Comparative Plasma Dispositions of Meloxicam and Carprofen Following Oral Administration in Dogs*

Umit Karademir[1], Cengiz Gokbulut[2] & Ferda Akar[1]

ABSTRACT

Background: Nonsteroidal anti-inflammatory drugs (NSAIDs) are used extensively in several domestic animal species for the treatment of a range of musculo-skeletal disorders and soft tissue injuries or inflammatory conditions. These drugs have antipyretic, anti-inflammatory and analgesic properties. Meloksikam (MLX) and carprofen (CRP) are two of the NSAIDs most commonly used by oral administration, which is the preferred route for the treatment of chronic pain and inflammation in dogs. The aim of the present study was to determine the pharmacokinetic properties of CRP and MLX in healthy dogs following oral administration at the doses of 2 mg/kg and 0.2 mg/kg bodyweight, respectively.

Materials, Methods & Results: A total of 12 client-owned, cross-bred bitches, 2-5 years old and weighing 15-20 kg were used in the study. The animals were allocated into two groups of six such that the mean weight of animals in each group was similar. In Group I, CRP was given orally at a dose of 2 mg/kg and in Group II, MLX was given orally at a dose of 0.2 mg/kg. Blood samples were collected one day prior to drug administration and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 32, 40, 48, 56, 72 and 96 h post-treatment. Samples were centrifuged at 3000 g for 20 min and plasma was transferred to plastic tubes. Heparinized drug-free blood samples for analytical method development and validation process were collected from dogs not included in the study. All the plasma samples were stored at -20ºC until estimation of drug concentrations with all analyses completed within one month of sampling and they were analysed by high-performance liquid chromatography (HPLC). The terminal half-life of MLX ($t_{1/2}$ = 37.91 ± 9.15 h) was significantly longer compared with that of CRP ($t_{1/2}$ = 17.02 ± 6.95 h). In addition, CRP was absorbed faster (t_{max} = 2.20 h) from gastrointestinal system and reached the peak plasma concentration significantly earlier than MLX (t_{max} = 5.00 h). Moreover, the results indicated that Cmax (1.31 ± 0.33 µg/mL) and AUC (33.73 ± 15.25 µg.h/mL) for CRP values were significantly higher and larger compared with those observed for MLX (C_{max} = 0.39 ± 0.13 µg/mL and AUC = 17.99 ± 4.97 µg.h/mL), respectively following dose-proportionality comparisons for C_{max} and AUC values of both molecules done after dose normalization.

Discussion: The pharmacokinetic parameters of CPR were significantly different from those of MLX following oral administration. The results of the present study suggest that, although the systemic availability of CPR was higher, its absorption and elimination phases were significantly faster compared with those of MLX. Though, as the earlier and higher C_{max} of CRP than MLX suggests that CRP seems to display anti-inflammatory effect faster than MLX in the treatment of the acute inflammatory disorders in dogs following oral administration, the anti-inflammatory effect is not necessarily directly related only to C_{max} and t_{max}, especially when the pharmacodynamic characteristics of the drugs may be different. The present study indicates that there are significant differences between pharmacokinetic parameters of MLX and CRP after oral administration in dogs. Because of the relatively slow absorption from gastrointestinal system after oral administration, MLX should be given intramuscular or subcutaneous routes in acute cases with respect of the time for onset of action.

Keywords: carprofen, dog, meloxicam, NSAIDs, pharmacokinetics.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are used extensively in several domestic animal species for the treatment of a range of musculo-skeletal disorders and soft tissue injuries or inflammatory conditions. These drugs have antipyretic, anti-inflammatory and analgesic properties. The common mechanism of action of this class of drugs can be attributed to a blockade of the biosynthesis of prostaglandins, resulting from inhibition of the cyclo-oxygenase (COX) enzyme [6,8,11].

MLX belongs to enolic acid class of NSAIDs with analgesic, antipyretic and anti-inflammatory activity. It preferentially inhibits COX-2, which is induced by inflammatory stimuli in pathophysiological conditions [4]. MLX is about 12 times more potent at inhibiting COX-2 than COX-1 enzyme activity [7].

CRP is one of the 2-aryl-propionic acid (profen) class that includes ketoprofen, ibuprofen, vedaprofen, naproxen and fenoprofen [3]. CRP contains an asymmetrical carbon atom and exists in two enantiomeric forms, (*R*)-CRP and (*S*)-CRP. The mechanism of action of CRP is not fully. Studies in a number of species have found that CRP is a weak inhibitor of cyclooxygenase (COX) at recommended dosages [9].

MLX and CRP are two of the NSAIDs most commonly used by oral administration, which is the preferred route for the treatment of chronic pain and inflammation in dogs.

The aim of the present study was to determine the pharmacokinetic properties of CRP and MLX in healthy dogs following oral administration at the doses of 2 mg/kg and 0.2 mg/kg bodyweight, respectively.

MATERIALS AND METHODS

Experimental animals

A total of 12 client-owned, cross-bred bitches, 2-5 years old and weighing 15-20 kg were used in the study. For the duration of the study, the animals in each group were housed in appropriate single pens and each dog was identified by natural markings. Water was supplied *ad libitum* and animals were fed a standard commercial diet once daily with an appropriate quantity of feed during the experiment period. This study was approved by Animal Ethics Committee of University of Adnan Menderes.

Treatments and sampling

The animals were allocated into two groups of six such that the mean weight of animals in each group was similar. All dogs were fed 1 h prior to the drug administration. Group I (CRP) and group II (MLX) received orally the commercially available tablet formulations of CRP and MLX at doses of 2 mg/kg and 0.2 mg/kg bodyweight, respectively. After the treatment, the animals were observed continuously for any adverse reactions within the first day.

Heparinized blood samples (5 mL) were collected by cephalic venipuncture using a 20 G catheter[1] 1 day prior to drug administration and 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 32, 40, 48, 56, 72 and 96 h post-treatment. Blood samples were centrifuged at 3000 *g* for 20 min and plasma was transferred to plastic tubes[2] (5 mL). Heparinized drug-free blood samples for analytical method development and validation process were collected from dogs not included in the study. All the plasma samples were stored at -20ºC until estimation of drug concentrations with all analyses completed within one month of sampling.

Analytical procedures

The parent compounds of CRP and MLX in dog plasma were analysed using validated high performance liquid chromatography (HPLC) following a liquid-liquid phase extraction procedure. Plasma concentrations of CRP and MLX were measured by minor modifications of the methods described by Nielsen-Kudsk [14] and Velpandian *et al*. [18], respectively.

Stock solutions (100 µg/mL) of analytical standards of CRP[3] and MLX[4] were prepared using water and ethanol as the solvents, respectively. These were diluted to give 0.05, 0.5, 1, 5 and 10 µg/mL standard solutions for calibration as standard curves and to add to drug-free plasma samples to determine the recovery.

For CPR analysis, drug-free plasma samples (0.25 mL) were spiked with CRP standards to reach the following final concentrations: 0.1, 0.5, 1, 5 and 10 µg/mL and after vortexing for 15 s. Acetonitrile (0.5 mL) was added, and after vortexing for 15 s. All tubes were centrifuged at 12.000 *g* for 10 min and then the organic phase (0.25 mL) was transferred to 250 µL-insert and 35 µL of this solution was injected into the chromatographic system. The mobile phase consisted of acetonitrile-buffer (99.5% deionized water and 0.5% phosphoric acid) (50:50, v/v) for CRP and was delivered[5] at a flow rate of 1 mL/min. A nucleosil C_{18}

analytical column[6] with nucleosil C_{18} guard column[6] was used for analysis of molecule. The eluate was continuously monitored using a photodiode array detector[5] at a wavelength of 300 nm.

For MLX analysis, drug-free plasma samples (0.5 mL) were spiked with MLX standards to reach the following final concentrations: 0.05, 0.1, 0.5, 1, 5 and 10 µg/mL. Hydrochloric acid (150 µL) was added, and after vortexing for 15 s, ethyl acetate (4 mL) was added and shaken on a slow rotary mixer for 15 min. After centrifugation at 2000 x *g* for 15 min, the organic phase (3 mL) was transferred to a thin-walled 10 mL-conical glass tube and evaporated to dryness at 45ºC in a sample concentrator[7]. The dry residue was dissolved in 250 µL of mobile phase and 35 µL of this solution was injected into the chromatographic system. The mobile phase consisted of acetonitrile-deionized water (65:35, v/v) for MLX and was delivered[5] at a flow rate of 1 mL/min. A nucleosil C_{18} analytical column[6] with nucleosil C_{18} guard column[6] was used for analysis of molecules. The eluate was continuously monitored using a photodiode array detector[5] at a wavelength of 355 nm.

The analytical method used for two molecules in dog plasma was validated prior to the start of the studies. The analytes were identified with the retention times of the pure reference standards. Recoveries of the two molecules under study were measured by comparison of the peak areas from spiked plasma samples with the areas resulting from direct injections of standards prepared ethanol and water. The inter- and intra-assay precisions of the extraction and chromatography procedures were evaluated by processing replicate aliquots of drug-free dog plasma samples containing known amounts of the drugs on different days. Calibration graphs were prepared (linear range 0.05-10 µg/mL for MLX and 0.1-50 µg/mL for CRP). The slope of the lines between peak areas and drug concentration was determined by least squares linear regression and showed correlation coefficients between 0.996 and 0.999. The detection limits of the two molecules were established with HPLC analysis of blank plasma fortified with the standard, measuring the baseline noise at the retention time of the peak. The mean baseline noise at the peak retention time plus three standard deviations was defined as the detection limit [15]. The mean baseline noise plus five standard deviations was defined as the limit of quantification.

Pharmacokinetic and statistical analysis of data

The plasma concentration vs. time curves obtained after each treatment in individual animals, were fitted with the WinNonlin software program[8]. Pharmacokinetic parameters for each animal were analysed using non-compartmental model analysis with extravascular input without applying of any weighting factor. The maximum plasma concentration (C_{max}) and time to reach maximum concentration (t_{max}) were obtained from the plotted concentration-time curve of each drug in each animal. The area under the plasma concentration time curve (AUC) and mean residence time (MRT) from time zero to infinity were calculated by trapezoidal rule. Terminal half-life ($t_{1/2\lambda z}$) was calculated as:

$$t_{1/2\lambda z} = -\ln(2)/\lambda_z$$

where λ_z represents the first-order rate constant associated with the terminal portion of the curve.

The pharmacokinetic parameters are reported as mean ± SD. Mean pharmacokinetic parameters were statistically compared by an analysis of variance (ANOVA). Mean values were considered significantly different at $P < 0.05$.

RESULTS

Clinically no adverse response was observed for any of the treatments in dogs during the study. The analytical methods used to extract and quantify the plasma concentration of CRP and MLX by chromatographic analysis were validated before analysis of experimental samples. The linear regression lines ranging from 0.1 to 10 µg/mL for CRP and from 0.05 to 10 µg/mL for MLX showed a coefficient of correlation of 0.996 and 0.999, respectively. The limits of detection of the assay for CRP and MLX were 0.021 and 0.011 µg/mL. The limits of quantification of the assay for CRP and MLX were 0.1 and 0.05 µg/mL. The mean extraction recoveries were 102.98% (inter assay CV = 6.43%) for CRP and 64.92% (inter assay CV = 4.43%) for MLX. The mean pharmacokinetic parameters of CPR and MLX after oral administration of 2 mg/kg and 0.2 mg/kg bodyweight are shown in Table 1, respectively. The mean plasma concentrations *vs.* time curves of CPR and MLX in dogs plotted on a semi-logarithmic scale are presented in Figure 1. The terminal half-life of MLX ($t_{1/2}$ = 37.91 ± 9.15 h) was significantly longer compared than that of CRP ($t_{1/2}$ = 17.02 ± 6.95 h). In addition, the results indicated that

time to reach peak plasma concentration (t_{max} = 5.00 ± 1.41 h) and mean residence time (MRT = 56.03 ± 13.62 h) for MLX were significantly longer compared with those observed for CRP (t_{max} = 2.20 ± 0.45 h and MRT = 25.35 ± 10.15 h). Moreover, following the statistical dose-proportionality comparisons for C_{max}

and AUC values of both molecules done after dose normalization across the dosages, C_{max} (1.31 ± 0.33 µg/mL) and AUC (33.73 ± 15.25 µg.h/mL) for CRP values were significantly higher and larger compared with those observed for MLX (C_{max} = 0.39 ± 0.13 µg/mL and AUC = 17.99 ± 4.97 µg.h/mL), respectively.

Table 1. Mean (±SD) pharmacokinetic parameters (with min and max ranges) of CRP (2 mg/kg) and MLX (0.2 mg/kg) following oral administration in healthy dogs (n = 6).

Kinetic parameter	CRP	MLX	*P* values
$t_{1/2\lambda z}$ (h)	17.02 ± 6.95[*]	37.91 ± 9.15	0.002
	(9.53-24.60)	(26.25-48.77)	
t_{max} (h)	2.20 ± 0.45[*]	5.00 ± 1.41	0.002
	(2.00-3.00)	(3.00-7.00)	
C_{max} (µg/mL)	13.10 ± 3.36[*]	0.39 ± 0.13	0.001
	(10.34-18.86)	(0.24-0.53)	
$AUC_{0\to\infty}$ (µg.h/mL)	337.34 ± 152.59[*]	17.99 ± 4.97	0.04
	(185.79-553.95)	(11.58-26.25)	
$AUMC_{0\to\infty}$ (µg.h^2/mL)	9537.05 ± 6444.03	1015.65 ± 416.75	0.85
	(2751.89-16839.46)	(664.70-1740.44)	
V_d/F (L/kg)	0.15 ± 0.03[*]	0.63 ± 0.24	0.001
	(0.11-0.19)	(0.37-0.95)	
Cl/F (mL/kg)	7.10 ± 3.28[*]	11.90 ± 3.23	0.037
	(3.60-10.90)	(7.90-17.30)	
$MRT_{0\to\infty}$ (h)	25.35 ± 10.15[*]	56.03 ± 13.62	0.003
	(14.12-35.94)	(40.88-75.92)	

$t_{1/2z}$: terminal half-life; t_{max}: time to reach peak plasma concentration; C_{max}: peak plasma concentration; $AUC_{0\infty}$: area under the (zero moment) curve from time 0 to infinity; $AUMC_{0\infty}$: area under the moment curve from time 0 to infinity; V_d: Volume of distribution; Cl: Body clearance; $MRT_{0\infty}$: mean residence time. [*]CRP is significantly different (*P* < 0.05) from MLX (The statistical comparisons for C_{max} and AUC values were done after dose normalization).
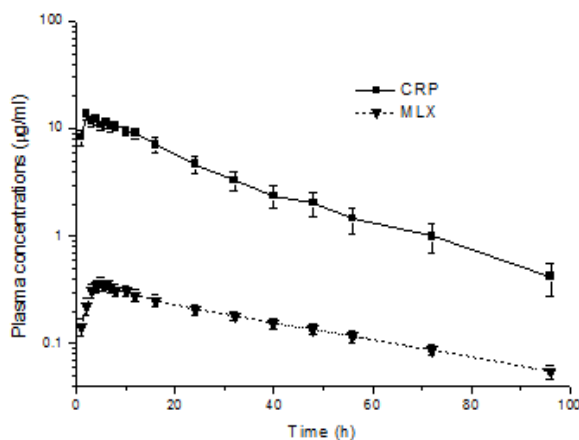

Figure 1. Semi log plot of mean (±SD) plasma concentrations of Carprofen (CRP) and Meloxicam (MLX) following oral administration in healthy dogs (n = 6) at a dose of 2 and 0.2 mg/kg, respectively.

DISCUSSION

MLX and CRP are NSAIDs that are currently used veterinary medicine. The pharmacokinetic parameters of CPR were significantly different from those of MLX following oral administration. The results of the present study suggest that, although the systemic availability of CPR was higher, its absorption and elimination phases were significantly faster compared with those of MLX. Though, as the earlier and higher Cmax of CRP than MLX suggests that CRP seems to display anti-inflammatory effect faster than MLX in the treatment of the acute inflammatory disorders in dogs following oral administration, the anti-inflammatory effect is not necessarily directly related only to Cmax and tmax, especially when the pharmacodynamic characteristics of the drugs may be different [10,17]. Moreover, the onset of anti-inflammatory effect depends on the transfer rate to tissues, which is also affected by the local modifications of the extracellular environment induced by inflammation, and, importantly, on the interaction of the drug with COX (or any other final mediators).

There are a number of limitations of this study - namely we did not examine the confounding effects of bodyweight, age, feeding regimen, feed type, drug interactions; as well as the possible effect of different formulations. The pharmacokinetic parameters of MLX ($t_{1/2}$ = 37.91h, t_{max} = 5.00 h, C_{max} = 0.39 µg/mL, AUC = 17.99 µg.h/mL and MRT = 56.03 h) in the present study were relatively similar to the parameters obtained by Busch *et al*. [2] ($t_{1/2}$ = 23.7 h, t_{max} = 7.5 h, C_{max} = 0.46 µg/mL and AUC = 22.9 µg.h/mL and MRT = 40 h), but different from those observed by Montoya *et al*. [13] ($t_{1/2}$ = 12.13 h, t_{max} = 8.5 h, C_{max} = 0.82 µg/mL and AUC = 14.61 µg.h/mL) and following same administration route and dose (0.2 mg/kg bodyweight) in dogs. These differences may in part be due to differences in methodology, namely different drug formulations and/or different feeding regime before drug administration. In the present study, the animals were fed 1 h before the drug administration and a commercial tablet formulation of MLX was used for treatment. Whereas, suspension formulation of MLX was administered to animals following fasting for 12 h and they were fed 8 h later of the drug administration in the study reported by Montoya and his colleagues [13]. Moreover, the present study was not omit a blinded randomised study with regard to selection of dogs and drugs.

In the present study, MLX displayed relatively slower absorption and longer tmax after oral administration compared with the previous studies conducted following subcutaneous [2] and intramuscular [5] routes in dogs. Oral administration could be a disadvantage in acute cases and subcutaneous or intramuscular routes should be preferred for MLX, because the onset time of the effect of the drug is longer for the oral route in dogs [2,5].

Large inter individual variations were observed in the kinetic parameters, especially for AUC values of two investigated drugs. The origin of these variations is unclear. Although, the animals were clinically healthy, possible chronic renal/hepatic problems that may have affected the kinetic parameters of the study; since any biochemical tests were not performed in the animals before the experiment in the present study.

The plasma pharmacokinetic parameters of CPR in dogs were different in this study when compared with those observed from previous studies [3,12]. The t_{max} (2.20 h), $t_{1/2}$ (17.02 h) and AUC (337.34 µg.h/mL) values of CRP were longer and larger in the presents study than those obtained by Clark and his colleagues [3] (t_{max} = 1.05 h, $t_{1/2}$ and AUC = 89.60 µg.h/mL). The origin of these differences is also unclear, but may be attributable to the differences in drug formulations, feeding regimes or animal breeds used in the studies.

CONCLUSION

The present study indicates significant differences between pharmacokinetic parameters of MLX and CRP after oral administration, MLX may be better given by the intramuscular or subcutaneous routes in dogs. Because of the relatively slow absorption from gastrointestinal system after oral administration, MLX should be given intramuscular or subcutaneous routes in acute cases with respect of the time for onset of action. Moreover the longer terminal half life of MLX may result in potential accumulation of drug after multi-dosing.

REFERENCES

1 **Botting R.M. 2006.** Cyclooxygenase: Past, present and future. A tribute to John R. Vane (1927-2004). *Journal of Thermal Biology*. 31: 208-219.

2 **Busch U., Schmid J., Heinze G., Schmaus H., Baier J., Huber C. & Roth W. 1998.** Pharmacokinetics of meloxicam in animals and the relevance to humans. *Drug Metabolism and Disposition*. 26: 576-584.

3 **Clark T.P., Chieffo C., Huhn J.C., Nimz E.L., Wang C. & Boy M.G. 2003.** The steady-state pharmacokinetics and bioequivalence of carprofen administered orally and subcutaneously in dogs. *Journal of Veterinary Pharmacology Therapeutics*. 26: 187-192.

4 **Engelhardt G., Homma D., Schlegel K., Utzmann R. & Schnitzler C. 1995.** Meloxicam: a potent inhibitor of adjuvant arthritis in the Lewis rat. *Inflammation Research*. 44: 548-555.

5 **Euller-Ziegler L., Vélicitat P., Bluhmki E., Türck D., Scheuerer S. & Combe B. 2001.** Meloxicam: a review of its pharmacokinetics, efficacy and tolerability following intramuscular administration. *Inflammation Research*. 50(Suppl 1): 5-9.

6 **Fiorucci S., Meli R., Bucci M. & Cirino G. 2001.** Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy. *Biochemical Pharmacology*. 62: 1433-1438.

7 **Kay-Mugford P., Benn S.J., LaMarre J. & Conlon P. 2000.** *In vitro* effects of nonsteroidal anti-inflammatory drugs on cyclooxygenase activity in dogs. *American Journal of Veterinary Research*. 61: 802-810.

8 **KuKanich B., Bidgood T. & Knesl O. 2012.** Clinical pharmacology of nonsteroidal anti-inflammatory drugs in dogs. *Veterinary Anaesthesia Analgesia*, 39: 69-90.

9 **Lees P., Giraudel J., Landoni M.F. & Toutain P.L. 2004.** PK-PD integration and PK-PD modelling of nonsteroidal anti-inflammatory drugs: principles and applications in veterinary pharmacology. *Journal of Veterinary Pharmacology Therapeutics*. 27: 491-502.

10 **Lees P., Landoni M.F., Giraudel J. & Toutain P.L. 2004.** Pharmacodynamics and pharmacokinetics of nonsteroidal anti-inflammatory drugs in species of veterinary interest. *Journal of Veterinary Pharmacology Therapeutics*. 27: 479-490.

11 **Mathews K.A. 2002.** Nonsteroidal anti-inflamatory analgesics: a review of current practice. *Journal of Veterinary Emergency and Critical Care*. 12: 89-97.

12 **McKellar Q.A., Delatour P. & Lees P. 1994.** Stereospecific pharmacodynamics and pharmacokinetics of carprofen in the dog. *Journal of Veterinary Pharmacology Therapeutics*. 17: 447-454.

13 **Montoya L., Ambros L., Kreil V., Bonafine R., Albarellos G., Hallu R. & Soraci A. 2004.** A pharmacokinetics comparison of meloxicam and ketoprofen following oral administration to healthy dogs. *Veterinary Research Communication*. 28: 415-428.

14 **Nielsen-Kudsk F. 1980.** HPLC-determination of some antiinflammatory, weak analgesic and uricosuric drugs in human blood plasma and its application to pharmacokinetics. *Acta Pharmacology and Toxicology*. 47: 267-273.

15 **Shrivastava A. & Gupta V.P. 2011.** Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles of Young Scientists*. 2: 21-25.

16 **Taketo M.M. 1998.** Cyclooxygenase-2 inhibitors in tumorigenesis. *Journal of the National Cancer Institute*. 90: 1609-1620.

17 **Toutain P.L., Cester C.C., Haak T. & Laroute V. 2001.** A pharmacokinetic /pharmacodynamic approach vs. a dose titration for the determination of a dosage regimen: the case of nimesulide, a Cox-2 selective nonsteroidal anti-inflammatory drug in the dog. *Journal of Veterinary Pharmacology Therapeutics*. 24: 43-55.

18 **Velpandian T., Jaiswal J., Bhardwaj K.R. & Gupta S.K. 2000.** Development and validation of a new high performance liquid chromatographic estimation method of meloxicam in biological samples. *Journal of Chromatography B: Biomedical Science Application Science*. 738: 431-436.