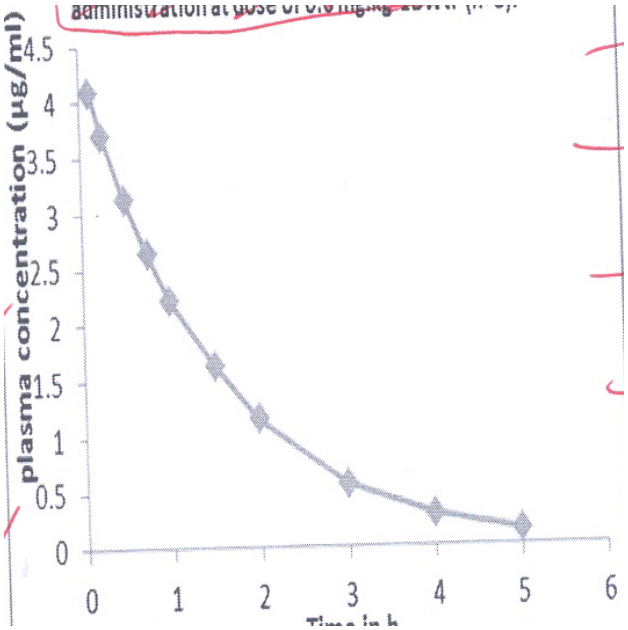

Fig. 1. Mean plasma concentration (µg/ml) versus curves of meloxicam in donkeys after intravenous administration at dose of 0.6 mg/kg body weight (n=8).

Table II.- Pharmacokinetic (PK) parameters of meloxicam in donkeys following intravenous administration of meloxicam at a dose of 0.6 mg.kg^{-1} BWt (n=8).

Pharmacokinetic Parameters	Range	Mean+SE	CV%
AUC(µg .h /ml)	5.72-6.28	6.01±0.08	4.0
Cl(l/hr/kg)	0.089-0.098	0.09±0.00	5.3
$VDss_{(}l/kg_{)}$	0.13-0.14	0.13±0.00	3.7
$t_{1/2}$ (h)	0.97-1.02	1.00±0.00	2.1
MRT (h)	1.04-1.47	1.40±0.05	10.6

AVC, area under curve; Cl, clearance; CV, coefficient of variation; VDSS, steady state volume of distribution; T½, half-life; MRT, mean residence time.

0.136±0.002 l/kg, 1.002±0.008 h, 1.404±0.053 h and 0.094±0.002 L/h/kg, respectively. These PK-values were comparable to the reported pharmacokinetic parameters of meloxicam in donkey found in other studies. The reported means PK- values in donkeys were AUC 4.6 µg/mL/h, MRT 0.6 h, Cl 0.188 mL/kg/h, VD_{SS} was 0.093L/kg. However, PK values reported for horse

were significantly different when compared to horse. The reported means PK- values in horses were AUC 18.8 µg/mL/h, MRT 9.6 hours, Cl 0.0347 L/kg/h ,VD_{SS} was 0.270 L/kg (Sinclair *et al.,* 2006).

The $t_{1/2}$ determined in donkey in the present study was different from other species. The half lives of meloxicam reported for sheeps and goats were 85±1.21 h and 6.73±0.58 h respectively (Shukla *et al.,* 2007). Relatively shorter elimination half-lives for meloxicam have been reported in ducks (0.72 h), turkeys (0.99 h) and ostriches (0.5 h) (Baert and Backer, 2003) whereas $t_{1/2}$ of 2.7 h was reported in piglets (Fosse *et al.,* 2008). The $t_{1/2}$, reported in horses was 8.54±3.02 h, (Toutain *et al*., 2004). However, meloxicam has longer half lives in albino rat (49.9 h), and human 15 to 20 h (Davies and Skjodt, 1999*).* The shorter $t_{1/2}$ observed in donkey was similar to vultures, who eliminate meloxicam extremely rapidly with a $t_{1/2}$ of 1 h (Naidoo *et al*., 2008).

The small value of AUC of meloxicam observed in donkeys may be due to higher rate of clearance. The lower value of Vd may be due to high protein binding of meloxicam which limit their ability to reach extra vascular compartments.

These PK-values determined in present study were comparable to the reported pharmacokinetic parameters of meloxicam in donkey. However, these pharmacokinetic parameters were different when compared with other species human, horses, goats, sheeps, piglets, ducks, vultures and turkeys.

The biological processes of absorption, distribution, metabolism and excretion (ADME) of drugs affects the level of drug and its movements towards site of action. Thus, ADME greatly influences pharmacological action of drugs (Balani *et al.,* 2005). Genetics and environmental factors affecting ADME are responsible for inter-individual indicated inter species and interethnic variations in clinical response to meloxicam (Lees *et al*., 1991 ; Rani *et al.,* 2004 ; Toutain *et al.,* 2004). Prior to the study the pharmacokinetics of meloxicam under local conditions of Pakistan has never been reported in donkeys.

We now know that donkeys and horses react differently to drugs. Pharmacokinetics of phenylbutazone and its metabolite oxyphenbutazone was different in clinically normal horses and donkeys (Matthews *et al*., 1997). The differences in pharmacokinetics between horse and donkey were also reflected in another comparative study with flunixin meglumine (MRT 0.92h±0.12 min and Cl 1.8±0.5 ml/kg/min, respectively, in donkeys vs. 1.84±0.4 h and 1.1±0.2 ml/kg/min, respectively, in horses). The value of Cl was greater in donkeys (Coakley *et al*., 1999).

The rapid plasma clearance leads to suggestion that use of meloxicam in donkey might not be as beneficial in comparison to the use of meloxicam to treat other species. Consideration should be given to administering higher doses or more frequent doses of meloxicam, in order for this drug to be clinically effective in the treatment of donkeys..

So, we need to conduct clinical trials in donkeys by using different doses and dosing intervals in order to evaluate effectiveness prior to give any final recommendation.

In conclusion, results of the present study indicate that variations exist in pharmacokinetics behaviour of meloxicam in donkeys when compared with other species. The use of meloxicam in donkey might not be beneficial at the dosing regimes that are recommended for treating other domestic ungulates. However, we need to carry out clinical trial in donkeys under disease state in order to make final recommendation**.**

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