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PHARMACOKINETIC OF TRAMADOL AND ITS MAJOR METABOLITES AFTER INTRAVENOUS AND INTRAMUSCULAR INJECTIONS IN ALPACAS (*Vicugna pacos*)

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ABSTRACT

The objective of this study is to test the intravenous and intramuscular administration of tramadol in alpacas (*Vicugna pacos*), to assess both its pharmacokinetic properties and its safety profile. The study design comprised a 2 groups, single dose, 2 treatments, 2 periods, randomised, open, balanced, cross-over design. Eight healthy male alpacas (*Vicugna pacos*), aged 5-9 years, and weighing 41-58 kg were selected. After both the administrations of tramadol (2.5 mg/kg), the concentration of tramadol and its main metabolites, M1, M2 and M5, were determined in plasma using an HPLC method. Moderate side-effects were shown after IV administration. The intramuscular bioavailability of the drug (81.5%) was within the generally accepted values for a positive bioequivalence decision of 80-125%. After the intramuscular injection the mean plasma drug concentration peak was reached after a Tmax of 0.16 h with a Cmax of 1.25 µg/mL. The minimal effective plasma concentration was reached after few seconds following intramuscular dosing and maintained for about 2-3 h in both administrations. The plasma concentrations of M1 and M5 were low and the amounts of the M2 produced were analogous in both routes of administration.

In conclusion, tramadol was rapidly and almost completely absorbed after IM administration and its systemic availability was equivalent to the IV injection. The differences in the observed onset time and duration of action were very small and probably therapeutically irrelevant. Hence, IM injection of tramadol is a useful and better alternative to IV injection in alpacas.

Key words: Alpaca, injection, metabolism, pharmacokinetic, tramadol

Tramadol is a centrally acting analgesic drug that has been used clinically for the last 2 decades to treat pain in humans. Tramadol displays a low affinity for the mu- and delta-opioid receptors, and weaker affinity for the kappa-subtype; it also interferes with the neuronal release and re-uptake of serotonin and norepinephrine descending inhibitory pathways (Raffa *et al*, 1992). The metabolism of this drug has been investigated in different species as rodents (Lintz *et al,* 1981), goats (De Sousa *et al*, 2008), cats (Pypendop and Ilkiw, 2008), dogs (KuKanich and Papich, 2004; McMillan *et al*, 2008; Giorgi *et al,* 2009a, b, d), dromedary camels (Elghazali *et al*, 2008), donkeys (Giorgi *et al*, 2009c) and horses (Giorgi *et al*, 2007; Shilo *et al*, 2008; Giorgi *et al*, 2010); similar metabolites are produced but in different amounts. The clinical response of tramadol is correlated to its metabolism, because of the different analgesic activities of its metabolites. O-desmethyl-tramadol hydrochloride (M1), the major active metabolite, is 200 times more potent at the mu-receptor than the parent drug tramadol (Raffa *et al*, 1992). The primary metabolites of phase I, namely M1 and N-desmethyl-tramadol (M2, inactive), may be further metabolised to 3 additional secondary metabolites, namely N-N-didesmethyl-tramadol (M3), N-N-O-tridesmethyl-tramadol (M4), and N-O-didesmethyl-tramadol (M5, poor active because not easily penetrating the blood-brain barrier). The lack of side effects, characteristic of opioid derivatives, shown by this drug, and the absence of typical side effects due to non-steroidal anti-inflammatory drugs, suggests tramadol is a potential molecule for long-term therapeutic use in chronic pain in animals. Recently, tramadol was reported to be metabolised faster to inactive metabolites in goats (De Sousa *et al,* 2008), dogs (KuKanich and Papich, 2004; McMillan *et al,* 2008; Giorgi *et al,* 2009a, b, d), donkeys (Giorgi *et al,* 2009c) and horses (Giorgi *et al*, 2007; Shilo *et al,* 2008) than in cats (Pypendop and Ilkiw, 2008). The clinical

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effectiveness of this drug has been questioned in the animal species that mainly allow the drug to be metabolised to inactive substances. It has been suggested that these species would not be suitable as an effective and safe treatment for pain if given tramadol (De Sousa *et al,* 2008; McMillan *et al,* 2008; Shilo *et al,* 2008; Giorgi *et al,* 2007, 2009a, b, d). The animal species seem to be related to both the bioavailability of tramadol and the metabolic patterns, leading to different amount of M1. In dromedary camels, only a single study on plasma concentrations of tramadol is available (Elghazali *et al*, 2008) and no data is accounted for its active metabolite M1 in plasma. Hence, the aim of the present study was to test the intravenous (IV) and intramuscular (IM) administration of tramadol in alpacas (domesticated species of South American camelid), to assess both its pharmacokinetic properties and its safety profile and to evaluate the plasma amount of M1.

Materials and Methods

Tramadol hydrochloride and atenolol hydrochloride (IS) were obtained from Sigma–Aldrich (St. Louis, MO, USA). O-demethyl-tramadol hydrochloride (M1), N-demethyl-tramadol (M2), and O,N-didemethyl-tramadol (M5) were purchased from LGC Promochem (Milano, Italy). High performance liquid chromatography (HPLC) grade acetonitrile, methanol, diethyl ether, di-isopropyl ether, dichloromethane, and 1-butanol were purchased from Merck (Darmstadt, Germany). Analytical grade sodium dodecyl-sulphate (SDS), sodium dihydrogen phosphate, and tetraethyl-ammonium bromide (TEA) were from BDH (Poole, UK). Deionised water was produced by a Milli-Q Millipore Water System (Millipore, MA, USA).

Animals and experimental design

Eight male clinically healthy alpacas (*Vicugna pacos*), aged 5-9 years, and weighing 41-58 kg, were used. The study protocol, conforming to the EU regulations (86/609/EEC), was approved by the ethics committee of the University of Pisa, Italy (authorisation n°9403). The test preparations were made according to an open balanced cross-over design: animals were assigned to 2 treatment groups, using an open, single dose, 2 treatments, 2 periods, and randomised cross-over design. Each subject received a single dose of 2.5 mg/kg of tramadol (Altadol; Formevet, Milan, Italy). A catheter was placed into the left jugular vein to facilitate IV drug administration by 2 minutes IV injection. The IM injection was placed in the upper outer quadrant of the buttocks. The wash-out period was 2 weeks. A catheter was placed into the right jugular vein to facilitate blood withdrawals.

Blood samples (5 mL) were collected at 0, 5, 15, 30, and 45 min, and 1, 1.5, 2, 4, 6, 8, 10 and 24 hours after administration of tramadol, and placed in collection tubes containing lithium heparin. The blood samples were centrifuged at 3,000 rpm within 30 min after collection, and the harvested plasma was stored at -20°C until used within 30 days from collection (Giorgi *et al*, 2007).

Preparation of plasma samples

Samples were prepared by placing 1.0 mL plasma into a 15-mL polypropylene tube (Sarsedt, Verona, Italy) followed by 100 µL internal standard solution (8 µg/mL) and 0.5 mL 0.2 M borate buffer (pH 9.3). After vortex-mixing, 7.0 mL extraction solvent (diethyl ether:dichloromethane:1-butanol 5:3:2) was added, then the tube was shaken for 20 minutes (100 oscillations/min), and centrifuged for 10 minutes at 3,400 rpm. Five millilitres of the organic layer were transferred to a clean 15-mL plastic conical tube, shaken with 200 µL back-extraction solvent (0.05 M H_2SO_4: acetonitrile 9:1) for 20 min (100 oscillations/min), and centrifuged for 10 min at 3,400 rpm. The aquous phase (20 µL) was injected into the HPLC system.

HPLC

The concentration of tramadol, M1, M2 and M5 in plasma were evaluated using HPLC, according to the method described by Giorgi *et al* (2007). Briefly, the HPLC system was an LC Workstation Prostar (Varian Corporation, Walnut Creek CA, USA) consisting of an LC-10ADvp pump, CTO-10Avp column oven, SCL-10Avp system controller, and RF-10A spectrofluorometric detector. Data were processed by an LC solution workstation (Varian Corporation). Chromatographic separation was performed on a Luna C18 ODS2 analytical column (150 mm x 2.1 mm inner diameter, 3-µm particle size, Phenomenex, Bologna, Italy) maintained at 25°C. The mobile phase consisted of acetonitrile:buffer (20 mM sodium dihydrogen phosphate, 30 mM SDS, and 15 mM TEA, adjusted to pH 3.9 with phosphoric acid) (40:60 v/v) at a flow rate of 0.8 mL/min. Excitation and emission wavelengths were 275 and 300 nm, respectively. Validation data were reported previously (Giorgi *et al*, 2007). Briefly, the concentrations of tramadol, M1, M2 and M5 in plasma were calculated from standard curves of blank plasma spiked with known concentrations

of tramadol, M1, M2 and M5 (1, 5, 10, 25, 50, 100, and 200 ng/µL). Quantification of tramadol, M1, M2 and M5 in plasma samples was accomplished by chromatographic analysis of unknown samples in parallel with standard curve and quality control samples. For each series of analysis, a standard curve was generated and in addition 9 quality control samples (3 different concentrations) were analysed together with the test samples. An acceptance criterion for analysis of the quality control samples should have a precision and accuracy equal to or better than 10% of the intended concentration. The selection of the correct method was verified by the chromatograms of the diluents and blank plasma samples having no peaks matching those of tramadol, M1, M2 and M5. The limits of detection (LOD) and quantification (LOQ) were determined as analyte concentrations giving signal-to-noise ratios of 3 and 10, respectively. Intra- and inter-day precision (expressed as relative standard deviation) and accuracy (expressed as percentage of the nominal value) were determined by analysis of replicates (n=3) of LOQ, and low, medium, and high quality control samples on 7 different days. The LOQ of tramadol, M1 and M2 was 5 ng/µL and 10 ng/µL for M5. The maximum value of the CV in intra-/inter-day assay precision for tramadol and its metabolites was 7 and 5%.

Pharmacokinetic Evaluation

The pharmacokinetic calculations were carried out with WinNonLin v. 5.2.1 program (Pharsight Corp., Cary, NC, USA). Changes in plasma tramadol and M2 concentrations were evaluated by use of standard non-compartmental analysis and the following pharmacokinetic parameters were determined with standard non-compartmental equations (Gabrielsson and Weiner 2002); plasma half life ($t_{1/2}\lambda_z$), systemic clearance (CL), volume of distribution (Vz), area under the first moment curve from zero to infinity ($AUMC_{0-¥}$) and mean residence time (MRT). The elimination rate constant (λ_z) was estimated on at least 4 points of the terminal part of the curve. Cmax, the highest observed plasma concentration, and Tmax, the time required to reach Cmax, were obtained from the individual plasma concentration/time curves. The $AUC_{0-¥}$ was calculated with the log-linear trapezoidal rule. Systemic availability (F%) was calculated from the ratio of the areas under the plasma tramadol concentration curve after IM and IV administration (Toutain and Bousquet-Melou, 2004):

$$F(\%) = [(AUC_{IM})/(AUC_{IV})] \times 100$$

Additionally, the intervals t_e and Δt_e characterising the onset time and duration of action, respectively, were determined by linear interpolation between plasma concentration/time curve at a relevant plasma concentration, derived from a clinical efficacy study in humans as the minimum effective concentration (MEC) in analgesia in moderate pain (Malonne *et al,* 2004). The t_e is equivalent to the time taken to reach the MEC, and Δt_e is the period of time during which this plasma concentration is exceeded.

Statistical analysis

The statistical analyses were evaluated using an ANOVA test. The results were presented as mean (± standard deviation). All the analysis were conducted using GraphPad InStat (GraphPad Software, Inc, La Jolla CA, USA). In all the experiments, differences were considered significant if the associated probability level was lower than 0.05.

Results

Few minutes after tramadol (2.5 mg/kg) IV administration, one of the subjects showed an epileptic crisis which lasted 3 minutes. When the same dose was administered slowly (within 2 minutes), only a mild sedation and tremors were shown by all the animals, lasting less than 30 minutes. No adverse effects were noted after IM administration of the same dose of drug.

The plasma concentrations/time curves of tramadol after IV and IM administrations are reported in Fig 1. A non-compartmental model best fitted the plasma concentrations of tramadol and M2 after IV and IM administration, respectively. The

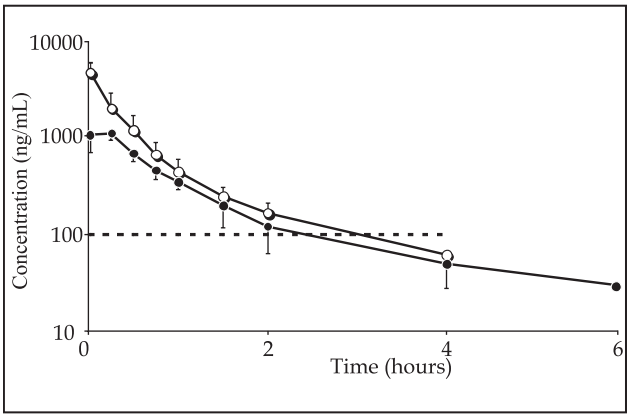

Fig 1. Observed mean concentrations of tramadol in plasma following single IV (○) and IM (·) administration of tramadol (2.5 mg/kg) in 8 alpacas (*Vicugna pacos*). Dash line represents the MEC calculated in humans as the plasma concentration tramadol at the time a patient required a supplementary dose for pain control (Malonne *et al*, 2004). Bars represent standard deviation.

Table 1. Mean ± SD values for T and M2 pharmacokinetic parameters after intravenous (IV) and intramuscular (IM) (2.5 mg/kg) administration of tramadol to eight adult male alpacas.

Parameter	Tramadol				M2			
	IV		IM		IV		IM	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
r^2	0.96	0.02	0.97	0.02	0.99	0.01	0.97	0.03
λz (1/h)	0.95	0.27	0.72	0.39	0.47	0.09	0.43	0.13
$t_{1/2}$ λz (h)	0.78*	0.25	1.20	0.61	1.58	0.26	1.69	0.46
Tmax (h)	/	/	0.16	0.13	0.26	0.24	0.35	0.28
Cmax (µg/mL)	4.71	1.31	1.25	0.21	0.41	0.19	0.39	0.13
$AUC_{0-\infty}$(h µg/mL)	1.46	0.54	1.19	0.22	0.81	0.16	0.86	0.12
Vz (L/kg)	1.54*	0.54	3.42	2.63	/	/	/	/
CL (L/h/kg)	1.29*	0.39	2.15	0.35	/	/	/	/
$AUMC_{0-\infty}$ (h h µg/mL)	1.43	0.46	1.52	0.81	1.76	0.33	1.95	0.32
MRT (h)	0.69	0.07	1.23	0.45	2.22	0.43	2.35	0.52
F%	/	/	81.5	14.3	/	/	/	/

*Values significantly different (P<0.05) between the administration groups. SD = standard deviation.

corresponding parameters are listed in Table 1. The elimination half life, the volume of distribution, and the systemic clearance of tramadol were 1.20±0.61 and 0.78±0.25 h, 3.42±2.36 and 1.54±0.54 L/kg, and 2.15±0.35 and 1.29±0.39 L/h/kg, following IV and IM administration, respectively. These values were significantly different (P<0.05) between the groups. The mean systemic bioavailability of tramadol administered IM was 81.5±14.3% with a range of values between 75-96%. Following IM administration, t_e and Δt_e were 1.0±0.2 min and 2.5±0.35 h, respectively. Following IV administration Δt_e was 3.15±0.30 h.

The M2 metabolite showed similar plasma concentration/time curves after either IV or IM administrations (Fig 2). M2 was detectable from 5 min up to 4 and 6 h, respectively. By contrast, M1 and M5

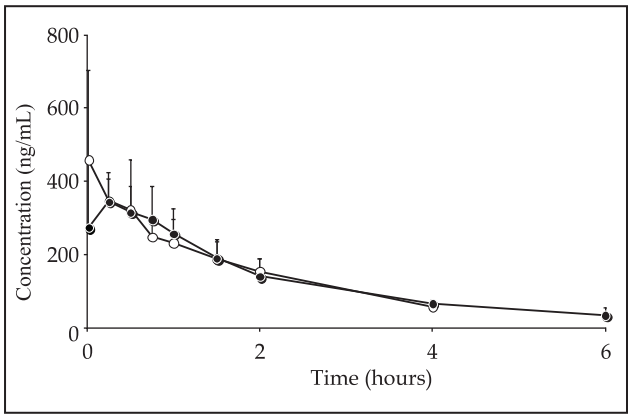

Fig 2. Observed mean concentrations of M2 metabolite in plasma following single IV (○) and IM (●) administration of tramadol (2.5 mg/kg) in 8 alpacas (*Vicugna pacos*). Bars represent standard deviation.

were detected after IV and IM administration in one and 2 alpacas, respectively. These metabolites were detected at concentrations close to the LOQ of the method. Their pharmacokinetic parameters were not calculated since only 3 or 4 concentration-time points were detected.

The chromatographic curves from both treatments showed some time dependent unknown peaks (data not shown). These peaks were eluted between the retention times of IS and M1.

Discussion

The administration of tramadol has been widely studied in the recent past in both equine (Giorgi *et al*, 2007; Shilo *et al*, 2008; Giorgi *et al*, 2009c; 2010) and ruminant species (De Sousa *et al*, 2008; Elghazali *et al*, 2008). Tramadol is frequently used in veterinary clinical practice, although the half-life of this drug has been reported shorter in several animal species than in humans. The main active metabolite M1 has been known as the major effective molecule in humans (Raffa *et al*, 1992). Unfortunately, in veterinary medicine, M1 is found low in plasma concentration with a supposed lack of effectiveness in pain therapy (De Sousa *et al*, 2008; Giorgi *et al*, 2007; McMillan *et al*, 2008; Shilo *et al*, 2008; Giorgi *et al*, 2009b; 2009d) but no data are present in camelids. The present study reports for the first time the plasma concentration of the main tramadol metabolites in a camelid species as alpaca (*Vicugna pacos*).

In alpacas, a non-compartmental model best fitted the tramadol concentration, according to early studies in horses (Shilo *et al*, 2008) and donkeys

(Giorgi *et al*, 2009c), but disagreeing with others in dromedary camels (Elghazali *et al*, 2008) and horses (Giorgi *et al,* 2007).

To estimate the onset time and the duration of action of IM and IV injection, the clinically relevant therapeutic parameters t_e and Δt_e were calculated for an assumed MEC. A target plasma concentration of tramadol of 100 ng/mL (derived from a single study in humans as the plasma concentration of summed tramadol enantiomers at the time a patient required a supplementary dose for pain control) was clinically effective in the treatment of mild to moderate pain in humans (Malonne *et al*, 2004). At a MEC of 100 ng/mL the t_e IM was fast (1.0±0.2 min). The Δt_e IM (2.5±0.35 h) and Δt_e IV (3.15±0.30 h) were similar. The small differences reported were not significant and probably due to differences in initial time course of absorption. These data assume that the MEC as calculated for humans is relevant for alpacas and should be integrated with further pharmacodynamic studies in this animal species. To determine the analgesic effect of tramadol administration, some authors, especially in human studies, use the plasma concentration of M1, because the evidence available indicates that this molecule rather than parent drug is responsible for most of the therapeutic effects (Garrido *et al*, 2003). In the present study, the metabolite M1 was detected at a concentration at or lower than the MEC (0.040±0.030 µg/mL) reported in humans (Grond *et al*, 2003) and the calculation of Δt_e for M1 was not possible. Hence, tramadol might be liable for the major clinical effectiveness in the alpaca as reported in the goat (De Sousa *et al*, 2008), equine (Giorgi *et al*, 2007; Shilo *et al*, 2008; Giorgi *et al*, 2010) and dogs (McMillan *et al*, 2008; Giorgi *et al*, 2009a, b, d). In the present study, no pharmacodynamic tests on analgesia were carried out, but following IM administration, the early Tmax (0.16 h) of tramadol, seems to induce the maximum analgesic effects in short time. Such data should be confirmed with clinical studies, because the time of plasma concentration and the time of effect could not be in phase (Toutain and Bousquet-Melou, 2004).

Although the IV and IM routes of administration are almost completely bioequivalent (F = 81.5%), the lower initial plasma concentrations after IM administration of tramadol, might be therapeutically beneficial. It has been suggested (Lintz *et al*, 1999) to have a lower incidence of side effects with a slightly longer onset of action.

The concentration of tramadol metabolites produced in plasma reports a higher production of M2, than M5 and M1 (active metabolite). The low concentration of M1 is in accordance with previous data in dogs (McMillan *et al*, 2008; Giorgi *et al*, 2009a, b, d), horses (Giorgi *et al*, 2007; Shilo *et al*, 2008), goats (De Sousa *et al*, 2008) and donkeys (Giorgi *et al*, 2009c) suggesting that in these species the effectiveness of tramadol might be lower than in cats (Pypendop and Ilkiw, 2008) and humans (Raffa *et al*, 1992). Since the M1 metabolite is a significant contributor to the analgesic effects of tramadol (Wu *et al*, 2001), this may significantly limit its usefulness as analgesic in alpacas. For the first time a low plasma concentration of M5 has been reported. This could be due to the remarkable glucuronidation process in camelids (Al Katheeri *et al*, 2005) leading to a faster rate of elimination M1 and M5 (as glucuronates), than their formation. This phase II enzyme could be mainly accountable for the plasma high speed disappearance of M1 and M5, according to previously data reporting tramadol undergoing extra-hepatic glucuronidation in dogs (Giorgi *et al*, 2009a).

Conclusion

In conclusion, tramadol is rapidly and almost completely absorbed after IM injection: peak plasma concentrations were already reached after an average of 0.16 h and, after few seconds, plasma concentrations adequate (in humans) for treatment of moderate pain, were achieved. Further studies need to evaluate if the MEC calculated in humans is applicable to alpacas. The systemic availability after IM injection was higher than 80% and therefore equivalent to the same dose administered by the IV route. Differences in the onset time and duration of action might be due to a slightly slower absorption after IM administration but these differences may be therapeutically irrelevant. Therefore, according to the data generated in this study, IM injection of tramadol is a useful and better alternative to IV injection in alpacas.

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