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TRAMADOL IN THE HORSE: A PRELIMINARY REPORT ON ITS DETECTION, PHARMACOKINETICS AND PHARMACODYNAMIC RESPONSES

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ABSTRACT

Tramadol,[(±)cis-2-[dimethylamino)methyl]-1-(3-methoxyphenyl)cyclohexanol hydrochloride, MW 363.4] is reportedly a potent analgesic in dogs with effects resulting from interactions between opiate, adrenergic and serotonin receptor systems. This paper reports on the analytical detection, pharmacokinetics, preliminary pharmacodynamics and analgesic responses to Tramadol in the horse. We developed a sensitive quantitative method for Tramadol in equine plasma using liquid/liquid extraction followed by silyl derivatisation and gas chromatographic mass spectrometric detection with an HP-5970 mass selective detector. This procedure had a limit of detection [LOD] of 2 ng/mL in plasma, was linear [R^2=0.9997] from 5–500 ng/mL and the coefficients of variation were 8.3% at 10 ng/mL and 6.8% at 500 ng/mL respectively. We then established the relationship between Tramadol dose, plasma concentrations and pharmacological effect in horses infused with Tramadol at dosages increasing stepwise every 20 min and recorded the increasing plasma concentrations of Tramadol and associated behavioural changes. Peak plasma concentration of Tramadol after this cumulative dose of 3.1 mg/kg was 619.5 ± 60.2 ng/mL and the terminal half-life was 114.3 ± 19.7 min. Analysis of behavioural responses showed that Tramadol infusion caused no significant changes in heart rate, step frequency or sweating score. On the other hand, Tramadol infusion produced dose-dependent increases in respiratory rate, trembling, head nodding and, somewhat unexpectedly, head height was increased. The animals appeared more alert and all study animals showed a Tramadol associated decrease in gut sounds. In an experiment specifically designed to evaluate analgesic responses following 2 mg/kg iv Tramadol, plasma Tramadol concentrations peaked at 1,512 ± 463 (SD) ng, and declined thereafter with a half-life of 2.5 h but with no statistically significant cutaneous analgesic response.

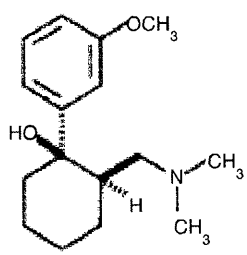
INTRODUCTION

Tramadol [(±)cis-2-[dimethylamino)methyl]-1-(3-methoxyphenyl) cyclohexanol hydrochloride, MW 363.4] is a centrally acting synthetic analogue of codeine (Fig 1) with analgesic effects resulting from interactions between opiate, adrenergic and serotonin receptor systems (Lewis *et al.* 1997; KuKanich *et al.* 2004). It is a weak mu-opioid agonist and its action is mostly mediated by inhibiting neuronal norepinephrine and serotonin reuptake (Lewis *et al.* 1997). In people, administration of the opioid antagonist naloxone can reverse approximately 30% of the analgesic effect of Tramadol (Raffa *et al.* 1992; Besson *et al.* 1994). Tramadol is used widely for treatment of chronic cancer and orthopaedic pain in people and in dogs, and it has the advantage of being a non-scheduled substance. In people, Tramadol has minimal effects on gastrointestinal motility and no significant effects on cardiovascular or respiratory functions (Scott *et al.* 2000) yet has the same

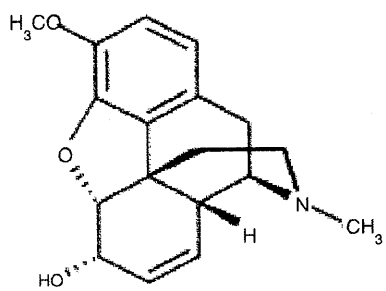
analgesic effects for mild to moderate pain as equipotent doses of morphine, with less respiratory depression (Lewis *et al.* 1997; Mastrocinque *et al.* 2003).

Published reports of Tramadol use in veterinary medicine are limited and these studies are mainly focused on the pharmacokinetic profile. One study in dogs undergoing ovario-hysterectomy following pyometra showed that the analgesic effects of pre-operative iv Tramadol (2 mg/kg) or morphine (0.2 mg/kg) were similar when assessed in the early post operative period (Mastrocinque *et al.* 2003). In dogs, the following doses have been suggested: 1–4 mg/kg PO every 6 h for cancer pain (Lascelles 2003) and 1–2 mg/kg PO every 12 h for degenerative joint disease and other chronic pain (Parker 2004). In one report, 500 mg of Tramadol was given iv to a 7-year-old Standardbred gelding to evaluate urine metabolites but there was no report of the effects of the drug on the horse (Russo *et al.* 2001). Two recent reports of the pharmacokinetics in horses following iv Tramadol administration evaluated doses of 2 mg/kg (Shilo *et al.* 2007) and 5 mg/kg (Giorgi *et al.* 2007) and a recent analysis of its pharmacokinetics in cats (Pypendop and Ilkiew 2007).

The optimal dose of Tramadol for use in horses has not been established. Epidural injection of 1 mg/kg Tramadol in horses has been shown to produce a moderate analgesic effect for approximately 6 h, with no adverse effects on behaviour (Natalini *et al.* 2003). It is not known if the effects of iv Tramadol in horses include the typical opioid-induced sympathetic stimulation, increased locomotion, CNS excitation and analgesic responses. If Tramadol produces analgesia without associated excitatory responses, it has the potential to be useful for analgesic therapy in horses.



Tramadol, m.w. 263.4



Codeine, m.w. 299.36

Fig 1: Structures and molecular weights of Tramadol and codeine.

The overall objectives of this study were to determine the effects of cumulatively increasing doses of iv Tramadol on behaviour, heart rate and respiratory rate, to assess the cutaneous analgesic effect of Tramadol on the response to a thermal stimulus, and to correlate these effects with plasma concentrations and pharmacokinetic data. The objectives were addressed in 3 phases. In Phase 1, a quantitative analytical method for Tramadol was developed, Phase II was a dose-finding study to determine the highest dose of Tramadol that could be administered safely and in Phase III, a dose based on results from Phase II was evaluated for analgesic efficacy using a cutaneous thermal stimulus model. This report details Phase 1 of the project, development of a quantitative analytical method for Tramadol and overviews the pharmacokinetic and clinical correlates of Phases II and III of this study.

Materials and Methods

Animals

The Michigan State University Institutional Animal Care and Use Committee approved the study. Sixteen horses were screened for inclusion in the study group based on the latency of their response to a thermal pain stimulus (methods described below). The 6 selected horses - 3 geldings and 3 mares with a mean age of 21 years (range 7–29) and a mean weight of 565 kg (490–623), were studied in 2 experiments. Physical examination, packed cell volume and total solids were within normal limits. Horses were brought in from pasture and housed in box stalls bedded with shavings for at least 12 h prior to each study. They had free access to fresh water and were fed a pellet diet. For the cutaneous pain portion of the study, the heat lamp utilised was provided by the University of Kentucky Gluck Equine Research Center. Horses were excluded if they demonstrated a response time of 6 s or greater to the thermal stimulus because it was anticipated that Tramadol would result in a prolongation of the baseline response time and the cut off time of exposure would be 10 s to

prevent tissue damage. Hoof withdrawal and skin twitch reflex latencies were measured in response to a thermal stimulus using the method first published by Kamerling *et al.* (1985). The skin over the left withers and left front fetlock was clipped and blackened with stamp pad ink to promote uniform absorption of light. The heat lamp was always operated by the same investigator. Before each use, the lamp was pointed away from the horse, turned on for 5 s, and was then allowed to cool for 1 min before it was used again. The lamp was held approximately 11 cm from the horse and the intense stimulus was applied to a focal area. The heat lamp had an automatic timer that was activated when the heat lamp was turned on, and shut off when the lamp was turned off. A sham light was randomly activated so as not to condition horses to expect the heat stimulus. Positive responses were skin twitch at the left wither or shoulder and withdrawal of the left front foot. Latency to response was determined at each site in triplicate and sites were alternated with at least 1 min between readings at a site.

Tramadol

A stock solution of 5% Tramadol (Sigma-Aldrich Chemical Corp., St Louis, Missouri, USA) was provided by Dr Tobin (Gluck Equine Research Center, University of Kentucky). Heparinised saline was prepared by adding 1 unit/mL of heparin to 0.9% NaCl. Coded syringes containing Tramadol or vehicle were prepared on the morning of each study by a technician who was not involved in data collection.

Tramadol administration

On the morning of the study, blinders were placed on the horses and they were fed as usual. After a period of 30 min, their jugular veins were catheterised aseptically with a 5 $^1/_4$ inch 14 gauge catheter (BD Angiocath, BD Medical, Utah, USA). The left jugular catheter was used for administration of treatment and the right jugular catheter was used for blood sampling. Each injection was at head height as determined from the height of the poll tape against a tape measure applied to a wall of the stall. The Tramadol infusion and sample/data collection schedule is presented in Figure 6.

Sample collection

10 mL of blood was drawn out of the right jugular catheter before the 20 mL sample was collected and placed into 2 x 10 mL vacutainer tubes. Tubes were spun for 15 min at 1,700 *g* in a Jouan CR4-12 centrifuge (Jouan Inc., Virginia, USA). The serum was removed and stored at -20°C until analysis.

Tramadol analysis

We developed a highly sensitive quantitative method for Tramadol in equine plasma using liquid-liquid extraction followed by silyl derivatisation and GC/MS analysis. 1.0 mL of plasma was adjusted to pH 7.8 with phosphate buffer. Pentazocine was used as an internal standard and the sample was extracted with dichloromethane for 45 min. Following centrifugation the dichloromethane layer was separated and evaporated under nitrogen at 45°C. To the dried residue, 80 µL of BSTFA 1% TMCS and 5 µL of dimethylformamide (DMF) were added, the tubes vortexed to dissolve the residue and the solutions transferred to an automatic sampler vial. The vials were sealed and placed in a 70°C oven for 20 min, cooled and transferred to the GC/MS. Gas chromatography was accomplished on a HP 5890 (Agilent) chromatograph fitted with an HP-5MS capillary column (10 m x 0.25 mm id x 0.25 mm film thickness), oven temperature initially was 70°C (2 min) then 20°C/min to 280°C (5 min). Mass spectral data were acquired on a HP 5970 (Agilent) mass selective detector in full scan mode to characterise the standards and in selected ion monitoring (SIM) mode for the analytical runs. The spectrometer was operated in the EI mode at 70 eV under standard autotune conditions, with the exception of the electron multiplier voltage which was set at 200 volts above autotune conditions. Injection volume was 1 mL. This procedure has a limit of detection (LOD) of 2 ng/mL at a signal/noise ratio of 3 with a sample size of 1 mL.

Pharmacokinetic analysis

Pharmacokinetic analyses were performed using a non-linear regression program (Winnonlin, version 5.1) (Pharsight Corporation, North Caroline, USA). Area under the curve (AUC) following iv administration was measured by use of a linear trapezoidal approximation with extrapolation to infinity, and slope of the terminal portion (β) of the log plasma drug concentrations versus time curve was determined by the method of least-squares regression (Gibaldi and Perrier 1982).

The compartmental model used is represented by general equation a where Cp is plasma

concentration of compound at any time (t), A and B are the Y intercepts associated with distribution and elimination phase, respectively, and a and b represent the rate constant of distribution and terminal elimination phase, respectively. The rate constant of distribution (α), and distribution half-life ($t_{1/2}\ \alpha$) were determined using the method of residuals (Gibaldi and Perrier 1975). The terminal half-life ($t_{1/2}\ \beta$) (Martinez 1998a,b) was calculated according to Equation 1:

$$Cp = A \times e^{-\alpha \times \tau} + B \times \varepsilon^{-\beta \times \tau} \quad (a)$$

$$t_{1/2}\ \beta = \ln 2 / \beta \quad (1)$$

Total body clearance (Cls) was calculated by use of Equation 2:

$$Cls = IV\ Dose / AUC_{0\text{-}inf}\ (iv) \quad (2)$$

The volume of distribution in central compartment (Vdc), volume of distribution in terminal elimination phase (Vdb) and volume of distribution at steady state (Vdss) were calculated according to Equations 3, 4 and 5, respectively (Martinez 1998a, b):

$$Vd_c = Dose\ (iv) / A+B \quad (3)$$

$$Vd_\beta = iv\ Dose / AUC_{0\text{-}inf} \times \beta \quad (4)$$

$$Vd_{ss} = iv\ Dose \times AUMC_{0\text{-}inf} / (AUC_{0\text{-}inf})^2 \quad (5)$$

AUMC is area under the first moment curve and calculated by the trapezoidal method and extrapolated to infinity (Gibaldi and Perrier 1982).

K_{10} is first order elimination rate constant which describes elimination of the drug from the central compartment. K_{12} and K_{21} are distribution rate constant from central to peripheral and from peripheral to central compartment, respectively. K_{10}, K_{12}, and K_{21} (Martinez 1998a, b) were calculated according to Equations 6, 7, 8, respectively:

$$K_{10} = \alpha \times \beta / K_{21} \quad (6)$$
$$K_{12} = \alpha + \beta - k_{21} - K_{10} \quad (7)$$
$$K_{21} = B \times \alpha + A \times \beta / (A+B) \quad (8)$$

RESULTS

Tramadol analysis

Figure 2a shows typical total ion chromatograms of our reference standard Tramadol as its trimethyl silyl derivative, Tramadol-TMS derivative and internal standard, Pentazocine, as Pentazocine-TMS. In the chromatographic conditions the retention time of Tramadol-TMS was 8.02 min, while the retention time of Pentazocine-TMS was 9.45 min, with good peak characteristics and chromatographic separation. Figures 2b and 2c show the full scan mass spectrum of Tramadol TMS with the base peak of 58 m/z and molecular ion of 335 m/z, and full scan EI spectrum of internal standard Pentazocine-TMS in agreement with the expected mass spectral characteristics of Tramadol-TMS. Tramadol-TMS and the fragmentation pattern are presented in Figure 3. Figure 4 shows the Tramadol standard calibration curve, the curve is linear between 5 and 500 ng/mL of Tramadol, and the correlation coefficient was R^2= 0.9997.

Tramadol/safety evaluation

We next performed a Tramadol safety evaluation in which we infused Tramadol at increasing dose rates over an approximately 2 h period. The experiment started with an infusion rate equivalent to a dose of 0.1 mg/kg infused over 20 min, and we subsequently doubled the infusion rate at each 20 min intervals, as indicated in the legend of Figure 5. Infusions were continued until the behavioural effects suggested that administration be terminated. As shown in Figure 5, serum concentrations of Tramadol increased with increasing infusion rate, peaking at about 619 ± 60 ng/mL at about 2 h after administration.

Clinical signs of Tramadol administration

During these infusions a selection of clinical signs were monitored carefully.

Preliminary analysis of associated behavioural responses showed that Tramadol infusion caused no significant changes in heart rate, step frequency or sweating score. Following Tramadol, there was a decrease in rectal temperature during recovery phase [about 0.5°F] which, although statistically significant, was not considered clinically significant. On the other hand, Tramadol infusion produced dose-dependent increases in respiratory rate, trembling, head nodding and, somewhat unexpectedly, head height. The animals appeared more alert and all horses showed a marked Tramadol associated decrease in gut sounds (Dhanjal *et al.* 2009).

Intravenous Tramadol:pharmacokinetic response

Figure 7 shows the plasma concentrations of Tramadol after rapid iv injection of a 2 mg/kg dose of Tramadol to 6 horses. As shown in Table 1,

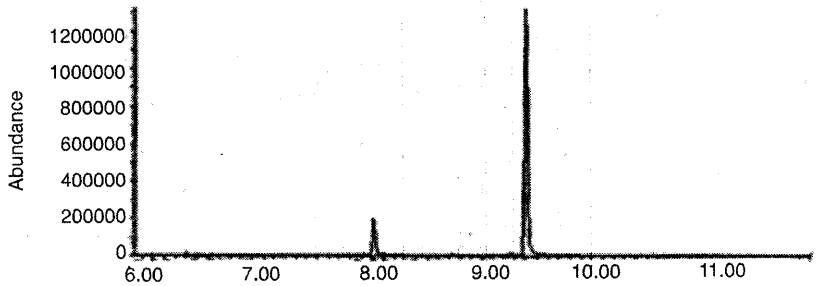


Fig 2a: Chromatogram of standards tramadol-TMS (8.02 min) and pentazocine-TMS (9.45 min).

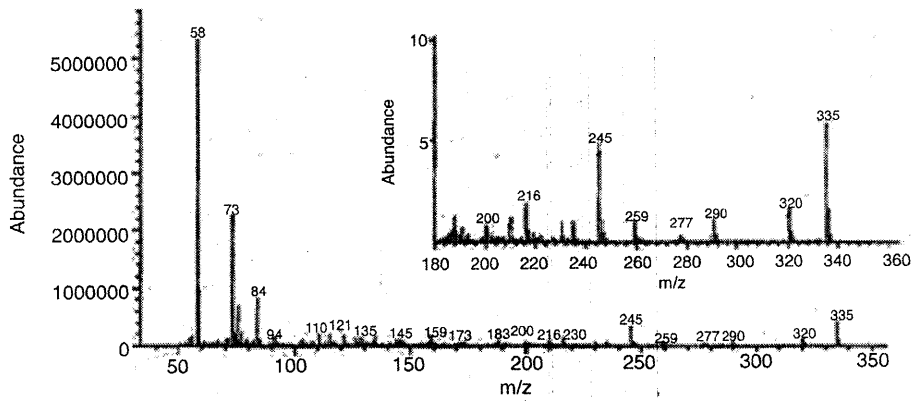


Fig 2b: Tramadol-TMS, full-scan EI spectrum.

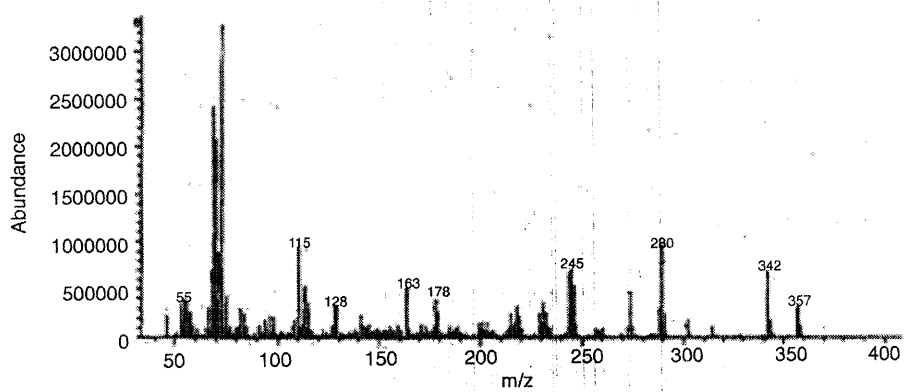


Fig 2c: Pentazocine-TMS, full scan EI spectrum.

plasma concentrations of Tramadol peaked at 1,512 ± 463 (SD) ng/mL 5 min after Tramadol administration. Thereafter the plasma concentrations of Tramadol declined with an alpha phase half-life of about 12 min, followed by a beta phase terminal half-life of about 2 h following a simple 2 compartment open system. The distribution half life ($T\ ^1/_2\ \alpha$) and terminal elimination half life ($T\ ^1/_2\ \beta$) were 0.210 ± 0.132 (SD)/h and 2.05 ± 0.929 (SD)/h respectively. The volume of distribution in the central compartment (Vd_c) was 1,016 ± 362 (SD) mL/kg. The volume of distribution in the terminal elimination phase ($Vd\beta$) was 3,359 ± 1196 (SD) mL/kg and the volume of distribution at the steady state (Vd_{ss}) was 2,484 ± 736 (SD) mL/kg. Plasma clearance of Tramadol averaged about 1,222 ± 342 mL/kg/h (Table 1).

Intravenous Tramadol:analgesic efficacy

A single bolus dose of 2 mg/kg of Tramadol iv did not prolong the hoof withdrawal or skin twitch reflex latencies to a thermal stimulus. Baselines were 4.16 ± 0.41 s and 3.06 ± 0.41 seconds, respectively, and were not significantly prolonged

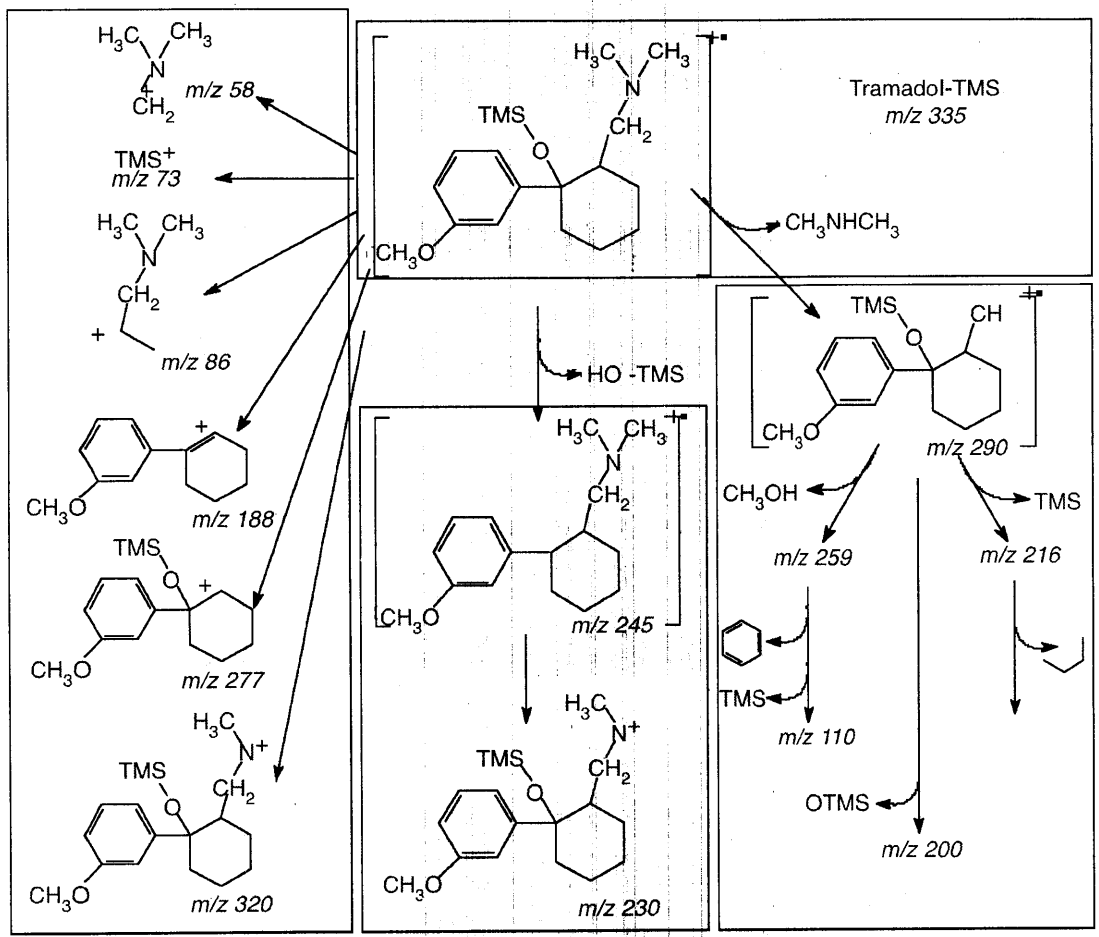


Fig 3: Pathways to tramadol-TMS fragmentation during electron impact mass spectrometry. The M+. molecular ion at m/z 335 can undergo loss of uncharged radicals to give m/z 58, 73, 84, 188, 277 and 320 (left side). It can also undergo loss of uncharged neutrals such as HO-TMS to give m/z 245 and dimethylamine to give m/z 290 (middle and right side). The latter radical cations can then undergo further cleavages as shown. M/z 216 can also undergo further cleavages to release m/z 121, 135 and 173 (not shown).

by Tramadol. Following the 2 mg/kg dose of Tramadol, trembling score increased from 0–2.2 ± 0.3 and head nodding score increased from 0–2.5 ± 0.34. These scores were back to baseline values of by 30 min after dosing (Dhanjal *et al.* 2009).

Discussion

Tramadol does not appear to produce the classical opiate responses in the horse. Behavioural effects that have been reported with opioids such as morphine, fentanyl, buprenorphine and butorphanol include pacing, pawing and ataxia (Sellon *et al.* 2001; Boscan *et al.* 2006; Carregaro *et al.* 2006) and the very well-characterised equine locomotor response to opiate administration (Tobin 1981). These effects did not occur in horses given Tramadol. Following Tramadol, horses tended to adopt a base wide stance and seemed to plant their feet. No ataxia was noted when the horses were moved laterally and no locomotor response was observed.

On the other hand, Tramadol produced obvious central nervous system (CNS) stimulant effects. Horses appeared to be more excited, more alert (head held higher), and more sensitive to noise and stimulation. Trembling was displayed by 5 of the 6 horses treated with Tramadol, and all of the treated horses exhibited head nodding (Dhanjal *et al.* 2009). Head nodding or shaking has been reported with butorphanol, buprenorphine and other non-opioid drugs such as alpha 2 agonists.

The results of our Phase II infusion study

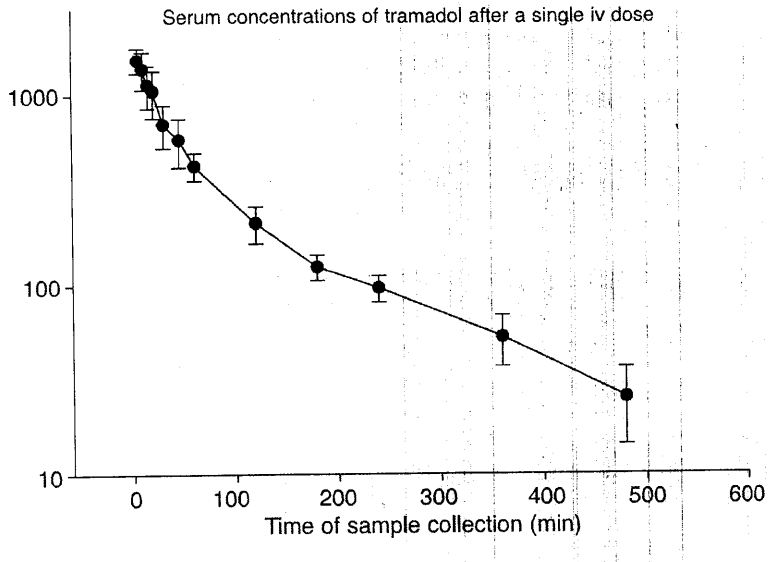


Fig 7: The solid circles show serum concentrations of Tramadol after a single bolus dose of 2 mg/kg iv injection at the indicated time points post administration. Elapsed time started from 5 min after injection to 480 min after injection, with elapsed time on X-axis and Tramadol concentration on Y-axis n = 6.

TABLE 1: Pharmacokinetic parameters of Tramadol following single 2 mg/kg iv bolus injection

Horse	Choice	Dash	Calvin	Shades	Little bit	Rusty	Mean ± SD
Weight (kg)	570	596	582	575	637	472	572 ± 55
A (ng/ml)	2908	978	1748	2897	813	1146	1748 ± 948
B (ng/ml)	323	796	284	666	651	258	496 ± 234
K_{10} (/h)	0.957	1.159	1.650	2.014	0.989	0.922	1.282 ± 0.45
α (/h)	1.735	5.377	4.752	8.26	4.685	1.956	4.46 ± 2.41
β (/h)	0.190	0.590	0.329	0.470	0.499	0.276	0.392 ± 0.152
K_{12} (/h)	0.624	2.069	2.484	4.789	1.833	0.725	2.088 ± 1.518
K_{21} (/h)	0.344	2.738	0.946	1.926	2.359	0.585	1.483 ± 0.993
$t_{1/2}$ K_{10} (h)	0.724	0.598	0.420	0.344	0.700	0.751	0.59 ± 0.171
$t_{1/2}$ α (h)	0.399	0.129	0.146	0.084	0.148	0.354	0.210 ± 0.132
$t_{1/2}$ β (h)	3.65	1.17	2.11	1.48	1.390	2.514	2.05 ± 0.929
Cls (ml/kg/h)	605	1307	1625	1131	1352	1313	1222 ± 342
Vdc (ml/kg)	632	1127	985	561	1366	1424	1016 ± 362
Vdβ (ml/kg)	3120	2214	4938	2407	2712	4762	3359 ± 1196
Vdss (ml/kg)	1778	1979	3570	1958	2428	3188	2484 ± 736
$AUC_{0\text{-inf}}$ (ngxh/ml)	3376	1530	1231	1769	1479	1523	1818 ± 782
R^2	0.991	0.997	0.952	0.992	0.985	0.971	0.981 ± 0.017

responses we decided that effects seen after a cumulative dose of 1.5 mg/kg were acceptable. In Phase III, moderate trembling and head nodding were appreciated for up to 10 min after the single dose of 2 mg/kg of Tramadol. With regard to higher doses, one study that administered 5 mg/kg iv to horses reported nausea, tremor, confusion, agitation and tachycardia 3–5 min after the dose with maximum effects at 15–20 min following dosing (Giorgi *et al.* 2007). Another study reported muscle twitching of the pectorals in 2 horses receiving 2 mg/kg iv but the authors attribute this to the rate of administration because they did not see this effect when the dose was given over 10 min versus 5–6 min (Shilo *et al.* 2007). In phase III of the present study, Tramadol was administered as a bolus dose over less than 1 min, and this rate of administration may have contributed to the degree of trembling exhibited by the horses.

Intravenous Tramadol does not induce sedation in horses; in fact it causes stimulation of the central nervous system. Data presented as an abstract indicated that 2 horses receiving 1 mg/kg Tramadol after 1 mg/kg xylazine for sedation for dental procedures had adverse reactions such as rearing up and falling over in the stocks (Roscoe *et al.* 2006).

Although iv Tramadol at 2 mg/kg does not prolong the response to a thermal stimulus, other models of analgesia should be evaluated. Two studies have indicated that Tramadol is bioavailable in horses following oral dosing but with conflicting results. Long-term effects on

gastrointestinal function in horses have not been evaluated. Single dose iv administration of Tramadol does not cause mania, increased spontaneous locomotor activity and has no adverse effects on fecal output. The incidence of trembling and muscle twitching may be decreased if iv Tramadol is given slowly over at least 10 min, as opposed to a bolus dose. Future studies should consider Tramadol's potential role for treatment of chronic pain in the equine patient.

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