PHARMACOKINETICS OF ECOFRIENDLY MELOXICAM IN HEALTHY HORSES

K.T. Mahmood and M. Ashraf

DTL, Health Department, Punjab, Lahore; Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences (UVAS), Lahore.
Corresponding author e-mail tahir7@nexlinx.net.pk

ABSTRACT: The main aim of the present study was to evaluate the pharmacokinetics (Pk) of meloxicam, a non-steroidal anti-inflammatory drug (NSAID), in healthy horses. Eight healthy horses, investigated, were administered meloxicam 0.6 mg.kg⁻¹ body weight, as intravenous bolus, into the jugular vein. Blood samples (5mL) were collected before medication and then 0.12, 0.25, 0.5, 1.0, 1.5, 2.0, 2.4, 3.0, 4.0, 6.0, 7.0, 8.0, 9.0, 12.0, 24.0, 36.0, 48.0, 60, 72 and 96 hours post medication. The plasma concentrations of meloxicam were determined in triplicate by the HPLC method developed at UVAS, Lahore and Lahore College For Women University (LCWU), Lahore, Pakistan. The plasma concentration versus time profile was prepared. The mean ± SEM values of pharmacokinetic parameters viz area under curve (AUC), steady state volume of distribution (VDSs), half-life (t₁/₂), mean residence time (MRT) and clearance (Cl), determined in horses were 25.43±1.07 μg h mL⁻¹, 0.267±0.014 L kg⁻¹, 8.17±0.03 h, 11.4±0.08 h and 0.023±0.005 L h⁻¹ kg⁻¹ respectively. The pharmacokinetic parameters were put in different PK-equations for calculations of dose. On the basis of these findings, we recommend a single IV dose of 0.6 mg.kg⁻¹ Body weight of meloxicam in horses.

Keywords: Diclofenac toxicity, Meloxicam; Pharmacokinetics, HPLC, Horse.

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 including rheumatic disorders, (Huskisson et al., 1996).

It has been established scientifically that relay toxicity of diclofenac sodium was responsible for dramatic fall in vulture population within Indian subcontinent. This freely available NSAID was extensively used in veterinary practice as an analgesic and anti-inflammatory agent, (Green et al., 2004). Another NSAID, meloxicam was found safe when administered directly to Gyps vulture in captivity at the highest dose of 2 mg kg⁻¹. It was recommended as a safe substitute of diclofenac sodium, (Swan et al., 2006). It can be said that diclofenac caused eco-damage whereas meloxicam is its ecofriendly alternate for use in veterinary practice.

Meloxicam is chemically designated as 4-hydroxy-2-methyl- N - (5-methyl-2-thiazalyl)-2 H -1,2-benzothiazine-3-carboxamide-1, 1-dioxide (BNF, 2003) and belongs to oxicam class of NSAIDs. It has the molecular weight of 351.4 Dalton and its formula is shown in fig 1.

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 (99.5%) to plasma proteins (Davies and Skjodt, 1999). The therapeutic index of meloxicam is higher when compared with other NSAIDs like piroxicam, diclofenac and indomethacin (Engelhardt et al., 1995). Meloxicam undergoes fast elimination, leading to a shorter t₁/₂ in comparison with piroxicam and tenoxicam. It has no capability for nephrotoxicity (Schoenfeld, 1999).

The clinical trial had indicated that meloxicam was effective and safe analgesic in horses, (Mathews et al., 1997).

Relatively shorter elimination half-lives for meloxicam have been reported in ducks (0.72 h), turkeys (0.99 h) and ostriches (0.5 h) by Baert and De Backer, (2003) whereas t₁/₂ of 2.7 h reported in piglets, (Fosse et al., 2008). However, meloxicam has longer half lives in albino rat (49.9 h), and human 15 to 20 h, (Davies and Skjodt, 1999). Vultures eliminate meloxicam extremely rapidly with a t₁/₂ of 1 h, (Naidoo et al., 2008).
The basic aim of the present study was to determine pharmacokinetics of meloxicam in horses under local conditions of Pakistan and to make some recommendation regarding its dose.

**MATERIALS AND METHODS**

**Experimental animals:** Eight healthy and clinically normal adult horses with average weight of 400 kg were used in the study. All the horses were tagged and acclimatized to the experimental environment at the animal sheds of Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Lahore, Pakistan. Standard food was provided with water supply ad libitum. Health status of these experimental animals was regularly monitored throughout the experiment.

**Experimental chemicals and drugs:** Meloxicam (Sigma) was purchased for use as external standard. HPLC grade water, phosphoric acid and acetonitrile (E. Merck Germany); 30 ml injections of meloxicam manufactured by INTAS Pharmaceutical Limited Ahmadabad, India and chemicals of analytical grade were used in this experiment.

**Design, drug treatment, sampling and analysis:** Experimental horses were administered an intravenous bolus of meloxicam 0.6 mg/kg body weight, via jugular vein. Blood samples (5 ml) were collected from all the eight horses in heparinized vacutainer test tubes before medication and then 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, 60.0, 72.0 and 96.0 hours post medication. The saline (0.9% NaCl) solution was used to wash IV cannula pre and post sampling. Plasma was separated from blood samples by centrifugation at 3000 rpm for 10 minutes and stored at −20°C till analyzed.

**HPLC analysis:** Meloxicam in plasma was measured in triplicate by a simple, specific, precise and accurate, HPLC method developed and validated previously, (Mahmood and Ashraf, 2008). In brief, HPLC grade acetonitrile (1 ml) was added to 1 ml plasma for extraction of meloxicam. The mixture was subjected to high speed vortex mixing at 1500 rpm for 3 minutes, followed by ultra centrifugation at 8000 x g for 15 minutes. The clear supernatant (1 ml) was mixed well with 1 ml of HPLC grade water and filtered through 0.22 μm filter. Ten micro litters (μL) of the aliquot were injected into 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 0.5 ml/minute. Separation was achieved by using a reversed 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°C. The meloxicam was detected at 352 by using a Water 2487 dual absorbance detectors. Meloxicam was used as external standard.

The distinct peak observed in chromatograms of meloxicam extracted from plasma of horses was 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 value 1.8% CV (RSD) had indicated precision of the method. The intraday and interday assays had shown that method was reproducible within acceptable variation of < 2% and < 3%, respectively. Five reading were taken. The limit of detection (LOD) and limit of quantification were 0.06 and 6 (µg) respectively. The plasma concentration (µg/ml) versus time profile of meloxicam in horses was prepared.

**Pharmacokinetics:** The computer software APO PC-Program, MWPHARM Version. 3.02, a MEDIWARE product, Holland. was used for calculation of pharmacokinetic parameters through standard noncompartmental analysis. Pharmacokinetic parameters were calculated for horses by use of statistical moment theory.

The following equations were used for different calculations:

\[
\text{Cl} = \frac{\text{Dose/AUC}}{}; \quad \text{AUMC} = \text{MRT x AUC} ;
\]

\[
\text{Dose} = \frac{\text{VDS}_{ss}\times \text{AUC}^2}{\text{AUMC}}
\]

Where, AU C = Area under the curve, CL = Clearance, MRT = Mean residence time and AUMC = Area under the first moment curve.

**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, SEM (standard error of means); median and standard deviation.

**RESULTS AND DISCUSSION**

Plasma concentrations (µg/ml) of meloxicam were determined at various time intervals after intravenous administration at dose of 0.6 mg.kg⁻¹ body weight in horses. The results are given in Table 1. The graphical representation of plasma concentrations (µg/ml) of meloxicam versus time is given in Figure 2. The dose of 0.6 mg.kg⁻¹ body weight was recommended on the basis of these finding.

The above results had indicated that PK-values determined in present study were comparable to the reported pharmacokinetic parameters of meloxicam in horse (Sinclair et al., 2006). These pharmacokinetic parameters were different when compared with reported
Table 1. The plasma concentration (µg/ml) versus time profiles of meloxicam in horses after intravenous administration at dose of 0.6 mg.kg⁻¹ body weight (n=8).

<table>
<thead>
<tr>
<th>Time(hours)</th>
<th>Range(µg/ml)</th>
<th>Mean + SEM</th>
<th>SD</th>
<th>CV%</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.12</td>
<td>3.030-3.510</td>
<td>3.202±0.078</td>
<td>0.203</td>
<td>6.34</td>
<td>3.09</td>
</tr>
<tr>
<td>0.25</td>
<td>2.870-3.078</td>
<td>2.956±0.028</td>
<td>0.079</td>
<td>2.67</td>
<td>2.94</td>
</tr>
<tr>
<td>0.5</td>
<td>2.330-2.720</td>
<td>2.573±0.045</td>
<td>0.128</td>
<td>4.97</td>
<td>2.61</td>
</tr>
<tr>
<td>1</td>
<td>1.894-2.450</td>
<td>2.219±0.084</td>
<td>0.238</td>
<td>10.73</td>
<td>2.27</td>
</tr>
<tr>
<td>1.5</td>
<td>1.643-2.270</td>
<td>2.028±0.099</td>
<td>0.279</td>
<td>13.76</td>
<td>2.12</td>
</tr>
<tr>
<td>2</td>
<td>1.520-2.110</td>
<td>1.861±0.094</td>
<td>0.265</td>
<td>14.24</td>
<td>1.91</td>
</tr>
<tr>
<td>3</td>
<td>1.387-2.090</td>
<td>1.750±0.107</td>
<td>0.304</td>
<td>17.37</td>
<td>1.77</td>
</tr>
<tr>
<td>4</td>
<td>1.293-1.740</td>
<td>1.529±0.775</td>
<td>0.219</td>
<td>14.32</td>
<td>1.54</td>
</tr>
<tr>
<td>5</td>
<td>1.209-1.552</td>
<td>1.380±0.064</td>
<td>0.182</td>
<td>13.19</td>
<td>1.38</td>
</tr>
<tr>
<td>6</td>
<td>1.080-1.412</td>
<td>1.230±0.470</td>
<td>0.133</td>
<td>10.81</td>
<td>1.23</td>
</tr>
<tr>
<td>7</td>
<td>0.970-1.280</td>
<td>1.130±0.054</td>
<td>0.154</td>
<td>13.64</td>
<td>1.73</td>
</tr>
<tr>
<td>8</td>
<td>0.807-1.169</td>
<td>1.020±0.044</td>
<td>0.124</td>
<td>12.12</td>
<td>1.05</td>
</tr>
<tr>
<td>12</td>
<td>0.595-0.801</td>
<td>0.750±0.027</td>
<td>0.078</td>
<td>10.46</td>
<td>0.78</td>
</tr>
<tr>
<td>24</td>
<td>0.176-0.550</td>
<td>0.310±0.038</td>
<td>0.109</td>
<td>34.94</td>
<td>0.28</td>
</tr>
<tr>
<td>36</td>
<td>0.050-0.120</td>
<td>0.089±0.011</td>
<td>0.031</td>
<td>34.83</td>
<td>0.08</td>
</tr>
<tr>
<td>48-96</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Pharmacokinetic parameters of meloxicam in horses following intravenous administration of meloxicam at a dose of 0.6 mg.kg⁻¹ body weight. (n=8)

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Range</th>
<th>Mean±SEM</th>
<th>S.D</th>
<th>CV%</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC µg.h mL⁻¹</td>
<td>20.49-27.97</td>
<td>25.43±1.07</td>
<td>3.015</td>
<td>11.86</td>
<td>26.85</td>
</tr>
<tr>
<td>VDss L/kg⁻¹ L/kg₀</td>
<td>0.2324-0.3321</td>
<td>0.267±0.014</td>
<td>0.0408</td>
<td>15.28</td>
<td>0.255</td>
</tr>
<tr>
<td>t₁/₂ hr (hr)</td>
<td>6.818-10.91</td>
<td>8.174±0.602</td>
<td>1.702</td>
<td>20.82</td>
<td>7.353</td>
</tr>
<tr>
<td>MRT hr (hr)</td>
<td>9.3720-15.060</td>
<td>11.4±0.821</td>
<td>2.321</td>
<td>20.36</td>
<td>10.445</td>
</tr>
<tr>
<td>Cl L h⁻¹ kg⁻¹ (l/hr/kg)</td>
<td>0.02115-0.0294</td>
<td>0.0225±0.0016</td>
<td>0.005</td>
<td>22.22</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Figure 2 Mean plasma concentration-time curves of meloxicam in horses after intravenous administration at dose of 0.6 mg/kg body weight (n=8).

Pk. values in other specie’s such as human, donkeys, 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. Genetics and environmental factors affecting ADME are responsible for inter-individual, inter-ethnic and inter-species variations to clinical response during any drug therapy. Previous studies have indicated inter species and interethnic variations in clinical response to meloxicam (Lees et al., 1991, Rani et al., 2004, Toutain et al., 2004). The pharmacokinetics of meloxicam under local conditions of Pakistan has never been reported in horses.

The IV dose 0.6 mg.kg⁻¹ body weight, of meloxicam had produced anti-inflammatory effects in carrageenan-sponge model of acute inflammation in horses, (Lees et al., 1991). So, this 0.6 mg.kg⁻¹ body weight dose of meloxicam for IV administration was chosen for the horses, to achieve plasma concentrations of meloxicam that were likely to have an effect against inflammation.

In horse mean ± SEM area under curve (AUC) was 25.43±1.07 µg h mL⁻¹, steady state volume of distribution (V₉₀) was 0.267±0.014 L.kg⁻¹, half-life (t₁/₂) was 8.17±0.602 h, mean residence time (MRT) was 11.4±0.08 and clearance (Cl) was 0.023±0.0016 L.h⁻¹ kg⁻¹. These pharmacokinetic parameters were
compared to the reported values in horse which were 
VDss was 0.27±.06Lkg⁻¹, MRT 9.6±3.26 hours and CI 0.0347± 0.0033Lh⁻¹kg⁻¹. The respective reported means 
PK-values in donkeys were AUC 4.6± 0.880μg h mL⁻¹, 
Vss 0.093± 0.012L . kg⁻¹, MRT 0.6 ±0.124hours and CI 0.188±0.0347+. The respective reported means 

The higher values of MRT and smaller values of 
clearance in horses as compared with donkeys had 
indicated that meloxicam was eliminated at a slow rate 
in horses as compared to closely related species of 
donkey . The smaller values of Vdss observed for 
meloxicam is typical of the NSAIDs. It may be due to 
high protein binding. However, protein binding was not 
measured in the present study.

CONCLUSION: Results of the present study indicate 
that variations exist in pharmacokinetics behaviour of 
meloxicam in horses when compared with other species. 
Meloxicam may be used as an ecofriendly substitute 
Keeping in view results in the present study, a single IV 
dose of 0.6 mg.kg⁻¹ body weight meloxicam once a day is 
recommended for horses in different pathophysiological 
conditions involving inflammation.

However, there is need to carry out trials for 
assessment of minimum effective plasma concentration 
of meloxicam in target animals in order to measure its 
benefits as an analgesic and anti inflammatory agent.
The interaction with antibiotics must be studied as 
that variations exist in pharmacokinetics behaviour of 
meloxicam in target animals in order to measure its 

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