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THE PHARMACOKINETICS AND DISPOSITION OF IBUPROFEN IN THE CAMEL

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ABSTRACT

The pharmacokinetics of ibuprofen was studied in six dromedary camels following intravenous (i.v.) and oral (p.o.) administration of a dose of 25 mg/kg body weight. The data, following i.v. administration, was best described by a bi-exponential equation. The data obtained (median and range in brackets) were as follows: the distribution half-life ($t_{1/2\alpha}$) was 0.56 (0.42 – 1.02) h, the elimination half-life ($t_{1/2\beta}$) was 3.50 (2.4 – 8.4) h, the area under the time-concentrations curve ($AUC_{0-\infty}$) was 0.56 (0.40 – 0.63) mg/h/ml. The volume of distribution at steady state (Vd_{ss}) was 0.19 (0.13 – 0.33) l/kg, the total body clearance (Cl_T) was 0.05 (0.04 – 0.06) L/h/kg, and volume of the central compartment of the two compartment pharmacokinetic model (V_c) was 0.12 (0.08 – 0.15) L/kg. Following oral administration the absorption half-life ($t_{1/2\alpha}$) was 1.7 (0.55 – 2.5) h, the elimination half-life ($t_{1/2}$) was 3.8 (2.2 – 8.4) h, and area under the time- concentrations ($AUC_{0-\infty}$) was 0.58 (0.29 – 0.86) mg/h/ml. Maximal plasma ibuprofen concentration (C_{max}) was 56 (39 – 67) µg/mL at a time (T_{max}), 3.0 (2.5 – 4.0) h. Ibuprofen is well absorbed in the camel after oral administration, with a median bioavailability of 104% and range of 73-107%. Two metabolites of ibuprofen were tentatively identified as, hydroxyl-ibuprofen and carboxy-ibuprofen.

Key words: Camels, disposition, ibuprofen, pharmacokinetics

Ibuprofen is a propionic acid derivative with nonsteroidal anti-inflammatory and analgesic properties (Adams and McCullough, 1969). Pharmacologic properties, pharmacokinetics, toxic effects, and therapeutic use of ibuprofen were studied in human beings and a variety of laboratory, domestic species, and horses (Lambert *et al,* 1979; Albert and Gernaat, 1984; Kantor, 1984; Vandenbossche *et al,* 1992; Breuhaus *et al,* 1999). Ibuprofen is reported to cause fewer adverse gastrointestinal effects than do other nonsteroidal anti-inflammatory drugs (NSAIDs) in human beings and laboratory animals (Kopcha *et al,* 1992; Walson and Mortensen, 1989), yet it is rarely used in veterinary medicine. The use of human drugs in horses and camels is a known practice in the United Arab Emirates (UAE) where we detected several cases of ibuprofen administration in racing camels. However, any published pharmacokinetic studies of ibuprofen in camels is not known so far.

Camel racing authorities in UAE have adopted a zero drug concentration regulation at the time of racing. Blood and urine obtained from camels after racing should be free of drugs. The purpose of the study was to characterise the pharmacokinetics properties of ibuprofen after a single intravenous bolus and oral administration in the camel and to determine a pharmacologically irrelevant plasma concentration. Another objective of the study was to determine ibuprofen phase I metabolites in camels. The data obtained should be of value to practicing veterinarians and camel trainers in advising when to withhold ibuprofen administration to camels before racing.

Materials and Methods

Experimental animals

Six 9-10 years old male dromedary camels weighing 400 - 450 kg were used in this study. Camels were healthy based on physical and clinical chemistry examination. These were housed in shaded, ventilated stalls and received hay and water *ad libitum*. This study was approved by the ethical committee in the Veterinary Department, Ministry of Agriculture.

Ibuprofen injection solution

Intravenous ibuprofen solution was prepared by dissolving 10.1 g of ibuprofen sodium salt (Sigma chemical Co, St Louis, MO, USA) equivalent to 10 g ibuprofen base into 50 ml of 0.9 % sterile sodium chloride solution (normal saline) for intravenous administration. Oral ibuprofen solution was prepared

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by diluting 50 ml of intravenous solution equivalent to 10 g ibuprofen base to 500 ml with deionised distilled water.

Study design

The study was carried out in a two period cross-over design with animals randomly divided into two groups of three camels each. In the first period one group received ibuprofen 25 mg per kilogram as a single intravenous injection into the jugular vein whilst the other group received the same single dose of ibuprofen orally. The first period was followed by washout period of 15 days. After which the second period was conducted where treatments were reversed in the two groups.

Sample collection

Blood samples (15 ml) were collected into heparinised vacutainers from the opposite jugular vein prior to ibuprofen administration (0 time), and at 5, 10, 15, 30, and 45 min and 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, and 24 h after i.v. drug administration. Blood samples were also collected from the opposite jugular vein at 10, 20, 30, and 45 min, and 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12, and 24 h after P. O. administration. Blood samples were immediately placed on ice; plasma was separated by centrifugation at 4500 x g at room temperature for 10 min. The harvested plasma was frozen at -20°C until analysis.

Assay procedure

Ibuprofen plasma concentrations were measured by gas chromatography/mass spectrometry (GC-MS). Liquid-liquid extraction was carried out as followed: to 200 µl of plasma in screw-capped test tubes was added 10 µl concentrated phosphoric acid, and 100 mL the internal standard (fenoprofen, 5 mg/ml). The tubes were vortexed for 1 min. Then 5 ml of n-hexane / ethyl acetate mixture (80: 20; v/v) was added to each tube. The mixture was then vortex again for 1 min and centrifuged at 3500 x g for 10 min. 400 µL of the top organic layer was separated into disposable test tubes (100 µl for the samples from 5 min till 2.5 h of the IV experiment) and was evaporated to dryness at 45°C under a low stream of nitrogen. The residue was re-dissolved in 100 µl methelute (methelute: methanol 1:1 v/v) and vortex. 2 µl were injected in the GC/MS system.

GC/MS Analysis

GC/MS analysis was carried out using a Hewlett-Packard 5972 Mass Selective Detector interfaced to a HP 5890 gas chromatography (GC) with HP 7673 auto-injector and sample tray. Injections were made in the splitless mode onto a 25 m x 0.25mm i.d. HP-5MS column (Hewlett-Packard). The initial column temperature was 80°C and it was programmed to rise to 300°C at 20°C min^{-1}. Helium was used as the carrier gas. Data were acquired in the selected ion mode (SIM) monitoring the ion m/z 161 for ibuprofen and the ion m/z 256 for fenoprofen. The linearity of the method was from 1 to 50 µg/ml of ibuprofen in spiked plasma (r^2) >0.999. The inter-assay coefficient of variation for 5 and 40 µg/ml (n=6), determined on three consecutive days, were 2.38% and 6.80%, respectively. The intra-assay coefficient of variation for 5 µg/ml and 40 µg/ml (n=10) were 5.23% and 3.98%, respectively. The percent recovery of spiked camel plasma at concentrations of 5 and 40 µg/ml was 76% - 80%. The lower limit of quantification (LLOQ) defined as the lowest concentration which can be quantified with adequate accuracy and precision, based on S: N^3 was 1 µg/ml.

Determination of metabolites

The metabolites of ibuprofen in camels were investigated as reported previously (Wasfi *et al,* 2003). Ibuprofen metabolites were tentatively identified from the chromatographic peaks obtained with the urine samples taken after administration of ibuprofen compared with urine samples taken before the administration of ibuprofen, and also by matching against a commercial mass spectral data base (Pfleger *et al,* 1992).

One of us also took one ibuprofen oral tablet (400 mg) once. Urine sample was collected 5h after drug administration and was extracted and analysed as above. The aim was to compare ibuprofen metabolites between humans and camels.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed using standard methods and equations (Gibaldi and Perrier, 1982) by the use of a computer programme (PC Modfit version 2.1; Cambridgeshire, UK) as reported previously (El Ghazali *et al,* 2007). The best fit was based on Akaike criterion (Yamoka *et al,* 1978). Maximum plasma concentration (C_{max}) and time of maximal plasma concentration (T_{max}) were determined directly from the concentrations versus time curves.

Statistical Analysis

Pharmacokinetic parameters estimates of ibuprofen were expressed as median and range. Wilcoxon and Kruskal-Wallis rank- sum tests were used for statistical comparisons of parameters.

Results

Pharmacokinetic parameter estimates (median and range) following i.v. and p.o. administration are shown in Table 1. Ibuprofen plasma concentrations versus time concentrations are presented in Fig 1. Following intravenous administration of ibuprofen, the plasma-time concentrations of the 6 camels were best described with two-compartment open model. The plasma drug profiles were characterised by fast distribution phase; a small volume of distribution at steady state, 0.19 (0.13-0.33) l/kg and slow total body clearance of, 0.05 (0.04-0.06) l/h/kg.

After oral drug administration ibuprofen plasma profile was best described by a 2-exponential equation (Fig 1). The C_{max} was 56 (39-67) µg/ml which was attained at T_{max} of 3.0 (2.5-4.0) h. Ibuprofen was well absorbed after oral administration with a mean systemic bioavailability (%F), of 104%. There was no significant differences between AUC and elimination half-lives between the two routes of administration.

Fig 2 shows the total ion chromatogram of a plasma extract (acidic fraction) of camel A before ibuprofen administration. Neither ibuprofen or its metabolites are present. The acid-neutral fraction of plasma sample extracts of camel A 5 h after i.v. administration of ibuprofen showed two major metabolites (Fig 3A). Theses metabolites were tentatively identified as hydroxy-ibuprofen (retention time 9.4 min and molecular ion 236, Fig 3B) and carboxy-ibuprofen (retention time 10.2 and molecular ion 264, Fig 3C). These metabolites were also detected from the human urine sample (data not shown). No metabolites were detected in the basic fraction.

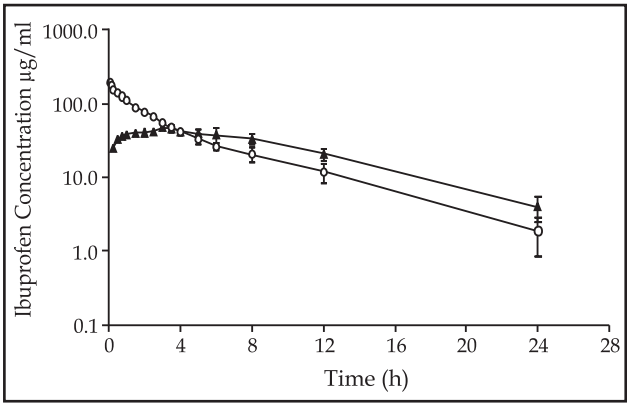

Fig 1. Mean ± SEM plasma concentrations vs time curves for intravenous, (○) and oral, (▲) ibuprofen administration to six camels (25 mg/kg body weight).

Discussion

Ibuprofen plasma concentrations data obtained following i.v. and p.o. administrations were best described by two-compartment pharmacokinetic model which agrees to what was reported in other species including horses, lactating dairy cows , goats and dogs (Scherkl and Fery, 1987; Vandenbossche *et al,* 1992; De Graves *et al,* 1993 a,b; Breuhaus *et al,* 1999). The steady state volume of distribution of ibuprofen in camels is similar to that reported in goats and dairy cattle, but the elimination half-lives

Table 1. Pharmacokinetic parameters (median and range) of ibuprofen following a single intravenous and oral administration of 25 mg/kg to six male camels.

Parameters	intravenous administration		oral administration	
	Median	Range	Median	Range
$t_{½a}$ (h)	0.56	0.42 – 1.02	–	–
$t_{½b}$ (h)	3.5	2.4 – 8.4	3.8	2.2 – 8.4
$t_{½ab}$ (h)	–	–	1.7	0.55 – 2.5
Vd_{ss} (l/kg)	0.19	0.13 – 0.33	–	–
V_c (L/kg)	0.12	0.08 – 0.15	–	–
Cl_T (l/h/kg)	0.05	0.04 – 0.06	–	–
$AUC_{(0-\infty)}$ (mg/h/Ml)	0.56	0.4 – 0.63	0.58	0.29 – 0.86
$AUMC_{(0-\infty)}$ (mg/h^2/ml)	2.2	1.2 – 3.6	5.8	2.2 – 8.8
MRT (h)	3.8	3.0 – 6.2	8.6	7.4 - 13
C_{max} (µg/ml)	–	–	56	39 - 67
T_{max} (h)	–	–	3.0	2.5 – 4.0
F%	–	–	104	73 - 107

$t_{½\alpha}$, the distribution half-life; $t_{½\beta}$, the elimination half-life; $t_{½abs}$, the absorption half-life; Vdss, volume of distribution at steady state; Vc, volume of the central compartment of the two pharmacokinetic model; ClT, total body clearance; $AUC_{0-\infty}$, the area under the concentration-time curves from zero to infinity; AUMC, area under momentum curve; MRT, mean residence time; C_{max}, maximum concentration at maximal time (T_{max}); %F, percentage bioavailability.

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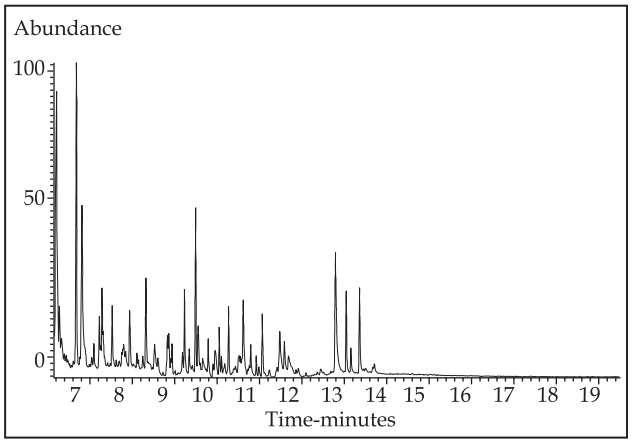

Fig 2. Total ion chromatogram of a plasma extract (acidic fraction) of camel A before ibuprofen administration. Neither ibuprofen nor its metabolites are present.

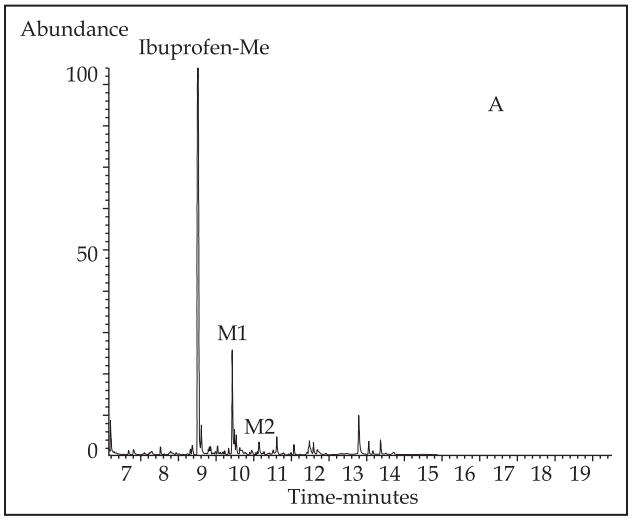

Fig 3A. Total ion chromatogram of camel plasma extract of camel A 5 h after i.v. administration of ibuprofen. Two metabolites could be seen (M1and M2) including ibuprofen (ibuprofen-Me) itself.

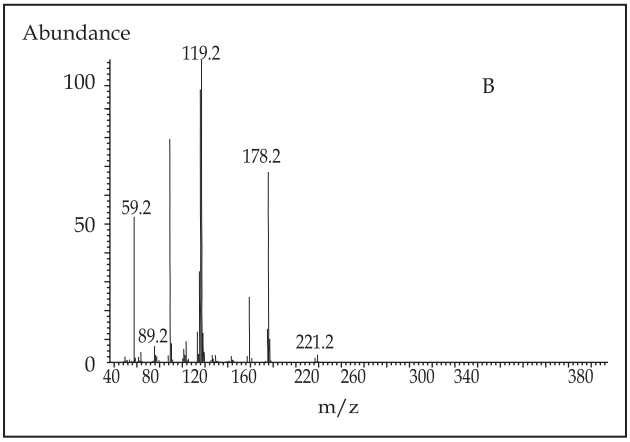

Fig 3B. Positive EI spectrum of metabolite I which is tentatively identified as hydroxy-ibuprofen. Methylation of hydroxyl- ibuprofen will result in a pseudo-molecular weight of m/z 236 with a typical loss of m/z 15, giving ion m/z 221.

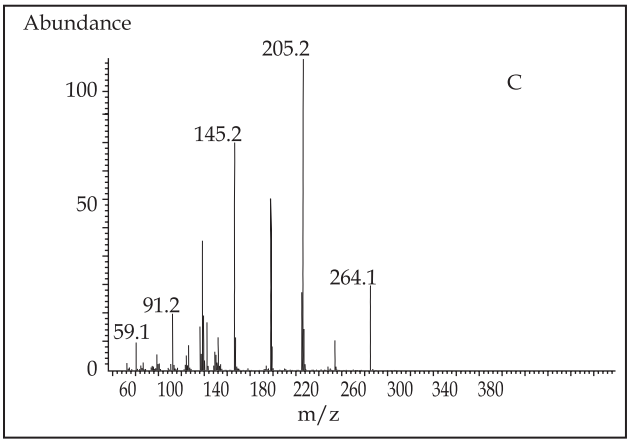

Fig 3C. Positive EI spectrum of metabolite II which is tentatively identified as carboxy- ibuprofen. Methylation of carboxy-ibuprofen will result in a pseudo-molecular weight of m/z 264.

in camels is much longer than of goats and cattle (De Graves *et al,* 1993 a,b). Normal dogs, however, have a much smaller volume of distribution (53 ml/Kg) when compared to camels (190 ml/Kg) while the terminal elimination half-life of ibuprofen is shorter in dogs (2.5 h vs 3.5 h). The low volume of distribution in camel (0.19 l kg^{-1}) may indicate that ibuprofen was highly protein-bound, as reported in other species (Lambert *et al,* 1979; Albert and Gernaat, 1984). Although, we have not determined the degree of plasma protein binding of ibuprofen in camel plasma, NSAID like flunixin, tolfenamic acid, ketoprofen, and diclofenac are characterised by a high degree of plasma protein binding in camels (Wasfi *et al,* 1998 a,b; Alkatheeri *et al,* 1999; Wasfi *et al,* 2003). Ibuprofen is well absorbed in camels with bioavailability ranging from 73 to 105% making oral administration an effective route. Large bioavailability of ibuprofen is reported in other species too (Breuhaus *et al,* 1999; De Graves *et al,* 1993 a,b). The clearance of ibuprofen in camels (50 ml/Kg/h) is slower than that reported in horses (120 ml/Kg/h; Dumacia *et al,* 2002) while the volume of distribution is smaller and the elimination half-life is longer in camels when compared to horses (Dumasia *et al,* 2002).

Using positive electron impact ionisation mode (EI) were able to identify two phase I metabolites of ibuprofen. The first one, hydroxyl ibuprofen (Fig 3, B) has a pseudo molecular ion of m/z 236 ($C_{13}H_{18}O_2$ + OH + CH_3). It is highly likely that hydroxylation resulted from aromatic hydroxylation, although it is not possible to determine

the exact position of hydroxylation from this study. Aromatic hydroxylation is a common route of drug biotransformation in camels. We have consistently found that aromatic hydroxylation is a major route of biotransformation for several compounds in camels, including tramadol, declofenac, tolfenamic acid and flunixin (Elghazali *et al,* 2007; Wasfi *et al,* 2003 and 1998a,b). The second metabolite identified from this study was the 2-carboxy ibuprofen which has a pseudo molecular ion of m/z 264 ($C_{13}H_{18}O_2$ + COOH + $2CH_3$). These are the major metabolites in humans (Han *et al,* 2004) and were also detected in the urine of one of us in this study. In humans, both metabolites were reported to be metabolised further into phase II glucuronide conjugates. We did not investigate phase II metabolites in this study.

This appears to be first report which describes the pharmacokinetics of ibuprofen in camels. The dose used in this study sustained the minimum therapeutic concentration of ibuprofen of 10 µg/ml (Doshi and Deshpande, 2007) for more than 12 hours without apparent adverse effects and seems to be appropriate for camels. However, accurate and safe dose regimens in camels should be established following controlled pharmacokinetics/pharmacodynamics (PK/PD) studies.

One of the objectives of the current study is to advise on withdrawal times following therapeutic treatments of ibuprofen. However, the developments of sensitive analytical instrumentations, has increased significantly the detection periods of therapeutic drugs and detected concentrations of prohibited substances may indeed be without pharmacological effect; no-effect or irrelevant concentrations (Houghton, 1995; Tobin *et al,* 1999). To determine a no-effect concentration experimentally, several time-consuming expensive PK/PD are required. Recently, however, using selected PK parameters, Toutain and Lassourd (2002) described a model for calculating irrelevant concentrations of veterinary drugs in biofluids. In their model, the effective plasma concentration (EPC) of the drug need to be calculated first as follows:

$$EPC = \frac{\text{Standard dose per dosing interval}}{\text{Plasma clearance per dosing interval}}$$

Then the EPC is converted to Irrelevant Plasma Concentration (IPC) by applying a safety factor (SF). IPC = EPC/SF

The SF is a regulatory decision and Toutain and Lassourd (2002) proposed 500. Applying these equations using the current PK data we have IPC of 42 ng/ml. This means that, although the concentration of ibuprofen following a standard dose of 25 mg could be detected for several days in plasma, yet the concentration after 2-3 days would be an irrelevant concentration without a pharmacological effect. It should be noted though, that ibuprofen is a chiral drug with its pharmacological activity residing in the S-(+)-enantomer. On the other hand the R-(-)-enantomer of 2-arylpropionic acid NSAFDs undergo chiral inversion, usually unidirectional, into the active form (Evans *et al,* 1990). This process is reported to be drug and species dependent (Tan *et al,* 1999). This should be put into consideration when calculating IPC of chiral drugs when the racemic form of the drug is used.

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