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ABSOLUTE BIOAVAILABILITY OF ORAL MELOXICAM IN HEALTHY DOGS

K. T. Mahmood and M. Ashraf*
DTL, Health Department, Punjab, Lahore
*Department of Pharmacology & Toxicology, University of Veterinary and Animal Sciences (UVAS), Lahore
Corresponding author email: tahir7@nextinx.net.pk

ABSTRACT

Meloxicam, a non-steroidal anti-Inflammatory drug has been registered in Pakistan as a safe substitute of diclofenac sodium which was banned for veterinary use, due to its relay toxicity associated with the catastrophic decline in vulture population of the Indian subcontinent. In Pakistan, Injection is the only dosage form registered for veterinary purposes whereas Boehringer Ingelheim are licensed to sell meloxicam liquid oral suspension in UK and USA for use in dogs. The aim of the present study was to determine absolute bioavailability in dogs under local conditions of Pakistan. Eight dogs were used in two groups A & B (4x4) in a cross over fashion. The dogs in-group A were administered an intravenous bolus of meloxicam 0.2 $mg.kg^{-1}$b.wt. and dogs in group B were administered an oral single dose of meloxicam 0.2 $mg.kg^{-1}$b.wt.After wash out period of 14 days, experiment was repeated by change of treatment to group A and B. The blood samples were drawn at pre defined time intervals up to 96h. An HPLC method developed/validated at laboratories of UVAS/ LCWU, Lahore was used for the measurement of meloxicam concentration in plasma. The AUC (area under the curve) of all the dogs was determined from the plasma concentration versus time data following I/V and oral administration of the drug. The result indicated that the absolute bioavailability of meloxicam following oral administration at the dose of 0.2 $mg.kg^{-1}$b.wt. was 90.24%. It is recommended that the oral dosage form like suspension must be developed / marketed in Pakistan in order to ensure easy administration of meloxicam at user end.

Key words: Meloxicam; HPLC, absolute bioavailability; dogs.

INTRODUCTION

Non-steroidal anti-Inflammatory drugs (NSAIDs) are agents having anti-inflammatory analgesic and antipyretic effects Brooks and Day (1991).These drugs are used in humans as well as in animals to reduce pain, fever, inflammation and for the treatment of different clinical conditions such as rheumatic disorders, Huskisson *et al*. (1996). The most common adverse reactions involve the gastrointestinal (GI) tract, Allison *et al*. (1992) and Wallace (1997) and renal system Perazella and Eras (2000).These drugs are also frequently prescribed and commonly used in Pakistan.

Relay toxicity of a non-steroidal anti-inflammatory drug diclofenac sodium was responsible for catastrophic drop in Asian vulture population within the Indian subcontinent Prakash *et al.* (2003), Green *et al.* (2004) and Oaks *et al*. (2006). Diclofenac sodium was withdrawn from veterinary use in Pakistan, India and Nepal during 2005-06. Meloxicam which is another NSAID, has been reported as a safe substitute of diclofenac sodium Swan *et al.* (2006).

Meloxicam ($C_{14}H_{13}N_3O_4S_2$) belongs to Oxicam class of NSAID. It selectively inhibits cyclooxygenase-2, responsible pathophysiological conditions rather than cyclooxygenase-1, which is responsible for physiological processes, e.g. in the stomach Churchill *et al.* (1996). Meloxicam is strongly bound to plasma proteins (99.5%) and displays linear pharmacokinetics, with a half-life of 20-24 hours in humans, Davies and Skjodt (1999). It has a favourable tolerability profile as compared to other NSAIDs and has a higher therapeutic index than that of other NSAIDs, including piroxicam, diclofenac, and indomethacin. It forms four metabolites that undergo fast elimination, leading to a shorter $t_{1/2}$ (half life) in comparison with piroxicam and tenoxicam. These pharmacologically inactive metabolites do not change renal blood flow and are therefore not capable of causing nephrotoxicity, Woolf and Radulovic (1989), Olkkola *et al.* (1994), Schmid *et al.* (1995), Huskisson *et al*. (1996), Schoenfeld (1999) and Tacca *et al.* (2002). It has been reported as an effective drug in acute synovitis and lameness improvements in dog, Cross *et al.* (1997).

The efficacy and toxicity of drugs depends on the biological processes of absorption, distribution, metabolism and excretion (ADME) of drugs. ADME affects the blood level of drug and its movement towards the site of action and thus greatly influence its pharmacological action Guttendorf *et al*. (1992), and Shargel and Yu (1999). The genetics and environmental factors affecting ADME are responsible for inter-individual, inter-ethnic and inter-species variations to clinical response during any drug therapy Kalow (1991), Castañeda *et al.* (1993), Vesell (1997) and Min *et al*. (2000). It is reported in many studies that inter species / interethnic variation exist in clinical response to meloxicam, Lees *et al*. (1991), , Dasandi *et al*. (2002), Mose and Bertone (2002), Rani *et al.* (2004) and Toutain *et al.* (2004)

Bioavailability is one of the principal pharmacokinetic properties of a drug and is defined as the measurement of the rate and extent of a therapeutically active drug that reaches the systemic circulation and is available at the site of action. It is expressed as the letter F. Absolute bioavailability compares the bioavailability (estimated as the area under the curve, or AUC) of the active drug in systemic circulation following non-IV route of administration (i.e., after oral administration), with the bioavailability of the same drug following intravenous administration. It is the fraction of the drug absorbed through non-intravenous administration compared with the corresponding intravenous administration of the same drug Shargel and Yu (1999). It is mandatory that in any study for absolute bioavailability of a drug, the drug be given intravenously.

The absolute bioavailability of meloxicam has not been studied in dogs in Pakistan. The basic aim of the present study was to determine absolute bioavailability of meloxicam in dogs under local conditions of Pakistan and to make some recommendation regarding its introduction in the dosage form of liquid oral suspension in Pakistan for convenience of the end-user.

MATERIALS AND METHODS

Experimental Animals: Eight healthy and clinically normal adult dog with average weight of 20kgs were included in the study. These were tagged and acclimatized to the experimental environment at the animal sheds of Department of Pharmacology & Toxicology, UVAS, Lahore. Standard food was provided with water supply ad libitum. Veterinarians regularly monitored the health status of these experimental animals throughout the study period. The protocol of the experiment was approved by the competent authority at UVAS, Lahore and the research work was carried out at the Department of Pharmacology and Toxicology, UVAS, Lahore.

Experimental Drugs / Chemicals: Meloxicam (Sigma), HPLC grade acetonitrile, water, and phosphoric acid (E. Merck Germany) were purchased from the local market. All other chemicals of analytical grade were used. Injections of meloxicam 5mg/ml manufactured by M/S INTAS Pharmaceutical Limited. Ahmadabad, India and Metacam oral suspension of 0.5mg/ml M/S Boeringer Ingelheim UK were used.

Design, drug treatment, sampling, storage and analysis: Eight dogs were divided into two groups A & B (4x4) which were used in a cross over fashion with a wash out period of 14 days. The drug treatment protocol was that the dogs in-group A were administered an intravenous bolus of meloxicam 0.2 mg/kg body weight, into the cephalic vein whereas the four dogs in group B were administered an oral single dose of meloxicam 0.2 mg/kg body weight using Metacam suspension (meloxicam 0.5mg/ml)

Blood samples (3-5 ml) were collected from all the eight dogs in both groups in heparinized vacutainer test tubes before medication and then at 0.12,0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 7.0 8.0,,9.0, 12.0, 18.0, 24.0, 36.0, 48.0, 60, 72 and 96 hours post – medication. The IV brannula was washed with saline (0.9% NaCl) I/V solution pre and post sampling. The blood samples were centrifuged at 3000 rpm for 10 minutes for separation of plasma. The plasma separated from blood was stored at temperature −20 °C till analyzed.

After wash out period of 14 days, the experiment was repeated by change of treatment to group A and B. The blood sampling, separation and storage of plasma were carried out in a similar manner as described above.

HPLC analysis of the samples: The measurement of meloxicam in the above samples of plasma was done in triplicate by a simple, specific, precise and accurate HPLC method developed and validated at Dept. Of Pharmacology and Toxicology, UVAS Lahore/ Quality Assurance Laboratory, Lahore College for Women University, Lahore, 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 vortex mixing at high speed for 3 minutes, and then ultra centrifuged at 8000 rpm for 15 minute. 1 ml of the clear supernatant was mixed well with 1 ml of HPLC grade water and filtered through 0.22 µm filter. Ten micro litres (µl) of the aliquot were injected into the HPLC system for the analysis through an injector valve with a 10 µl sample loop. The mobile phase comprising of phosphate buffer and acetonitrile (38:62, v/v) was pumped into Water 1525 Binary HPLC Pump 1525 at the rate of 0.5ml/min Separation was achieved by using a reverse phase C18 column (Phenomenex, particle size 5 µm; 4.6 mm × 150 mm) at retention time of 7.4 minutes. Oven temperature was set at 25^{o}C. Meloxicam was detected at 352 by using a Water 2487 dual absorbance detector. Meloxicam (Sigma) was used as a external standard.

The distinct peaks observed in chromatograms of meloxicam extracted from plasma of dog were similar to the peak in chromatogram of external standard at retention time of 7.4 minutes. Similarity between peaks indicated specificity. The recovery of meloxicam from the plasma spiked with the drug > 92 % had indicated accuracy. The C.V% (RSD) 1.8% indicated precision of the method. The intraday and interday assays showed that the method was reproducible within acceptable variation of < 2% and < 3%, respectively. Five readings were taken. The limit of detection (LOD) and limit of quantification were 0.06 and 6 micrograms respectively.

RESULTS AND DISCUSSION

The plasma concentration of meloxicam versus time profile after intravenous and oral administration of single oral dose of 0.2 mg.kg^{-1}b.wt. was prepared and mean values of concentrations at different time intervals were used for calculation of absolute bioavailability. The software SPSS 13.0 was used for statistical analysis and the values in the raw data were expressed as range, mean, S.E.M (standard error of means), median and standard deviation The AUCs (area under the curve) were determined by using computer software APO. The AUC data, following oral and I/V administration of drug is presented in Tables 1 and 2 respectively. The graphic comparison of mean plasma concentration-time curve of 0.2 mg.kg^{-1}bwt, meloxicam in dog (n=8) is shown figure 1.

Table 1. Area under the curve (µg.h/ml) of meloxicam in dogs following intravenous administration at dose of 0.2 mg.kg^{-1}bwt (n-8)

AUC-IV	µg .h /ml
Range	25..28---27.60
Mean	26.970
SEM	0.085
St.Dev	0.724
Median	27.080

Table2. Area under the curve (µg.h/ml) of meloxicam in dogs following oral administration at dose of 0.2 mg.kg^{-1}bwt (n-8)

AUC-Oral	µg .h /ml
Range	23.09--26.49
Mean	24.294
SEM	0.699
St.Dev	0.960
Median	23.980

The software SPSS 13.0 was used for statistical analysis and the values in the raw data were expressed as range, mean, S.E.M (standard error of means), median and standard deviation .

As the same dose was given through IV as well as oral rout of administration, the absolute bioavailability was calculated by using the formula.

$$F_{bioavailability} = \frac{AUC.Oral}{AUC.IV} \times 100$$

$$F = \frac{24.2936}{26.97} \times 100 = 90.076\%$$

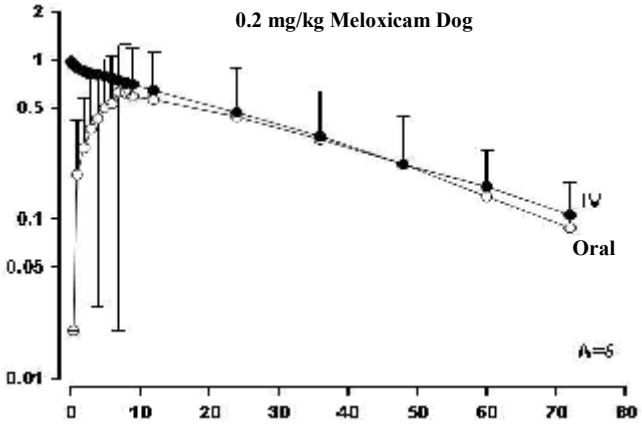

Figure. 1. Absolute bioavailability of meloxicam in healthy dogs. Comparison Mean (±SD) plasma concentration-time curve of 0.2 mg.kg^{-1}bwt, meloxicam in dog (n=8).

Meloxicam is an effective NSAID with better safety profile. In the oral dosage form (tablets), it is approved for human use in more than 70 countries including the UK, USA, Europe, Canada and Pakistan. Boehringer Ingelheim Pharmaceuticals were licensed in the early 1990s, to sell meloxicam liquid oral suspension in UK for use in dogs for alleviating inflammation and pain in both acute and chronic musculo-skeletal disorders such as osteoarthritis.

The dose of meloxicam 0.2 mg.kg^{-1}b.wt. IV was selected for investigation of absolute bioavailability and characterization of the pharmacokinetic parameters of meloxicam in dog as per recommendation of the pharmaceutical company Boehringer Ingelheim Vetmedica GmbH, Germany which sells meloxicam suspension for dogs in UK and USA (Boehringer's" professional insert" NADA 141-213, approved by FDA).

Conclusion: The result of experiment showed that meloxicam had an absolute bioavailability of 90% if given through oral route as a single dose 0.2 mg.kg^{-1}b.wt. This value is different but comparable to the value of >99% provided to the USA regulatory authorities by the manufacturing company Boehringer Ingelheim (US Patent 6,184,220).

Injection is the only dosage form of meloxicam registered for veterinary use in Pakistan. It is recommended that oral dosage form like liquid suspension must be developed and marketed for veterinary use in Pakistan in order to ensure easy administration of meloxicam at user end.

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