Clinical pharmacokinetics of tramadol and main metabolites in horses undergoing orchiectomy

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ORIGINAL ARTICLE

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Background: Tramadol is a synthetic codeine analogue used as an analgesic in human and veterinary medicine. It is not approved for use in horses, but could represent a valid tool for pain treatment in this species.
Objectives: The serum pharmacokinetic profile and urinary excretion of tramadol and its metabolites (*O*-desmethyltramadol [M1], *N*-desmethyltramadol [M2] and *N,O*-desmethyltramadol [M5]) was investigated in a multidrug anaesthetic and analgesic approach for orchiectomy in horses. The evaluation of the degree of cardiovascular stability, the intraoperative effect and postoperative analgesia obtained by the visual analogue scale are also reported.
Animal and methods: Tramadol (4 mg/kg BW) was administered intravenously to eight male yearlings as a bolus over 60 seconds, 5 min after intubation and 15 min prior to surgery. Drug quantification was performed in serum and urine for tramadol, M1, M2 and M5 by high-performance liquid chromatography with fluorimetric detection.
Results: Mean tramadol concentration was 14.87 ± 11.14 μg/mL at 0.08 h, and 0.05 ± 0.06 μg/mL at 10 h. Serum concentrations of M1 and M2 metabolites were quite limited. For M1 and M2, median maximum concentration (C_{max}) and time to achieve maximum concentration (T_{max}) were 0.05 μg/mL and 0.75 h, and 0.08 μg/mL and 2 h, respectively; M5 was never detected. In urine, tramadol was the most recovered compound, followed by M1, M2 and M5.
Conclusions and clinical relevance: Showing no adverse events and based on the kinetic behaviour, pre-operative tramadol IV at a dose of 4 mg/kg BW might be useful and safe as analgesic in horses undergoing surgery.

Keywords: horse; equine; tramadol; tramadol metabolites; anaesthesia; surgery; orchiectomy

1. Introduction

Tramadol is a synthetic codeine analogue that acts centrally as an analgesic and has received widespread acceptance in human medicine since its first introduction in 1977 in Germany. The analgesic effects of the drug result from complex interactions with the opiate, adrenergic and serotonin receptor systems (Scott & Perry 2000). Tramadol affinity for μ-opioid receptors is lower than that of codeine and morphine (Grond & Sablotzki 2004). However, the *O*-demethylated metabolite, *O*-desmethyl tramadol (M1), has a higher affinity for μ-opioid receptors than tramadol itself (Poulsen et al. 1996; Gillen et al. 2000).

Tramadol undergoes extensive first-pass metabolism in the liver *via* two main metabolic pathways involving cytochrome P450 (Scott & Perry 2000). The major metabolites present in human plasma are M1 and *N*-desmethyltramadol (M2), and to a minor extent *N,N*-didesmethyltramadol (M3), *N,N,O*-tridesmethytramadol (M4) and *N,O*-desmethyltramadol (M5) (Grond & Sablotzki 2004). Tramadol and its metabolites are almost completely excreted *via* the kidneys (Scott & Perry 2000). Hepatic demethylation to M1 occurs at different rates in veterinary species (Kukanich & Papich 2004; Giorgi et al. 2007; Vettorato et al. 2010; Cagnardi et al. 2011; Knych et al. 2013).

In humans, the analgesic effect of tramadol following parenteral administration is about 10% that of morphine

and tramadol provides postoperative pain relief comparable to that of pethidine (Grond & Sablotzki 2004). The analgesic properties of tramadol have been investigated in horses (Natalini & Robinson 2000; Dhanjal et al. 2009) and cats (Steagall et al. 2008), whereas its clinical efficacy has been investigated in dogs (Mastrocinque & Fantoni 2003; Vettorato et al. 2010) and cats (Brondani et al. 2009; Cagnardi et al. 2011).

In horses, pain is treated with few classes of drugs and opioid analgesics, except butorphanol, and have not been frequently employed because of substantial sympathetic stimulation and excitation of the central nervous system (Combie et al. 1979; Kamerling et al. 1985). Nonetheless, some more recent studies have indicated a widening role for opioids in horses and have encouraged their use (Clutton 2010). Tramadol could be added to this list, although its activity and efficacy has to be clarified in horses. Pharmacokinetic studies recently carried out are quite conflicting for metabolite detection, particularly for M1 (Zonca et al. 2006; Giorgi et al. 2007; Shilo et al. 2008; Dhanjal et al. 2009; Cox et al. 2010; Stewart et al. 2011; Knych et al. 2013). Only in one of them, the effects and nociceptive properties of tramadol were studied (Dhanjal et al. 2009), evidencing few adverse effects, but also limited nociceptive properties at a dose of 2 mg/kg BW intravenously (IV).

Principal aims of this study were the determination of the serum pharmacokinetic and excretive profiles of

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tramadol and its M1, M2 and M5 metabolites in horses after IV administration at 4 mg/kg BW in a multidrug anaesthetic and analgesic approach for orchiectomy to generate information for a rational use of this drug in horses.

2. Materials and methods

2.1. Animals

The study was performed on eight healthy colts (six Arabian horses, one Thoroughbred and one Quarter Horse cross breed), two years of age, weighing between 292 and 490 kg and undergoing orchiectomy. All animals came from the same farm, were bred at pasture and not started under saddle. Horses were kept in the hospital barn for four days before surgery. All animals were judged healthy (ASA status I) on the basis of physical examination and results of routine blood tests and were enrolled in the study after written consent from the owner, as required by Italian law (D.L. 116/1992). The study protocol was approved by the ethical committee of the University of Milan.

2.2. Anaesthetic, surgical and post-surgical procedures

All animals received acepromazine maleate (0.05 mg/kg BW) and detomidine (range 0.01−0.02 mg/kg BW) intramuscularly (IM), as pre-anaesthetic medications. Anaesthesia was induced by IV ketamine (2.2 mg/kg BW) and diazepam (0.05 mg/kg BW) mixed in the same syringe. After intubation, anaesthesia was maintained with isoflurane in oxygen (100%) in intermittent positive-pressure ventilation (IPPV) to maintain end-tidal carbon dioxide values between 39 and 42 mmHg. Tramadol (4 mg/kg BW, Altadol, Formevet, Italy) was administered IV as a bolus over 60 seconds through a jugular catheter (14 gauge), 5 min after intubation and 15 min prior to surgery, as pre-emptive analgesic. During surgery, lactated Ringer's solution was administered at 3 mL/kg BW/h through the same catheter. During anaesthesia, variations of isoflurane concentration were performed to maintain an appropriate depth of anaesthesia based on clinical assessment; signs monitored included degree of nystagmus, movement, muscle relaxation, response to surgery, invasive blood pressure (IBP), and heart rate (HR). All horses underwent closed orchiectomy according to standard surgical procedures. During surgery, IBP, HR electrocardiogram (lead II), oxyhaemoglobin saturation (SpO_2), end tidal carbon dioxide ($EtCO_2$), invasive systolic arterial

pressure (SAP), invasive mean arterial pressure (MAP) and invasive diastolic arterial pressure (DAP) were recorded every 5 min using a UT4000F Pro monitor (Goldway Inc, USA).

After extubation, postoperative pain assessment was performed by observations of pain responses (signs of pain present vs. absent) together with assessment of the severity of pain. The severity of pain was evaluated by a visual analogue scale (VAS, Hubbell 1999) that provides a subjective scoring method for evaluating pain in horses. Pain was assessed at extubation (0) and at 0.5, 1, 2, 3, 4, 6, 8 and 12 h. The trained evaluator places a time-dated mark on a 10-cm line: from 0 to 3 cm was considered 'No pain', from 3 to 6 cm 'Moderate pain' and from 6 to 10 cm 'Worst pain' (Table 1). Pain was judged to be unacceptable if a score $\geq$ 5 cm was achieved using VAS. The 'rescue analgesia' protocol was 0.1 mg/kg BW of butorphanol (Dolorex, Intervet) IV. At 12 h after extubation, 1 mg/kg BW of flunixin meglumine was administered IV in all horses to control signs of inflammation.

2.3. Collection, purification and analysis of serum and urine samples

Due to dangerous recalcitrance of horse number 4, it was not possible to obtain blood samples after its recovery; thus, this subject was excluded from kinetic analyses. For all the other seven animals, venous blood samples (10 mL) were collected from the contralateral jugular vein catheter into non-heparinised tubes before tramadol administration (time 0) and at 0.08, 0.16, 0.33, 0.5, 0.75, 1, 1.5, 2, 4, 8 and 10 h after tramadol administration. After clotting at room temperature for about 20 min, serum samples were prepared by centrifugation ($1500g$, 10 min at room temperature) and stored at −80 °C pending high-performance liquid chromatography (HPLC) assay. Considering both animal welfare and an easy sampling of urine, each horse was provided, before complete recovery from anaesthesia, with a specific collecting bag, similar to what is used for doping control in racehorses. Urine samples were collected after spontaneous urination using the mentioned bags, every 12 h or when full, for a maximum of 3.5 days after treatment. The samples collected within every 12-h interval were combined to obtain the sample of each time interval and the urine volumes registered. The aliquots (10 mL) of each sample were centrifuged (3500 g for 5 min) soon after collection and stored at −80 °C pending assay.

Serum and urine samples were purified by solid phase extraction (SPE) and residues of tramadol, M1, M2 and

Table 1. Description of the visual analogue scale used to determine the severity of pain in horses.

Score	Criteria
0−3	Horse is alert, active, playful, interactive, healthy, performs work normally, eats, drinks and defecates normally
3−6	Horse is anxious/uneasy, less attentive, uncomfortable, shows reduced activity, mouths food, plays in water, lays down, is reluctant to perform work, looks at flank (colic), lame and elevated respiratory rate
6−10	Horse is focused-glazed staring, depressed, tense/trembling, frozen, hypersensitive, restless, grunting, kicking (pawing, stomping), inappetent, shows abnormal posture, thrashing/rolling (colic), tachycardia and tachypnea

M5 were analysed by HPLC with the method previously reported by Cagnardi et al. (2011). Briefly, samples were purified on Isolute SPE C2 cartridges (International Sorbent Technology Ltd., UK). The eluate was evaporated to dryness and the residue dissolved in mobile phase. The HPLC system included: binary pump, auto sampler, Peltier column oven at 20 °C (all PerkinElmer Series 200, Italy) and fluorescence detector (PerkinElmer LC240, Italy) with excitation and emission wavelengths of 200 and 301 nm, respectively. The column was a Hypersil ODS C18 250×4.6 mm $5\,\mu$ equipped with Hypersil $5\,\mu$ 4.6 mm pre-column (Supelco, Italy). The mobile phase was 15 mM aqueous sodium hydrogen phosphate dodecahydrate with 45 mM triethylamine pH 3 and acetonitrile (82:18, v:v). Solutions for the calibration curve were prepared by diluting stock solutions of tramadol, M1, M2 and M5 (1 mg/mL) to obtain concentrations in the ranges $0.02-5\,\mu$g/mL and $0.02-10\,\mu$g/mL in blank horse serum and urine, respectively. Tramadol hydrochloride, M1, M2 and M5 were purchased from LGC Standards (London, UK). Other reagents and solvents were purchased from J.T. Baker (Milan, Italy).

Serum protein binding of tramadol, M1, M2 and M5 in the range $0.1-20\,\mu$g/mL was determined *in vitro*. The serum-bound molecules were removed by ultrafiltration using a disposable device (Amicon, Millipore, Milan, Italy) and free substances in the filtrate were analysed by HPLC as described above.

2.4. Pharmacokinetic analysis and statistics

Pharmacokinetic parameters were deduced from serum concentration-time data using WinNonLin 6.1 software (Pharsight Corporation, USA) which allows both compartmental and non-compartmental analyses of experimental data. Minimum information criterion estimation (MAICE; Yamaoka et al. 1978) was used to choose the best fitting model for the data. All data points were weighted by the inverse square of the fitted value. Serum concentrations after IV tramadol administration were fitted to a standard bi-exponential curve describing a two-compartment model with elimination from the central compartment, whereas the kinetics of M1, M2 and M5 were determined by non-compartmental analysis (Gibaldi & Perrier 1982).

A normality test (Kolmogorov−Smirnov test, InStat 3.0, GraphPad Software, USA) was performed on intraoperative variables and pharmacokinetic parameters. The first are reported as means and standard deviations, whereas pharmacokinetic parameters as median and range.

3. Results

3.1. Intra-anaesthetic and post-surgical evaluation

No adverse effects were observed after IV tramadol administration during the whole observation period. Mean age, body weight, duration of surgery, duration of anaesthesia and intraoperative variables are shown in Table 2. The results of the subjective pain evaluation over the 12 h

Table 2. Mean (±SD) values of general characteristics and selected surgical variables in eight horses undergoing surgical orchiectomy.

	Mean ± SD ($n = 8$)
Age (months)	24 ± 1.5
Body weight (kg)	351.7 ± 70.2
Anaesthesia time (min)	47.1 ± 2.17
Surgery time (min)	19.4 ± 1.19
Time from tramadol injection to start of surgery (min)	15
Heart rate (per minute)	36.8 ± 4.23
Invasive systolic arterial pressure (SAP)	111.9 ± 9.8
Invasive mean arterial pressure (MAP)	89.7 ± 12.5
Invasive diastolic arterial pressure (DAP)	79.4 ± 12.5
End tidal CO_2 (mm Hg)	40.45 ± 1.2
Oxyhaemoglobin saturation (%)	98.6 ± 0.6

after extubation are given in Figure 1. Pain score was max 2 cm in any observation time and no animal required rescue drugs.

3.2. Serum and urine concentrations

The HPLC methods in serum and urine were validated in our laboratory according to the European Commission decision (European Commission 2002) and the European guidelines for validation of analytical method for residue depletion studies (VICH GL 49 2012) and found to be linear (r^2 value > 0.98) in the range of $0.02-5\,\mu$g/mL and $0.02-10\,\mu$g/mL, respectively. Validation results of the methods and percentages of serum protein binding are reported in Table 3.

Mean serum concentrations of tramadol, M1 and M2 after IV administration are shown in Figure 2. In all serum samples, M5 was never detected (<LOD). Mean tramadol
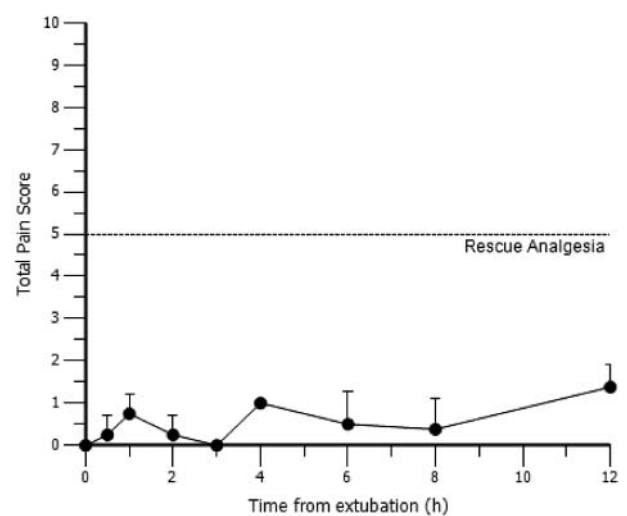

Figure 1. Mean total score (SD) of postoperative pain evaluations in the horses ($n = 8$) assessed at various times after extubation using visual analogue scale (VAS).

Table 3.   Intra-laboratory validation of analytical methods and protein binding for tramadol, *O*-desmethyl tramadol (M1), *N*-desmethyltramadol (M2) and *N,O*-desmethyltramadol (M5) in serum and urine.

Parameter (units)	Tramadol	M1	M2	M5
Serum				
LOQ (μg/mL)	0.02	0.02	0.02	0.02
LOD (μg/mL)	0.0009	0.002	0.0008	0.001
Recovery (%)	77 ± 2	73 ± 19	74 ± 10	76 ± 5
Intra-day repeatability (CV%)	2.48−9.5	5.7−15.6	3.8−11.8	5.11−8.61
Accuracy (%)	−7.7 to 0.31	−3.88 to 0.44	−12.19 to 0.62	−3.62 to 5.74
Protein binding (%)	19.5	14.7	26.1	18.7
Urine				
LOQ (μg/mL)	0.02	0.02	0.02	0.02
LOD (μg/mL)	0.002	0.003	0.001	0.002
Recovery (%)	56 ± 21	49 ± 18	52 ± 20	54 ± 17
Intra-day repeatability (CV%)	9.5−10.3	8.8−17.7	7.8−15.8	8.5−11
Accuracy (%)	−3.9 to 0.04	−12 to 0.15	−19 to 0.24	−17 to 0.22

Note: LOQ = limit of quantification; LOD = limit of detection; recovery is reported as mean ±SD; intra-day repeatability and accuracy are reported as range values; intra-day repeatability is measured as coefficient of variation (CV%) on six replicates of three concentrations; and accuracy (%) is measured as closeness to the concentration added on the six replicates.

concentration in serum was 14.87 ± 11.14 μg/mL at first sampling (0.08 h), decreased to 1.13 ± 0.31 μg/mL at 1-h post-treatment and subsequently declined more slowly to 0.05 ± 0.06 μg/mL at 10 h. M1 was detected at the first sampling point only in two horses with a mean value of 0.036 ± 0.01 μg/mL; at 0.16 h, five horses presented quantifiable concentrations with a mean value of 0.045 ± 0.02 μg/mL. From 0.33 h, M1 was always detected in all horses and reached a peak plateau of about 0.05 μg/mL that attained to 0.033 ± 0.02 μg/mL at 10 h. At 0.08 and 0.16 h, M2 concentrations were below LOQ and at 0.33 and 0.5 h were detected only in one horse with 0.08 and
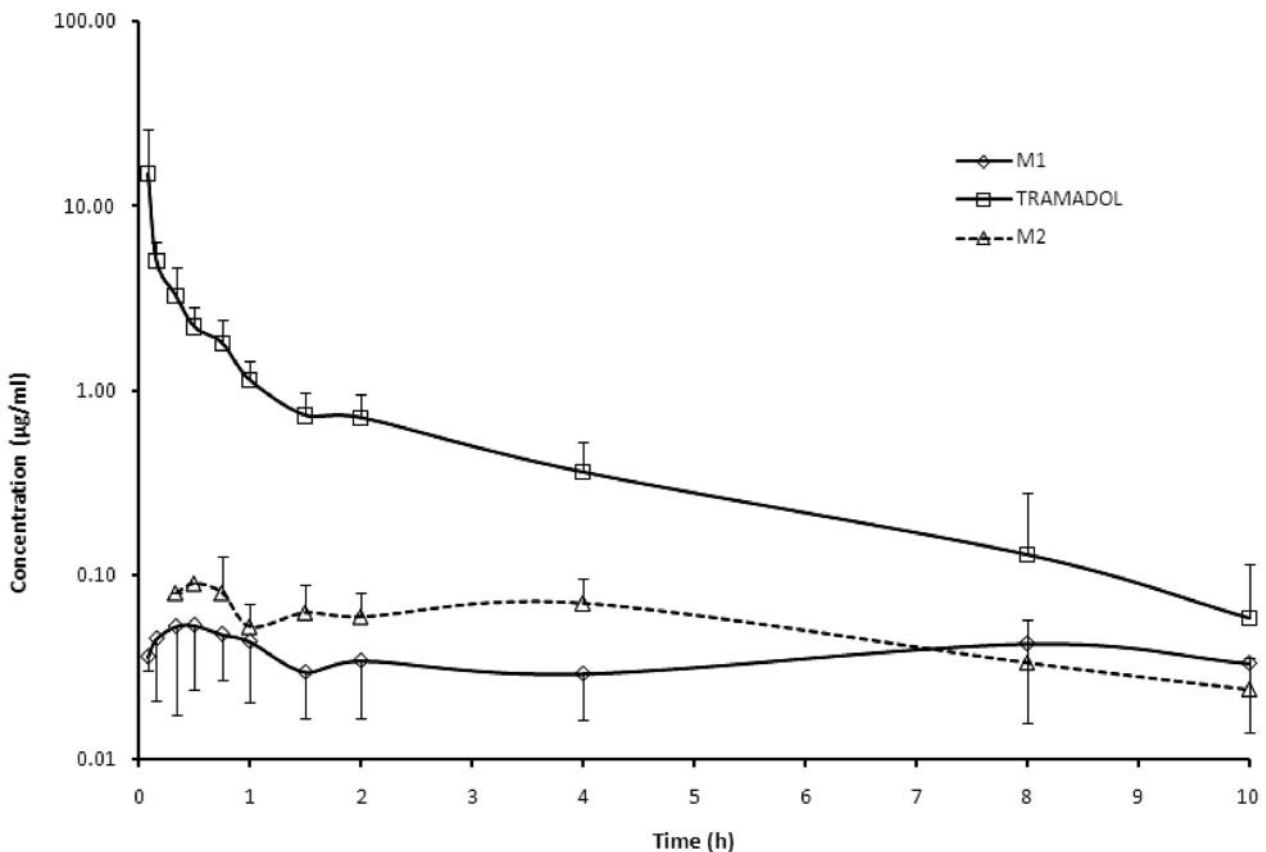

Figure 2.   Mean (SD) serum concentrations (μg/mL) of tramadol, M1 and M2 in horses (*n* = 7) after IV administration of tramadol at 4 mg/kg BW.
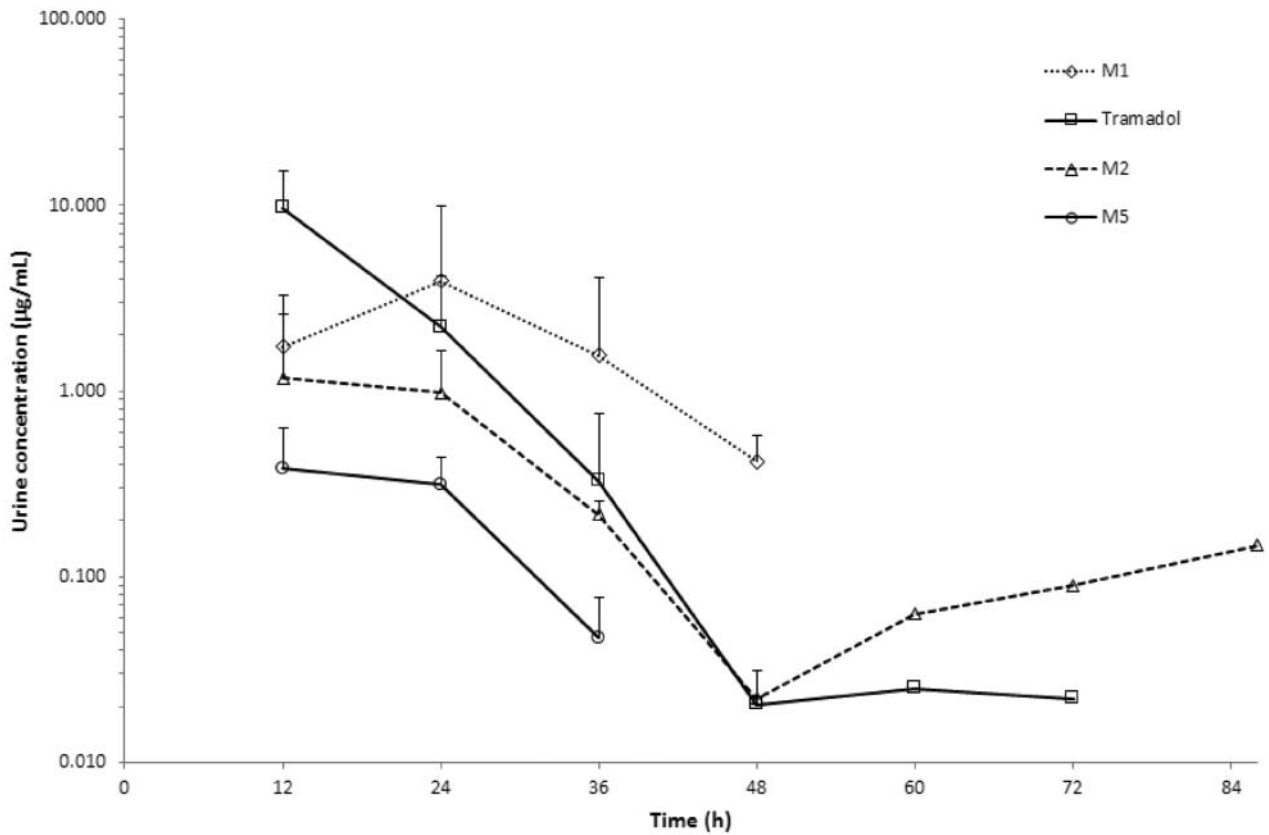
Figure 3.   Mean urinary concentrations of tramadol, M1, M2 and M5 in five horses after tramadol IV administration at 4 mg/kg BW.

0.09 μg/mL, respectively. At 0.75 h, M2 concentrations of 0.08 ± 0.05 μg/mL were detected in three horses; at 1 h, concentrations of 0.052 ± 0.02 μg/mL were quantified in four horses; and at 2 h, all horses showed detectable concentrations of 0.059 ± 0.02 μg/mL. At 10 h, M2 levels were quantified in four horses with a mean value of 0.025 ± 0.01 μg/mL.

The complete urine collection of 3.5 days was obtained from five horses. The amount of tramadol and its metabolites quantified in the combined samples collected at 12 h intervals are reported in Figure 3. The excretive profile of tramadol and its metabolites was rather variable, thus the kinetic analysis was not possible. Tramadol concentrations were above LOQ until 36 h in all horses and in one horse until 72 h. M1 was eliminated until 36 h in all horses and until 48 h in two horses. M2 was present until 24 h in all horses and until 86 h only in one. M5 was recovered until 24 h in all horses and until 36 h in three. The most recovered compound was tramadol, followed by M1, M2 and M5.

3.3.   Pharmacokinetics

The time courses of tramadol and its metabolites (M1 and M2) concentrations in serum were best described by a two-compartment open model and a non-compartmental model, respectively. Results are summarised together in Table 4.

4.   Discussion

The tramadol dose (4 mg/kg BW) was adopted on the basis of previous studies performed with 2.5 mg/kg BW IV, where a very small production of M1 compared to other species was reported (Zonca et al. 2006), and with 5 mg/kg BW IV, where adverse effects as tremors and fasciculation were observed (Cagnardi, unpublished data). Our study is the first that evaluates the pharmacokinetics of tramadol in a clinical setting, when it is administered, for analgesic purposes, in a multimodal pre-emptive analgesic protocol and would improve the current knowledge on the pharmacokinetics of tramadol and its metabolites when therapeutically administered in young horse undergoing surgery.

In our study, no adverse effects were observed, whereas IV administration of 3 and 5 mg/kg BW in pain-free horses had been reported to cause excitatory reaction, as tremor, confusion, agitation and tachycardia (Giorgi et al. 2007; Roscoe et al. 2007; Knych et al. 2013; Cagnardi, unpublished data). In the enrolled horses, drug administration was carried out slowly over 60 seconds, before surgery, when the animals were already under anaesthesia, thus tremors or fasciculation were probably hidden. However, all physiological parameters monitored during surgery were stable after tramadol administration. IPPV and normocapnia were maintained throughout the procedure in all animals (Table 2).

Table 4. Pharmacokinetic parameters [median (range)] of tramadol, *O*-desmethyl tramadol (M1) and *N*-desmethyltramadol (M2) after IV administration in seven horses at the dose of 4 mg/kg BW.

Parameter (units)	Tramadol median (range)	M1 median (range)	M2 median (range)
$t_{1/2\lambda z}$ (h)		20.52[a] (1.04−74.34)	4.34[b] (1.97−6.36)
$AUMC_{(0-last)}$ (h.h.μg/mL)	11.11 (6.79−13.61)	1.07 (0.08−3.39)	1.65 (0.36−3.08)
$MRT_{(0-last)}$ (h)	1.76 (0.63−2.10)	4.78 (1.05−5.60)	3.60 (2.07−4.89)
$AUC_{(0-last)}$ (h. μg/mL)	7.13 (17.69−4.19)	0.23 (0.08−0.64)	0.46 (0.12−0.76)
$t_{1/2\lambda 1}$ (h)	0.15 (0.02−0.36)		
$t_{1/2\lambda 2}$ (h)	1.95 (1.73−3.88)		
C_0 (μg/mL)	9.52 (5.49−409.31)		
V_{dss} (mL/kg)	1283.51 (166.52−1632.2)		
V_c (mL/kg)	420.12 (9.77−728.32)		
Cl_B (mL/h/kg)	579.86 (209.53−695.48)		
$C_{max\ obs}$ (μg/mL)	7.4 (4.88−33.29)	0.05 (0.04−0.13)	0.08 (0.05−0.13)
T_{max} (h)		0.75 (0.33−8)	2 (0.75−4)

Note: $t_{1/2\lambda z}$ = elimination half-time; AUMC = area under moment curve; $MRT_{(0-last)}$ = mean residence time; $AUC_{(0-last)}$ = area under serum concentration-time curve; $t_{1/2\lambda 1}$ = distribution half-time; $t_{1/2\lambda 2}$ = elimination half-time; C_0 = serum concentration at time 0; V_{dss} = volume of distribution at steady state; V_c = volume of distribution in central compartment; Cl_B = serum clearance; $C_{max\ obs}$ = maximum concentration observed; T_{max} = observed time for C_{max}.
[a]Calculated on six horses.
[b]Calculated on five horses.

A multimodal analgesic approach was carried out in this study with three different drugs: detomidine (range 0.01−0.02 mg/kg BW), ketamine (2.2 mg/kg BW) and tramadol (4 mg/kg BW) with the aim to achieve analgesia before noxious stimuli began. Detomidine was administered as pre-anaesthetic about 10 min before induction and intubation, ketamine was administered for anaesthetic induction about 20 min before surgery and tramadol 15 min before surgery. All three drugs can have contributed to analgesia during surgery. Duration of analgesia with detomidine is reported to last for 90 min at higher doses in horses (0.03 mg/kg BW; Mama et al. 2009); duration of analgesia with ketamine is not reported for horses, although ketamine is used to prevent sensitisation of the nociceptive pathways in spinal cord (Hellyer et al. 2007). For tramadol, Shilo et al. (2008) describes the presence of tramadol effective concentration for 2.5 h after IV injection of 2 mg/kg BW. Based on this, tramadol was included in the protocol assuming an analgesic efficacy also in the postoperative period. In fact, after recovery all horses were quiet, awake, interactive and comfortable and no signs of anxiety or depression were observed.

At the first time point (0.08 h), tramadol concentrations varied considerably between animals (range 4.88−33.29 μg/mL), whereas from the second time point, more homogenous results were obtained. The high variability observed was also reflected in the metabolites' production. Except for M5, that was never detected in serum, M1 and M2 were found variably in all subjects. In general, M1 was detected earlier, mainly starting from 0.16 h, while M2 started from 1h. This reflected also on M1 and M2 C_{max} and T_{max}. In fact, maximum concentrations of M1 were slightly lower and obtained a little earlier (0.05 μg/mL at 0.75 h vs. 0.08 μg/mL at 2 h). Tramadol and all metabolites (including M5) were detected in urine, even though significant variability characterised both the amounts and duration of excretion. The large inter-individual variability observed was quite unexpected considering that the selected animals were rather homogenous for breed, sex, age and breeding farm. Notwithstanding these similarities within the animals, it has to be stressed that our horses underwent anaesthesia because of surgery. Thus, the different times elapsed between pre-anaesthesia, anaesthesia and the administration of tramadol, due to different behaviour of the subjects and the technical procedures, could have influenced significantly the hemodynamic parameters and consequently the kinetics of tramadol soon after administration. Likewise, detomidine could have variably decreased cardiac output in individual horses and have influenced tramadol pharmacokinetics. Moreover, the drugs administered for pre- and anaesthetic purposes could have interfered with the distribution, metabolism and elimination of tramadol and its metabolites, although additional studies are necessary to elucidate this interaction. The influence of this interaction could have also been enhanced by the immature and variable metabolic pool typical of young horses. As reported by Nebbia et al. (2004), total CYP450 content in young horses is much lower and variable than that of other herbivorous species and CYP3A activity exhibits an age-dependent increase of more than 50% when young animals are compared to adults of more than 12 years. In the horse, the metabolic pathway of tramadol is still not known, whereas in man, the M1 production is mainly due to the CYP2D6 activity, M2 is produced by CYP2B6 and CYP3A, and M5 by CYP2D6, CYP2B6 and CYP3A4 (Poulsen et al. 1996; Grond & Sablotzki 2004). In horses, the comparative expression of CYP in the liver showed that CYP2D amount is low, if compared with CYP2B6 and 3A (Nebbia et al. 2003). Furthermore, DiMaio Knych and Stanley (2008) reported in the horse the presence of a member of the CYP2D family (named CYD2D50) able to metabolise selective substrates for CYP2D6. The CYP2D50

enzyme could be responsible for M1 production in horses, but further studies are necessary to elucidate the importance of CYP2D50 activity in this species.

The pharmacokinetic analysis produced quite variable and different results when compared to previously published papers with IV doses of 2 mg/kg BW (Shilo et al. 2008; Dhanjal et al. 2009), 3 mg/kg BW (Knych et al. 2013) or 5 mg/kg BW (Giorgi et al. 2007; Stewart et al. 2011). These differences could be attributed to the different doses of tramadol administered, but also to the different analytical method used (HPLC with UV, fluorimetic or mass [MS] detection) and thus to different analytical limits in the quantification of the compounds. Main kinetic parameters from all the above reported studies in horses are summarised in Table 5. The elimination half-life of tramadol (1.95 h) was similar to Dhanjal et al. (2009) (2 ± 0.9 h), longer than that published by Giorgi et al. (2007) (0.69 ± 0.1 h) and Shilo et al. (2008) (1.4 ± 0.2 h), but shorter than the latest published by Knych et al. (2013) (3 ± 2.2 h). The volume of distribution (1.28 L/kg BW) in the present study was smaller to that reported by all other authors. The clearance of tramadol in the present study (9.66 mL/min/kg BW) was higher than that reported by Giorgi et al. (2007) (1.16 ± 0.1 mL/min/kg BW) following a dose of 5 mg/kg BW, but lower than those reported by all other authors. The most relevant difference obtained in our study is the production of the metabolites. M1 production was high enough to carry out a kinetic analysis, as also reported by Knych et al. (2013), but conversely to all other authors (Giorgi et al. 2007; Shilo et al. 2008; Stewart et al. 2011). M2 production was higher than that of M1 and comparable to that reported by Stewart et al. (2011) and Knych et al. (2013), but lower to that reported by Giorgi et al. (2007). We found that M1:tramadol area under the curve (AUC) ratio was >0.03 in horses, whereas this ratio was approximately 0.3 in dogs and >1 in cats (Vettorato et al. 2010; Cagnardi et al. 2011), indicating apparently lower M1 production in horses than in cats and dogs, while M2:tramadol area under the curve (AUC) ratio was >0.06, indicating a slightly greater M2 production in horses. Mean residence time (MRT) was longer for M1 and M2 compared to tramadol, and also elimination half-lives for M1 and M2 were longer, although highly variable and not calculated in all horses. It has to be underlined that horses are highly efficient in glucuronidation and are able to glucuronidate M1 very quickly (Knych et al. 2013), thus this rapid and efficient glucuronidation of M1 could explain the difference observed in M1 concentration among the studies in horses, cats and dogs, since only the free fraction of M1 was evaluated.

Pharmacokinetic−pharmacodynamic data for tramadol and M1 associated with a clinical response in horses has not been available in literature yet. Although the correspondence of analgesic concentrations in humans and horses has not been evaluated, a minimally analgesic concentration is described for man and it varies considerably from 0.02 to 0.986, 0.065 to 2.169 and 0.272 to 1.9 μg/mL for tramadol and from 0.036 to 0.084 μg/mL for M1 (reported by various authors and reviewed by Grond & Sablotzki 2004). Ranges of C_{max} for tramadol

Table 5. Comparative pharmacokinetics of IV tramadol, O-desmethyl tramadol (M1) and *N*-desmethyltramadol (M2) in horses.

Compound (dose)	AUC_{0-inf} (h.μg/mL)	V_{dss} (L/kg)	Cl_B (mL/min/kg)	$t_{1/2,z}$ (h)	MRT (h)	C_{max} (μg/mL)	T_{max} (h)	Reference
Tramadol (4 mg/kg)	7.22 (4.27–17.4)	1.28 (0.16–1.6)	9.66 (3.5–1.6)	1.95 (1.73–3.88)	1.76 (0.63–2.1)			present study
M1	0.89 (0.1–7.7)			20.52 (1.04–74.34)	4.78 (1.05–5.6)	0.05 (0.04–0.13)	0.75 (0.33–8)	
M2	0.73 (0.2–0.96)			4.34 (1.97–6.36)	3.6 (2.07–4.89)	0.08 (0.05–0.13)	2 (0.75–4)	
Tramadol (2 mg/kg)	1.31 ± 0.2	2.17 ± 0.52	26 ± 3	1.36 ± 0.16	1.38 ± 0.16			Shilo et al. (2008)
Tramadol (2 mg/kg)	1.82 ± 0.78	2.48 ± 0.74	20.3 ± 5.7	2.05 ± 0.93				Dhanjal et al. (2009)
Tramadol (3 mg/kg)	2.07 ± 0.3	3.33 ± 0.67	24.6 ± 3.91	3.05 ± 2.18		1.7 ± 0.4		Knych et al. (2013)
M1	0.05 ± 0.01			3.83 ± 0.86		0.12 ± 0.05	0.56 ± 0.49	
M2	0.52 ± 0.08			2.59 ± 0.73		0.1 ± 0.02	1 ± 0.21	
Tramadol (5 mg/kg)	4.47 ± 0.35*	1.42 ± 0.08	1.16 ± 0.1	0.69 ± 0.1		0.25 ± 0.01	1.25 ± 0.18	Giorgi et al. (2007)
M2	1.12 ± 0.1*							
Tramadol (5 mg/kg)	2.7 ± 0.27	4.02 ± 1.35	30.1 ± 2.56	2.55 ± 0.88	2.81 ± 1.28			Stewart et al. (2011)
M2	0.27 ± 0.026				4.08 ± 0.47	0.07 ± 0.13	0.71 ± 0.12	

*AUC 0–8 h.

(4.88−33.29 μg/mL) and M1 (0.04−0.13 μg/mL) in our horses were higher than analgesic concentrations of tramadol and M1 and these analgesic levels were maintained for the whole observation period of 10 h (Figure 2). Except Knych et al. (2013), all other studies performed in horses recorded an M1 scarce production (Giorgi et al. 2007; Shilo et al. 2008; Dhanjal et al. 2009; Cox et al. 2010). Only Dhanjal et al. (2009) studied the antinociceptive effect of tramadol finding that at a dose of 2 mg/kg BW, it was quite unsatisfactory. As suggested by Cox et al. (2010), doses greater than 2 mg/kg BW would be necessary to achieve targeted plasma concentrations associated with analgesia in humans. In our study, a dose of 4 mg/kg BW IV recorded the achievement of human analgesic concentrations for tramadol and M1 for the whole observation period of 10 h and would be responsible of the smooth recovery and postoperative comfort of all horses.

Preoperative administration of tramadol (4 mg/kg BW IV) to eight horses undergoing orchiectomy did not show the adverse effects reported for this route of administration. This finding, together with the kinetic behaviour, can suggest that 4 mg/kg BW of tramadol IV might be useful and safe as postoperative analgesic in a multimodal analgesic approach for orchiectomy in horses.

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