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Pharmacokinetics and Metabolism of Meloxicam in Camels after Intravenous Administration Using Orbitrap Mass Spectrometry

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Abstract

The pharmacokinetics and urinary elimination of meloxicam were studied in camels (*Camelus dromedarius*) following a single intravenous (IV) administration at 0.5 mg/kg (body weight). Blood samples and urine were collected at different time intervals for30 days. Meloxicam was extracted from plasma and urine using solid phase extraction and the concentrations measured using liquid chromatography-orbitrap mass spectroscopy (LC-MS/MS). The results show thatPhaseI biotransformation of meloxicam led to the formation of hydroxyl and carboxy metabolites. The parent drug and the metabolites were detected unconjugated in urine and performing enzymatic hydrolysis did not increase the yield of the drug and its metabolites appreciably. The plasma drug concentrations were best fitted by a two compartment model.The results (mean ± SD) revealedthe distribution half-life ($t_{1/2\alpha}$) was0.863 ± 0.129 h, the elimination half-life ($t_{1/2\beta}$) was 46.952 ± 4.694 h and the total body clearance (Cl_T) was 5.587 ± 1.655 mL/kg/h. The apparent volume of distribution (Vd) was 371.940 ± 86.658 mL/kg.Two metabolites of meloxicam were identified unconjugated in urine as 5'-hydroxymethylmeloxicam and 5'-carboxymeloxicam.

It was concluded from this study that the pharmacokinetics of meloxicam in camel was characterized by a slow clearance, small volume of distribution and very long half-life, which resulted in a long detection time following the IV administration. Meloxicam and its two metabolites namely 5'-hydroxymethylmeloxicam and 5'-carboxymeloxicamcould be detected for 17, 25 and 27 days respectively in urine mainly unconjugated using high resolution accurate mass liquid chromatography-orbitrap mass spectrometry (LC-MS/MS) method.

Keywords: Camels; Clearance; Meloxicam; Metabolism; Pharmacokinetics

Introduction

Meloxicam [4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1, 2-benzothiazine-3-carboxamide-1,1-dioxide] is a potent non-steroidal anti-inflammatory drug (NSAID) of the oxicam group.These structurally related drugspossess analgesic, antipyretic and anti-inflammatory properties, with low ulcerogenic potency and less local tissue irritation in comparison to other NSAIDs such as diclofenac, indomethacin and piroxicam[1]. Meloxicam is a drug of choice in the management of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis by relieving pain and reducing inflammation in humans[2]. Meloxicam inhibits prostaglandin synthesis via selective inhibition of cyclooxygenase-2 enzyme and thus shows superior gastrointestinal tolerability than other NSAIDs[3]. The drug is available as either injectable or oral formulations in many countries worldwide[4].

Meloxicam has considerably varied pharmacokinetic parameters among species. For example, the elimination half-life ranged from 20-24 hours in humans[5] to about 8.54 hours in horses[6]. Recently, many positive findings were reported for meloxicam in racing camels in U.A.E. Veterinary records showed that the animals received a therapeutic dose of meloxicam 20 days before the race events. A previousstudyhas highlighted a detection

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period of 10 daysfor meloxicam in camel plasma[7]. It has been noted that no more blood samples were collected after 10 days and the reported analytical method lacks the sensitivity required to detect meloxicam at lower trace levels.

Therefore, the objective of the present study was to characterize the plasma pharmacokinetics, urinary excretion profile and metabolism after administration of a single IV doseof meloxicamto camels. Another goal of the study was to advise the field veterinarians about the detection and withdrawal time period for meloxicam and its metabolites in camels used in racing employing the latesthigh resolution bench-top orbitrap mass spectrometry (LC-MS/MS) with an in-house developed solid phase extraction method.

Materials and Methods

Animals

Four healthy male camels (*Camelus dromedaries*) aged 5 – 10 years and weighing approximately 500 kg were used in this study. The camels were tagged, dewormed and housed in shaded pens. They were fed with hay andlucerne, with water allowed ad *libitum*. The health status of these experimental animals was regularly monitored throughout the experiment by project veterinarians. The study had the relevant ethical clearance by the local animal ethics committee to undertake the work.

Experimental chemicals

Meloxicam at 20 mg/mL IV solution (Metacam, Boehringer Ingelheim Vetmedica) was purchased as the administration drug under investigation. All organic solventswere of HPLC grade and purchased from Fisher Scientific. Meloxicam as pure reference standard, d_3-flunixin and β-glucuronidase from *Helix pomatia* (type HP2) were purchased from Sigma Chemical Co.Ltd.

Treatment and sample collection

Meloxicam was administered as an intravenous single dose of 0.5 mg/kg (body weight) into the right jugular vein. Venous blood samples were collected from the jugular vein at 0 min (pre-injection), 5, 10, 15, 30, and 45 min and 1, 2, 4, 6, 8, 12, 24 hours post medication through an in-dwelling cannula on the opposite side. Blood samples were collected once daily for the next 30 days. The samples were centrifuged at 4000g at room temperature for 10 min and plasma was separated and stored at -20 °C until analysis. In addition, urine was collected as morning and evening samples as the animal disposes during the same period.

Sample preparation

Plasma and urine samples were analyzed by use of liquid chromatography-orbitrap mass spectrometry. Solid phase extraction (SPE) was carried out using automated extraction modules (RapidTrace, Biotage) using mixed-mode extraction cartridges that combine both non-polar (C_8) and strong anion exchange retention ($-NR_3+$) mechanisms (Isolute HAX, Biotage). A volume of 2 mL of plasma was combined with 20 µl internal standard (flunixin-d3 [1 µg/mL]). The extraction cartridge was conditioned with methanol (2 mL) and 0.05 M ammonium acetate (2 mL). The mixed sample of plasma and internal standard was loaded on to the cartridge and allowed to pass through at a slow rate. The cartridge was then washed with a mixture of methanol and 0.05M ammonium acetate (1 mL [1:9]) and allowed to dry under nitrogen for 5 min. Meloxicamwas eluted with 2% acetic acid in methanol (5 mL) into clean test tubes. The organic solvent was evaporated under nitrogen at 50 ºC. Samples werereconstituted with the analytical method mobile phase and 10 µL is injected into the LC-MS/MS system.

Similarly, a qualitative analysis to study the metabolites and excretion of meloxicam in urine was performed. Duplicate aliquots of 5 mL of camel urine were used for extraction. One aliquot was adjusted to pH 5 and enzyme hydrolyzedusing β-glucuronidase by incubating at 56 ºC for 2 hours while the other aliquot was not subjected to enzymatic hydrolysis. To both aliquots, 50 µL internal standard (flunixin-d_3 [1 µg/mL]) was added, pH was adjusted to 7.0, and centrifuged at 4000 g at room temperature for 10 min. Thereafter, 4 mL of the clear supernatant sample from each aliquot was extracted seperately with the described SPE procedure.

LC-MS/MS analysis

Detection of meloxicam was performed by an in-house developed and validatedLC–MS⁄MS using high pressure liquid chromatography system (Ultimate3000, Thermo Dionex) coupled with orbitrap high resolution mass analyser (Q Exactive, Thermo Scientific). The mobile phase consisted of acetonitrile and 1% formic acid at a flow rate of 0.6 mL/min, run in a multi-step gradient mode. Separation was achieved by usingreversed phase C18 column 4.6 mm x 150 mm, 3.5 micron (Zorbax Eclipse, Agilent Technologies). The mass spectrometer was operated in positive ion mode and the total run time was 8.4 minutes.

Calibration curves were prepared for the quantitative determination of meloxicam in plasma samples obtained afterIV administration. The calibrators ranged from 1.0 to 2000 ng/mL. Two quality control sample spiked at 10 and 50 ng/ml of meloxicam in plasma were also prepared. Calibrators and quality controls were prepared independently from separate weightings of the drug standard. All calibrators, quality control sample and test samples were analyzed in duplicate by LC-MS/MS. The peak area ratios of meloxicam to the internal standard versus the concentration of the calibrators were fitted using linear regression. The response for the assay method was linear over the designed calibration range (r^2=0.998). The limit of quantitation was 1 ng/mL. The within-day and day to day precision were < 10%.

Identification of metabolites

Aliquots of urine samples taken before and after the IV administration were extracted by SPE and analyzed using LC-MS/MS system applying Full MS, All-Ion-Fragment (AIF) and Data-Independent-Acquisition (DIA) scan functions of the mass analyzer. Meloxicam metabolites were identified by comparison of the obtained chromatographic peaks and spectra against reported spectra with the aid of commercial available spectral data-base (Association of Official Racing Chemists).

Pharmacokinetic Analysis

Pharmacokinetic parameters were determined for each animal individually from plasma drug concentration versus time profile using a computerized pharmacokinetic and drug disposition program (Kinetica Version 5.1 SP1, Thermo). The best fitting

was achieved by two compartment open model based on the statistical moments theory[8,9]. All data were analysed using SPSS package. For each parameter, the mean and its standard deviation (SD) were obtained from the parameters estimates of four animals. The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal method up to the last measured concentration.

Results

The pharmacokinetics (PK) of meloxicamafter a single dose IV administration (0.5 mg/kg) in camels was best fitted with two compartmental open model and followed the first order rate kinetics.The graphical representation of the mean ± SDplasma drug concentration (µg/mL) of meloxicam in camels (*n = 4*) versus time is given in (Figure.1). Pharmacokinetic parameters estimates of meloxicam (mean ± SD) are presented in Table 1.

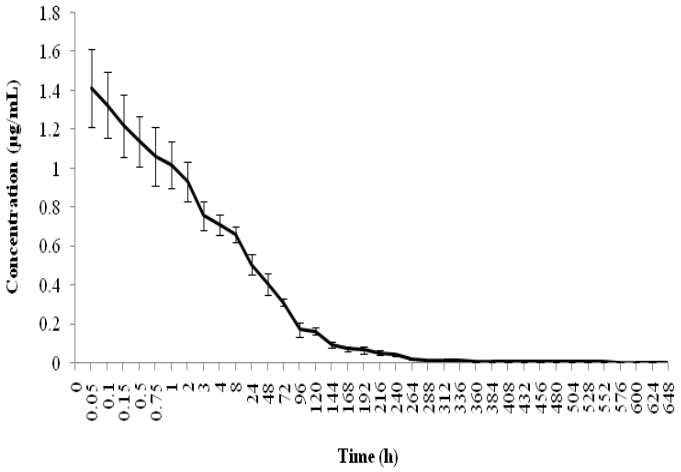

Figure1: Time course graph showing plasma concentrationprofile of meloxicam in camels following an intravenous dose of 0.5 mg/kg (body weight). Values are presented as mean ± standard deviation.

Table 1: Pharmacokinetic parameters of meloxicam in camels (*n = 4*) after a single intravenous administration at a dose of 0.5 mg/kg (body weight). Data are presented as mean ± SD.

Pharmacokinetic parameter	Mean ± SD
Rate of distribution (α) h^{-1}	0.816 ± 0.131
Distribution half-life ($t_{1/2\alpha}$) h	0.863 ± 0.129
Rate of elimination (β) h^{-1}	0.015 ± 0.001
elimination half-life ($t_{1/2\beta}$) h	46.953 ± 4.694
Volume of distribution (Vd) mL/kg	371.940 ± 86.658
Clearance (Cl_T) mL/kg/h	5.588 ± 1.655
AUC_{0-t} µg.h/mL	57.665 ± 14.788
Extrapolated initial drug concentration (C_0) µg/mL	1.572 ± 0.391

The plasma drug profile (Figure.1) indicates an initial distribution phase of meloxicam with a relatively long distribution half-life ($t_{1/2\alpha}$) of 0.863 ± 0.131 h. The distribution phase was followed by a very slow elimination phase with a very long terminal elimination half-life ($t_{1/2\beta}$) of 46.952 ± 4.694 h. The mean plasma drug concentration levels of meloxicam were still above the detection limit (>100 pg/mL) after 25 days of administration. The limit of quantitation (LOQ) of melociam in plasma was found to be 1ng/mL. The estimate of the extrapolated initial drug concentration (C_o) was 1.572 ± 0.391 µg/mL. The body clearance (Cl_T) was 5.587 ± 1.655 mL/kg/h and area under curve (AUC_{0-t}) was 57.665 ± 14.788 µg.h/mL.

The urine samples evidenced the presence of two metabolites: 5'-hydroxymethylmeloxicam (m/z 368.0369) and 5'-carboxymeloxicam (m/z 382.0162), separated and characterized by the proposed LC-MS/MS method in positive ion mode, at retention times (RT) 5.86 min and 6.26 min respectively, as shown in (Figure.2.A,B). Meloxicam could be detected up to 25 days after administration in plasma with the limit of detection of 100 pg/mL. The metabolites 5'-carboxymeloxicam and 5'-hydroxymethylmeloxicam could be detected upto 17 days and 27 days in urine samples respectively. No significant changes were observed in the concentration of meloxicam and its metabolites in urine after enzymatic hydrolysis, indicating that meloxicam and its metabolites are excreted unconjugated.

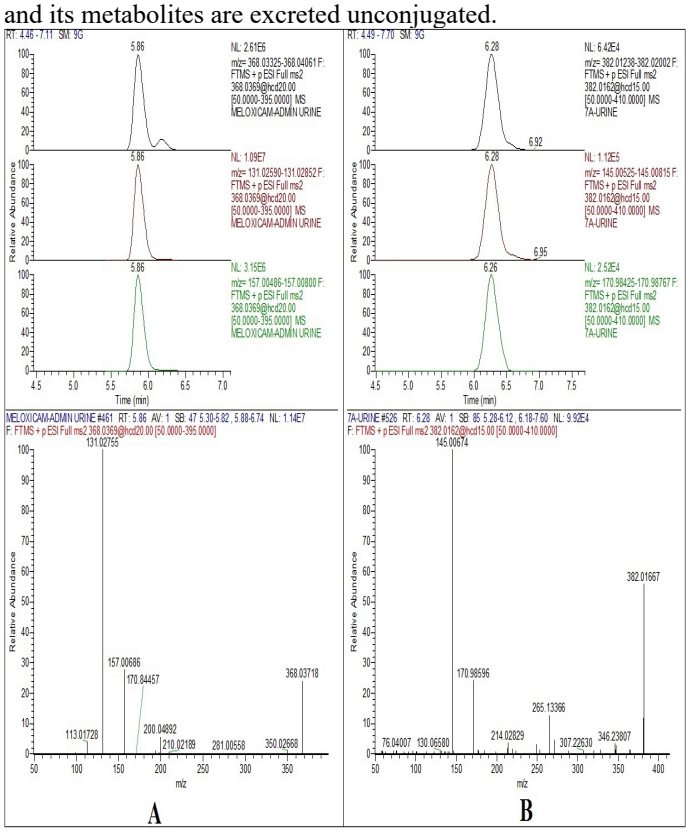

Figure 2: Original chart recordings of LC-MS/MS extracted ion chromatogram and mass spectra of (A) 5'-hydroxymethylmeloxicam (RT = 5.86 min) and (B)5'-carboxymeloxicam (RT = 6.28 min) obtained from extracted camel urine after intravenous administration of meloxicam.

Discussion

Meloxicam is a commercially available anti-inflammatory drug widely used to suppress pain[10]. Meloxicam has been found to be well absorbed and cleared almost exclusively by metabolic pathways in mice, rats, mini-pigs, baboons and human[11,12]. Meloxicam pharmacokinetics in camels has been previously reported[7]; however, the present study employed more frequent and longer blood sampling up to 30 days after drug administration. The sensitive analytical method developed enabled quantitation of meloxicam at very low levels (1 ng/mL) and is about ten times lower than the reported LOQ of 10 ng/mL.

The mean ± SD plasma level of meloxicam was 1.41 ± 0.20 µg/mL at 0.05 h after IV administration, which declined rapidly to 0.7 ± 0.33 µg/mL after 3 hours, and thereafter the drug concentration declinedto a steady state level. The distribution half-life ($t_{1/2\alpha}$) and the elimination half-life ($t_{1/2\beta}$) of meloxicam in this study were 0.863 ± 0.131 h and 46.952 ± 4.694 h respectively, and are found to be much longer in camels compared to other species. The reported half-life of meloxicam in sheep and goat were 10.85 ± 1.21 and 6.73 ± 0.58 h, respectively[13]. Relatively shorter elimination half-life has been reported in avian species[14] while a half-life of 20 h has been reported in human[5]. In a short period experiment with less sampling intervals at the elimination phase, a shorter half-life of 40.2 ± 16.8 h for meloxicam has been reported in camels[7].

The volume of distribution (Vd) in the present study was 371.940 ± 86.658 mL/kg.This is more than the Vd reported in goat 276 mL/kg[15] and cattle 171 mL/kg[16,17]. These findings indicate that the drug is well distributed in camels emphasizing a therapeutic treatment of camels with meloxicam by a daily single dose administration.

The present results have demonstrated that the proposed analytical method was highly sensitive that it could detect meloxicam in plasma at low levels at about 100 pg/mL after 25 days of administration. The calculated total body clearance (Cl_T) was 5.587 ± 1.655 mL/kg/h and it is found to be lower than that reported for horses (34.7 mL/kg/h)[6], piglets (61 mL/kg/h)[18], dogs (10 mL/kg/h)[9] and goats (22.6 mL/kg/h)[12], indicating a slower elimination in camels in comparison to other species. This is also evidenced with a comparatively high AUC0-t in this study (57.665 ± 14.788 µg.h/mL) which also implies that the drug is eliminated at a lower rate in camels in comparison to other reported species[15-17,19].

The ESI-MS/MS product ion spectrum of meloxicam (m/z 352.0420) shows two diagnostic fragment ions (Figure. 3). The protonation on the benzothiazine nitrogen heteroatom and cleavage of the benzothiazine-3-carboxamide bond without any proton transfer leads to formation of distinct m/z 141.0117. Further more, protonation of the amide nitrogen atom, two proton transfer and cleavage of amide linkage leads to the formation of m/z 115.0328[20]. The pharmacokinetics of meloxicam has been well studied in horses and the drug and twoof its metabolites could be detected for 72 hours[21]. In a previously published study on camels, only one metabolite of meloxicam namely, 5'-hydroxymethyxlmeloxicam has been reported and detected up to 10 days in plasma[7]. The present study shows that 5'-hydroxymethylmeloxicam (m/z 368.0369) was detected in urine samples up to 27 days after administration. In addition, further oxidation to this metabolite was observed resulting in the formation of 5'-carboxymeloxicam (m/z 382.0162) that could be detected up to 17 days. The two metabolites have been previously detected in humans, rats, mice, mini-pigs[9], and thoroughbred horses[20,22].

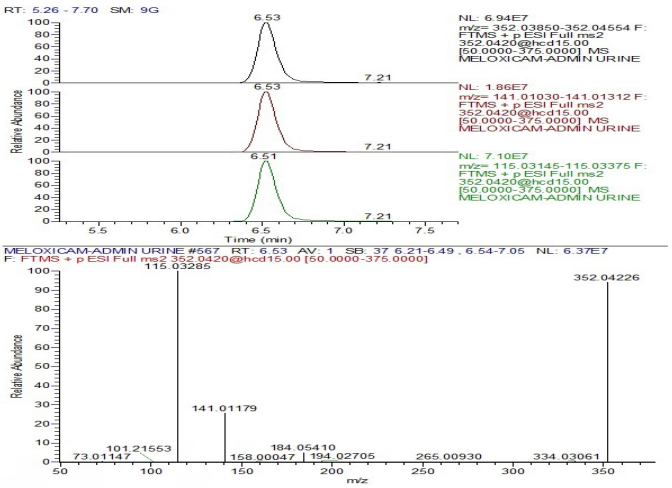

Figure 3: Original chart recordings of LC-MS/MS extracted ion chromatogram and mass spectra of meloxicam obtained from extracted camel urine after intravenous administration of meloxicam.

Conclusions

The results obtained in the current study revealed that meloxicam followed the first order rate kinetics and it could be best fitted to a two compartmental open model.Two main metabolites could be isolated and detected from camel urine samples following IV administration of meloxicam. The main objective of thisstudy was to investigate the pharmacokinetics of meloxicam and more over to find a withdrawal time following therapeutic administration for the practicing veterinarians. The proposed LC-MS/MS method was highly sensitive, with a limit of detectionof 100 pg/mL, which could easily detect meloxicam in camel plasma for 25 days after administration. As such, a very long elimination half-life of meloxicam was observed in the present study. Hence, it is advised to withhold the use of meloxicam in camels for at least 30 days prior to racing.

Conflict of interest statement

Boehringer Ingelheim Middle East & North Africa (Scientific Office) supplied Metacam(20 mg/mL solution for injection) used in this study. Boehringer Ingelheim Middle East and North Africa (Scientific Office) played no role in the study design nor in the collection, analysis and interpretation of data, nor in the decision to submit the manuscript for publication. None of the authors have any financial or personal relationships that could inappropriately influence or bias the content of the paper. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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