Veterinary Anaesthesia and Analgesia
Formerly the Journal of Veterinary Anaesthesia

Veterinary Anaesthesia and Analgesia, 2009, **36**, 581–590 doi:10.1111/j.1467-2995.2009.00492.x

RESEARCH PAPER

Intravenous tramadol: effects, nociceptive properties, and pharmacokinetics in horses

Jusmeen K Dhanjal* DVM, MS, **Deborah V Wilson*** BVSc (Hons), MS, Diplomate ACVA, **Edward Robinson*** BVetMed, MRCVS, PhD, Diplomate ACVIM, MRCVS, **Thomas T Tobin†** PhD, Diplomate ABVT, MRCVS & **Levent Dirokulu‡** DVM, MVSc, PhD
*Michigan State University College of Veterinary Medicine, East Lansing, MI, USA
†University of Kentucky Gluck Equine Research Center, Lexington, KY, USA
‡University of Illinois, College of Veterinary Medicine, Urbana, IL, USA

Correspondence: Jusmeen K Dhanjal, Veterinary Specialty Center, 1515 Busch Parkway, Buffalo Grove, IL 60089, USA.
E-mail: jdhanjal@vetspecialty.com

Abstract

Objective To determine the optimal dose, serum concentrations and analgesic effects of intravenous (IV) tramadol in the horse.

Study design Two-phase blinded, randomized, prospective crossover trial.

Animals Seven horses (median age 22.5 years and mean weight 565 kg).

Methods Horses were treated every 20 minutes with incremental doses of tramadol HCl (0.1–1.6 mg kg^{-1}) or with saline. Heart rate, respiratory rate, step frequency, head height, and sweating, trembling, borborygmus and head nodding scores were recorded before and up to 6 hours after treatment. In a second study, hoof withdrawal and skin twitch reflex latencies (HWRL and STRL) to a thermal stimulus were determined 5 and 30 minutes, and 1, 2, 4 and 6 hours after bolus IV tramadol (2.0 mg kg^{-1}) or vehicle. Blood samples were taken to determine pharmacokinetics.

Results Compared to saline, tramadol caused no change in heart rate, step frequency or sweating score. Respiratory rate, head height, and head nodding and trembling scores were transiently but significantly increased and borborygmus score was decreased by high doses of tramadol. Following cumulative IV administration of 3.1 mg kg^{-1} and bolus IV administration of 2 mg kg^{-1}, the elimination half-life of tramadol was 1.91 ± 0.33 and 2.1 ± 0.9 hours, respectively. Baseline HWRL and STRL were 4.16 ± 1.0 and 3.06 ± 0.99 seconds, respectively, and were not significantly prolonged by tramadol.

Conclusion and clinical relevance IV tramadol at cumulative doses of up to 3.1 mg kg^{-1} produced minimal transient side effects but 2.0 mg kg^{-1} did not provide analgesia, as determined by response to a thermal nociceptive stimulus.

Keywords horse, nociception, pharmacokinetics, tramadol.

Introduction

Currently, analgesics for horses are comprised mainly of two classes of drugs, alpha-2 adrenergic agonists and nonsteroidal anti-inflammatory drugs (NSAIDs). The former are used mostly for acute, visceral pain but cause considerable sedation at doses used for analgesia (Pippi & Lumb 1979; Muir & Robertson 1985). NSAIDs are the corner stone for treatment of chronic somatic and orthopedic pain but have side effects on the gastrointestinal, renal, and coagulation systems. Opioids are not widely used in horses because they can cause central nervous system (CNS) excitation, sympathetic stimulation, and can stimulate locomotion (Combie et al. 1981). In addition, the

regulatory controls on opioids make their practical use difficult.

Tramadol is a centrally acting synthetic analog of codeine but is not a controlled substance. It is a weak mu-opioid agonist but administration of naloxone does not fully block the antinociceptive effects, implicating a nonopioid component. The remainder is likely mediated by inhibiting neuronal norepinephrine and serotonin reuptake (Raffa et al. 1992). Tramadol is widely used for treatment of chronic cancer and orthopedic pain in people and in dogs. It has minimal effects on gastrointestinal motility and no significant cardiovascular or respiratory effects (Scott & Perry 2000), yet it has the same analgesic effects for mild to moderate pain as equipotent doses of morphine (Lewis & Han 1997; Mastrocinque & Fantoni 2003).

The analgesic and other effects of IV tramadol in horses are unknown. If it does not cause the typical opioid-induced sympathetic stimulation, increased locomotion, and CNS excitation, it has the potential to be a useful analgesic in horses. By epidural injection, tramadol (1 mg kg^{-1}) produced moderate analgesia, with no adverse effects on behavior (Natalini & Robinson 2000). In studies of the pharmacokinetic profile of tramadol in horses, IV doses ranging from 500 mg (Russo & Wynne 2001) to 5 mg kg^{-1} (Giorgi et al. 2007; Shilo et al. 2008) have been evaluated.

The objectives of the present study were to: 1) determine the effects of cumulatively increasing doses of IV tramadol on behavior, gastrointestinal function, and heart and respiratory rates; 2) assess the effect of tramadol on the response to a thermal stimulus; and 3) investigate the pharmacokinetics of tramadol. These objectives were addressed in two phases. Phase I was a dose-finding study to investigate the highest dose of tramadol that caused the least cardiopulmonary and behavioral changes. In Phase II, a dose based on results from Phase I was evaluated for analgesic efficacy using a thermal stimulus.

Materials and methods

Animals

The study was approved by the Michigan State University Institutional Animal Care and Use Committee. Sixteen horses were screened for inclusion in the study based on the latency of their response to a thermal pain stimulus (methods described below). The six selected horses – three geldings and three mares with a median age of 22.5 years (range 7–29 years) and a mean weight of 565 kg (490–623 kg) were studied in two experiments. Horses were healthy as determined by physical examination, packed cell volume, and total solids. Horses were brought in from pasture and housed in a heated barn, in box stalls bedded with shavings for at least 12 hours prior to each study. They had free access to fresh water and were fed a pelleted diet. Horses were restrained with a halter and lead rope during data collection.

Screening process

Horses were positioned in the middle of a large quiet room in the heated barn and held with a halter and lead rope. A 6 × 6 cm area over the left withers was clipped and blackened with stamp pad ink and a 2 × 3 cm area over the left front lateral fetlock was similarly prepared. Blinders were placed on the horses so that they could not see the light of the lamp. The latency of response to a focused thermal stimulus was determined at the withers and the fetlock. The heat lamp was provided by the Gluck Equine Research Center at the University of Kentucky (courtesy T. Tobin). Horses were excluded if they demonstrated a response time of 6 seconds or greater to the thermal stimulus. This was done because we anticipated tramadol would result in a prolongation of the baseline response time and the cutoff time of exposure would be 10 seconds to prevent tissue damage.

Instrumentation for Phases I and II

On the morning of the study, blinders were placed on the horse and it was fed as usual. Thirty minutes later, horses were instrumented. Each jugular vein was catheterized aseptically with a 5¼ inch 14-gauge catheter (BD Angiocath, BD Medical, Sandy, UT, USA). The left jugular catheter was used for administration of treatment and the right for blood sampling. Each injection was followed with 10 mL of sterile heparinized saline.

Respiratory rate was obtained by counting thoracic wall excursion for 1 minute. A digital thermometer was used to measure rectal temperature. Level of sedation was judged by the height of the horse's head from the ground. A bright orange piece of tape was affixed to the mane at the poll. After the horse had been instrumented and allowed to stand

undisturbed in the stall for at least 15 minutes, baseline head height was determined from the height of the head tape against a tape measure applied to a wall of the stall.

Sweating, excitement, trembling, and head nodding were scored using numerical rating scales (Derksen et al. 1999). Sweating was scored as follows: no sweat, cool flanks = 0; warm humid flanks = 1; flanks warm, hand wet after stroking = 2; flanks visibly wet = 3; sweat dripping from flanks = 4. Level of excitement was scored as follows: calm, no change from pre-treatment = 0; restless = 1; anxious appearance, pinnae retracted back, eyes wide open = 2; kicking and pawing, distressed = 3; uncontrollable, kicking violently, biting flanks = 4. Trembling was scored as follows: none = 0; intermittent trembling of flanks = 1; constant trembling of flanks = 2; sustained trembling of flanks and some shaking of whole body = 3; sustained shaking of whole body = 4. Head nodding was scored as follows: none = 0; intermittent subtle nodding of head = 1; constant mild nodding of head = 2; obvious constant nodding of head = 3.

To evaluate intestinal sounds, the right upper, right lower, left upper, and left lower abdominal quadrants were each ausculted for 30 seconds and intestinal sounds were scored using a modification of a previously published scale (Sellon et al. 2001). More than two sounds in 30 seconds scored 2, one to two sounds scored 1 and no sounds scored 0. The cumulative score from all four quadrants could therefore range from 0 to 8. The number of fecal piles was also counted.

Instrumentation for Phase I

A stepcounter (Cyma StepWatch Activity Monitor SAM3; Cyma Corporation, Seattle, WA, USA) was placed on the lateral side of the left lower forelimb, just proximal to the fetlock. It was secured in place using the Velcro strap provided by the manufacturer, and was covered with a light bandage. The stepcounter was programmed and calibrated by the manufacturer. A step was counted when the animal lifted its leg off the ground even if there was no forward or backward motion. At the end of each study day, the step counter was removed from the horse, and docked to a computer using the equipment and software provided by the company.

The haircoat was clipped over the left side of the body at the withers, the neck, and caudal to the elbow for application of ECG patches. A receiver for the telemetric heart rate monitor (Hewlett Packard M1401A, Hewlett Packard, Andover, MA, USA) was secured to the horse's neck using a Velcro strap and the leads were attached to the ECG patches.

Tramadol preparation

A stock solution of 5% tramadol (Sigma-Aldrich Chemical Corp., St Louis, MO, USA) was prepared by Cornerstone Pharmacy and Compounding Laboratory (Versailles, KY, USA). Heparinized saline was prepared by adding 1 unit mL^{-1} of heparin to 0.9% NaCl. Coded syringes containing tramadol or sterile water were prepared on the morning of each study by a technician who was not involved in data collection.

Blood sampling and tramadol assay

Ten milliliters of waste was drawn out of the right jugular catheter before the 20 mL sample was collected and placed into two 10 mL vacutainer tubes (BD). Tubes were spun for 15 minutes at 1700 ***g*** in a Jouan CR4-12 centrifuge (Jouan Inc., Winchester, VA, USA). The serum was removed and stored at −20 °C until analysis.

The concentration of tramadol in each sample (i.e., calibrators, quality control and unknowns run simultaneously) was determined by an internal standard method using the peak area ratio and linear regression analysis. The response for tramadol was linear and gave correlation coefficients (R^2) of 0.99 or better. The technique was optimized to provide a limit of detection at 2.0 ng mL^{-1} and limit of quantitation at 4.0 ng mL^{-1}. Intra-day precision (% of nominal concentration) was 93 and 98% for 10 and 500 ng mL^{-1}, respectively. Intra-day accuracy (% relative SD) was always less than 10% (Tobin et al. 2009).

Experimental design

Phase I (dose–response)

In a blinded, randomized crossover design, six horses were treated with 5% tramadol intravenously or with a similar volume of IV saline at each treatment time point. Evaluation of the effects of saline followed evaluation of tramadol in three horses, with treatments separated by 40 hours (two horses) and 100 hours (one horse). In the other three horses saline was evaluated first. Based on the

half-life of tramadol in other species, this dosing interval was thought sufficient for drug clearance.

Measurements of heart rate, respiratory rate, steps taken (counted over one 2 minute period), level of sedation (head height), excitement, trembling, and head nodding were made from outside the stall. The stall was entered to assess gut sounds, sweating, and rectal temperature, and then the injection was administered. Following baseline (time zero) measurements, the first dose was tramadol (0.1 mg kg^{-1}) or an equivalent volume of saline. Subsequent doses of tramadol were serially doubled (0.2, 0.4, 0.8, and 1.6 mg kg^{-1}) and administered every 20 minutes (Fig. 1). This cumulative dose was based on some pilot work that showed that a dose of 6.2 mg kg^{-1} produced unacceptable trembling and head nodding. Ten minutes after each dose, measurements were taken. After the final dose, data were collected as depicted in Fig. 1. Intestinal sounds were scored at time 0, and at the times indicated (after the final dose of tramadol or saline). The number of fecal piles was counted at time zero, at each data collection time, and then 24 hours after time zero (Fig. 1). Blood was collected for measurement of serum tramadol concentration at time 0, 20 minutes after each dose, and 80, 140, 200, and 380 minutes after the final dose of tramadol or saline (Fig. 1). Horses were monitored for signs of colic and other potential adverse effects (excitement, restlessness) for a further 24 hours and then returned to pasture.

Phase II (single dose)

In a blinded, randomized crossover design, six horses were treated IV with a single dose of 2 mg kg^{-1} of 5% tramadol or with a similar volume of sterile water, and the responses to a thermal stimulus were evaluated (described below). The treatments were separated by at least 7 days. Phase II began 1 month after completion of Phase I. One horse that was not studied in Phase I was studied in Phase II, and the study environment was the same. Horses were instrumented with bilateral jugular catheters and blinders as in Phase I. The same observer, unaware of treatment status, collected all the data. Horses were restrained by use of halter and lead rope and a single handler at data collection time points. In addition, horses were restrained periodically throughout the study period to prevent the association of being handled with the thermal stimulus. Baseline readings for respiratory rate, level of sedation, sweating, excitement, trembling, head nodding, hoof withdrawal reflex latency (HWRL), and skin twitch reflex latency (STRL) were taken, horses were dosed, and then readings were taken 5, 30, 60, 120, 240, and 360 minutes following dosing. Twenty mL of blood was collected (collection and sample handling as described in Phase I) 30 minutes prior to dosing, and 5, 10, 15, 20, 30, 45, 60, 120, 180, 240, 360, and 480 minutes after dosing. If other variables were being assessed, blood collection was performed first.

Response to a thermal stimulus

Hoof withdrawal and skin twitch reflex latencies were measured in response to a thermal stimulus (Kamerling et al. 1988). 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 seconds, and was then allowed to cool for 1 minute 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 periodically activated independent of the heat stimulus so as not to condition horses to expect the heat stimulus. Positive responses were skin twitch at the left

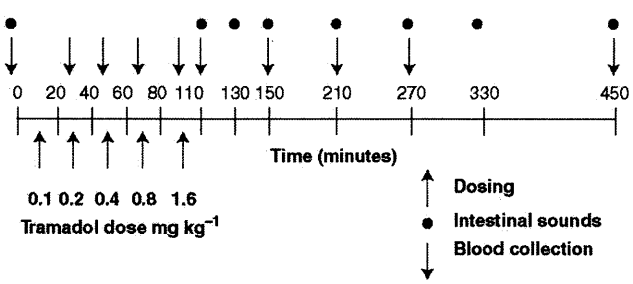

Figure 1 Protocol for Phase 1. Time (minutes) is on the horizontal axis. The times for dosing of tramadol, scoring of intestinal sounds, and blood collection are indicated.

withers 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 minute between readings at a site.

Pharmacokinetic analysis

Pharmacokinetic analyses were performed, using a nonlinear regression program (Winnonlin, version 5.1; Pharsight Corporation, Cary, NC, USA). Area under the curve (AUC) following intravenous administration was measured by use of a linear trapezoidal approximation with extrapolation to infinity. 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 & Perrier 1982).

The compartmental model used is represented by Eqn (1) where C_p is plasma concentration of compound at any time (t), A and B are the Y intercepts associated with the distribution and elimination phases, respectively, and α and β represent the rate constants of distribution and terminal elimination phases, respectively. The rate constant of distribution (α) and distribution half-life ($t_{1/2}\alpha$) were determined using the method of residuals (Gibaldi & Perrier 1975). The terminal half-life ($t_{1/2}\beta$) (Martinez 1998a,b) was calculated according to Eqn (2).

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

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

Total body clearance (Cl_s) was calculated:

$$Cl_s = \text{IV Dose}/\text{AUC}_{0-\text{inf}}\,(\text{IV}) \quad (3)$$

The volume of distribution in central compartment (Vd_c), volume of distribution in terminal elimination phase (Vd_β) and volume of distribution at steady state (Vd_{ss}) were calculated according to Eqns (4–6), respectively (Martinez 1998a,b).

$$\text{Vd}_c = \text{Dose (IV)}/A + B \quad (4)$$

$$\text{Vd}_\beta = \text{IV Dose}/\text{AUC}_{0-\text{inf}} \times \beta \quad (5)$$

$$\text{Vd}_{ss} = \text{IV Dose} \times \text{AUMC}_{0-\text{inf}}/(\text{AUC}_{0-\text{inf}})^2 \quad (6)$$

AUMC is area under the first moment curve and calculated by the trapezoidal method and extrapolated to infinity (Gibaldi & Perrier 1982). K_{10} is first order elimination rate constant which describes elimination of 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 Eqns (7–9), respectively.

$$K_{10} = \alpha\beta/K_{21} \quad (7)$$

$$K_{12} = \alpha + \beta - K_{21} - K_{10} \quad (8)$$

$$K_{21} = B\alpha + A\beta/(A + B) \quad (9)$$

Statistical analysis

Heart rate, respiratory rate, temperature, step frequency, and head height were analyzed by means of a three-factor analysis of variance. Fixed factors were treatment and time and horse was a random factor. Bonferroni's correction was used for multiple measurements over time. Within specific time points and between groups, trembling, head nodding, excitement, sweating, and intestinal sounds were analyzed using Wilcoxon Signed-Rank test. Significance was determined at $p < 0.05$. Results are presented as mean (standard deviation) except for the pharmacokinetic results from Phase II, which are presented as mean ± standard deviation.

Results

Phase 1 (cumulative dose–response)

Tramadol had no effect on heart rate (mean value = 35 beats minute^{-1}), step frequency (mean value = 1.5 steps minute^{-1}), sweating (mean score = 0.18), excitement (mean score = 0.35) scores, and fecal output. Following tramadol administration, horses tended to adopt a base-wide stance and seemed to plant their feet. No ataxia was noted when the horses were moved laterally.

Body temperature

Following tramadol administration, there was a significant decrease in rectal temperature during the recovery phase. Beginning 40 minutes after administration of the highest dose, rectal temperature in tramadol-treated horses was significantly lower than rectal temperature in saline-treated horses. Mean temperature for saline and tramadol treated horses during recovery was 37.4 (0.08) °C and 37.2 (0.09) °C, respectively.

Respiratory rate

For 30 minutes after the final cumulative dose, respiratory rate was significantly greater after tramadol than after saline treatment (Fig. 2).

Borborygmus score

Borborygmus score decreased significantly from 4.16 (2.22) at baseline to less than 2 at cessation of tramadol administration. The decrease persisted for 40 minutes. Saline had no effect. There was no difference in the number of fecal piles between saline and tramadol treatments (Fig. 2).

Head height

Head height was significantly increased to 67.6 (4.72) cm following the highest dose of tramadol, compared to 60.0 (7.69) cm after the corresponding dose of saline (Fig. 3).

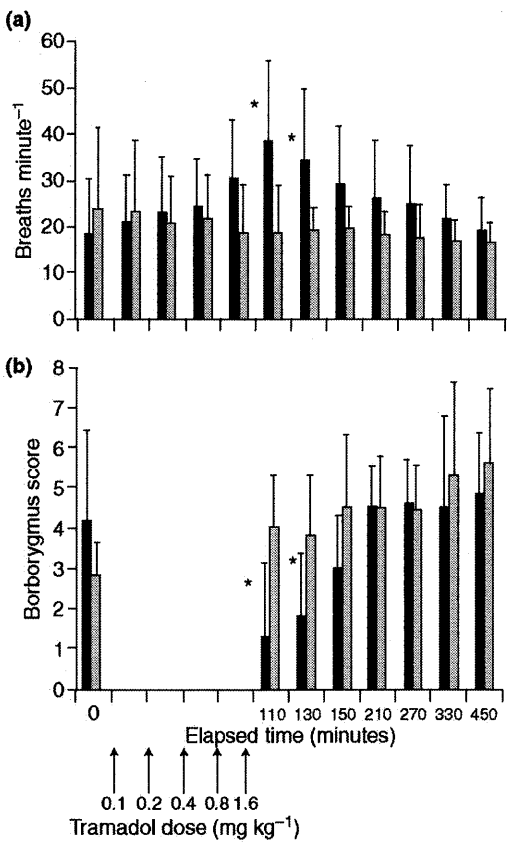

Figure 2 Effect of tramadol (black bars) and saline (gray bars) on respiratory rate (a) and borborygmus score (b). Doses of tramadol are indicated at the bottom. Borborygmus score was not recorded during tramadol administration. Values are mean ± SD, n = 6. *Significant difference between tramadol and saline treatment.

Head nodding

During tramadol treatment, there was a dose-dependent increase in head nodding score from 0 at baseline to 2.5 (0.84) after 1.6 mg kg^{-1}. The increase became significant after a dose of 0.4 mg kg^{-1} and remained significantly increased for 40 minutes after cessation of tramadol administration. Saline treatment had no effect on head nodding score (Fig. 3).

Trembling

During administration of tramadol, there was a dose-dependent increase in trembling score from 1 (1.26) after 0.8 mg kg^{-1}, to 2.5 (1.64) after 1.6 mg kg^{-1} (Fig. 3). This increase resolved by 20 minutes after the highest dose. Trembling was pronounced in neck muscles, pectorals, triceps, and gluteal muscles.

Phase II (single dose)

A single IV bolus dose of 2 mg kg^{-1} of tramadol did not prolong the HWRL or STRL to a thermal stimulus. Baseline hoof withdrawal and skin twitch latencies were 4.16 (1.0) and 3.06 (0.99) seconds, respectively, and were not significantly prolonged by tramadol (Fig. 4). Following the 2 mg kg^{-1} dose of tramadol, trembling score increased from 0 to 2.2 (0.75) and head nodding score increased from 0 to 2.5 (0.84). These scores were back to baseline by 30 minutes after dosing.

Pharmacokinetic data

Following a cumulative dose IV of 3.1 mg kg^{-1} tramadol (Phase I), the serum concentration 10 minutes after the final dose was 619.5 (60.2) ng mL^{-1}. The elimination half-life averaged 1.91 hours (0.33), based on the time from the final cumulative dose.

Following a single IV dose of 2 mg kg^{-1} (Phase II), tramadol serum concentration peaked at an average C_{max} of 2.2 ± 0.9 (SD) μg mL^{-1} (Table 1). Serum concentration decreased rapidly with an elimination half-life of 2.1 ± 0.9 hours (Fig. 5). Based on examination of a residual plot and the R^2 of 0.98, the pharmacokinetic equation was a good model for our observed data. Computer-derived parameters from the pharmacokinetic equation for each horse are presented in Table 1.

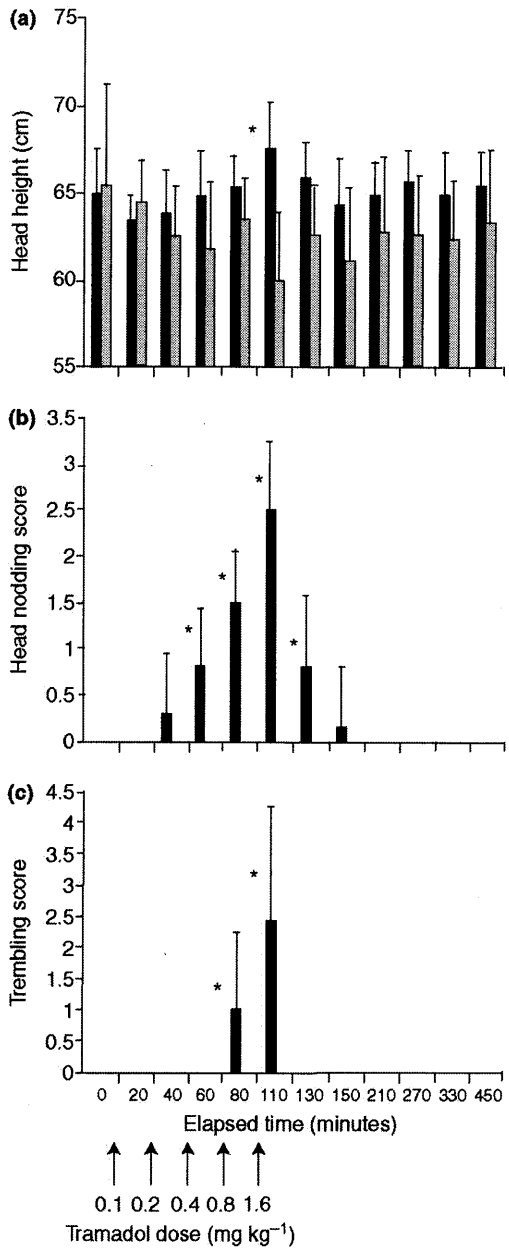

Figure 3 Effect of tramadol (black bars) and saline (gray bars) on head height (a), head nodding (b), and trembling score (c). Doses of tramadol are indicated at the bottom. Head nodding and trembling score was zero during saline treatments. Values are mean ± SD, $n = 6$. *Significant difference between tramadol and saline treatment.

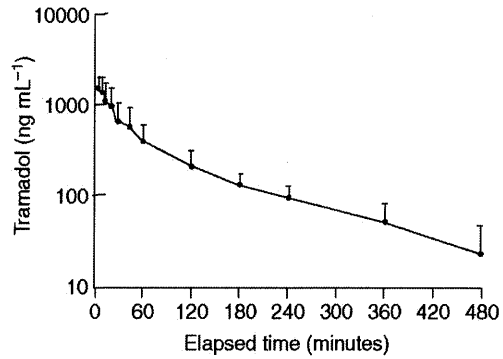

Figure 4 Serum concentrations of tramadol after a single bolus dose of 2 mg kg^{-1} IV. Samples were collected beginning 5 minutes after injection and ended 480 minutes after injection, $n = 6$.

Discussion

This study demonstrates that IV tramadol administration to horses does not produce the classical effects such as pacing, pawing and ataxia that have been reported with other opioids up to the highest dose used (Combie et al. 1981; Sellon et al. 2001; Carregaro et al. 2007). Despite the lack of locomotor effects, tramadol had other CNS-stimulant effects; horses appeared more excited and alert (head held higher), and more sensitive to noise and stimulation. Trembling occurred in five of the six horses, and all exhibited head nodding as has been observed with buprenorphine (Carregaro et al. 2006).

The short-lived decrease in borborygmus score, and absence of effect on fecal output gives tramadol an advantage over other opioids. By comparison, an IV bolus of butorphanol (0.1 mg kg^{-1}) decreased borborygmus score for up to 1 hour and the number of fecal piles passed in the first 24 hours (Sellon et al. 2001). Horses administered buprenorphine (10 μg kg^{-1}) IV had decreased borborygmus scores for 4 hours (Carregaro et al. 2006), and those receiving IV morphine (0.5 mg kg^{-1}) every 12 hours for 6 days had decreased gastrointestinal motility and fecal moisture content for 4–6 hours after dosing (Boscan et al. 2006).

Unlike morphine, tramadol did not produce a decrease in respiratory rate. In fact, respiratory rate increased following tramadol as it does after IV buprenorphine (Carregaro et al. 2006). The cause of the increase in respiratory rate is unknown but it may have been secondary to CNS stimulation. We do not know whether this dose of tramadol is associated with respiratory depression or stimulation as we did not measure arterial blood gases on these animals.

Table 1 Pharmacokinetic parameters of tramadol following single 2 mg kg^{-1} IV bolus injection. Data are expressed as mean ± SD with the exception of the half-lives that are expressed as the harmonic means ± pseudo-SD

Horse	1	2	3	4	5	6	Mean ± SD
Weight (kg)	570	596	582	575	637	472	572 ± 55
A (ng mL^{-1})	2908	978	1748	2897	813	1146	1748 ± 948
B (ng mL^{-1})	323	796	284	666	651	258	496 ± 234
K_{10} ($hour^{-1}$)	0.957	1.159	1.650	2.014	0.989	0.922	1.282 ± 0.45
α ($hour^{-1}$)	1.735	5.377	4.752	8.26	4.685	1.956	4.46 ± 2.41
β ($hour^{-1}$)	0.190	0.590	0.329	0.470	0.499	0.276	0.392 ± 0.152
K_{12} ($hour^{-1}$)	0.624	2.069	2.484	4.789	1.833	0.725	2.088 ± 1.518
K_{21} ($hour^{-1}$)	0.344	2.738	0.946	1.926	2.359	0.585	1.483 ± 0.993
$t_{1/2}$ K_{10} (hour)	0.724	0.598	0.420	0.344	0.700	0.751	0.59 ± 0.171
$t_{1/2}\alpha$ (hour)	0.399	0.129	0.146	0.084	0.148	0.354	0.210 ± 0.132
$t_{1/2}\beta$ (hour)	3.65	1.17	2.11	1.48	1.390	2.514	2.05 ± 0.929
Cl_s (mL kg $hour^{-1}$)	605	1307	1625	1131	1352	1313	1222 ± 342
Vd_c (mL kg^{-1})	632	1127	985	561	1366	1424	1016 ± 362
Vd_β (mL kg^{-1})	3120	2214	4938	2407	2712	4762	3359 ± 1196
Vd_{ss} (mL kg^{-1})	1778	1979	3570	1958	2428	3188	2484 ± 736
$AUC_{0\text{-inf}}$ (ng hour mL^{-1})	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

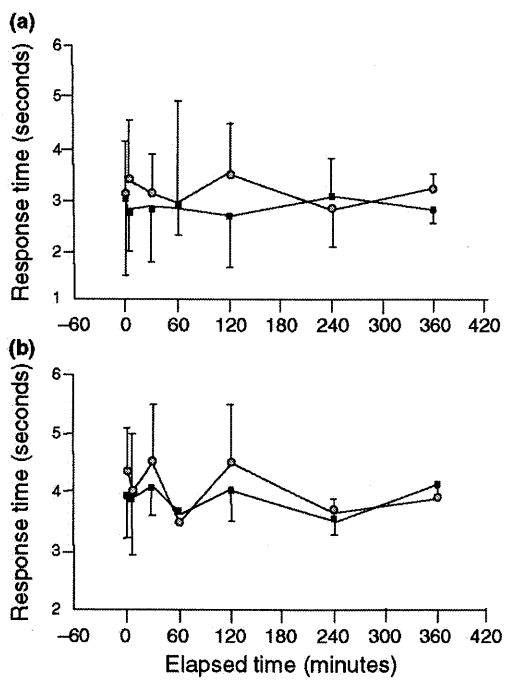

Figure 5 Effect of a single IV dose of tramadol (2 mg kg^{-1}; black squares) and vehicle (gray squares) on the skin twitch reflex latency (a) and hoof withdrawal reflex latency (b). Baseline responses were measured at time = 0 and tramadol or vehicle was administered immediately thereafter. Values are mean ± SD, n = 6.

Unfortunately, despite its lack of serious side effects, tramadol did not prolong the response to an intense thermal stimulus. The heat lamp stimulus is a proven method of testing analgesics in horses and has been used to demonstrate the antinociceptive activity of alpha-2 agonists and buprenorphine (Kamerling et al. 1988; Carregaro et al. 2007). Tramadol has antinociceptive efficacy in a thermal model of pain in mice (Raffa et al. 1992), so it is possible that the dose of tramadol we used was not high enough to blunt the response to the heat lamp. The tramadol dose of 2 mg kg^{-1} was selected for Phase II to avoid most of the undesirable effects observed during the dose–response investigation (Phase I). Even so, moderate trembling and head nodding were observed for up to 10 minutes after this dose. With regard to use of other doses in horses, administration of 5 mg kg^{-1} IV has been reported to cause tremor, confusion, agitation, and tachycardia (Giorgi et al. 2007), while 2 mg kg^{-1} administered IV over 10 minutes (Shilo et al. 2008) (compared to a bolus in the present study) caused no undesirable effects. Perhaps higher doses could be used if administered slowly.

In laboratory animal studies thermal nociception is modulated by mu receptors (Martin et al. 2003). Most models of nociception test responses that are dominated by activation of myelinated nociceptors

such as Aδ nociceptors (Yeomans & Proudfit 1996). Clinical doses of mu agonists primarily depress C fiber activity, having no effect on Aδ activity (Jurna & Heinz 1979). In the present study, it is possible that the preferential stimulation of Aδ fibers by the heat lamp was unaffected by tramadol. Intense stimulation predisposes a reaction triggered by Aδ fibers, especially when radiant heat is applied abruptly (LeBars et al. 2001).

Furthermore, in people, genetic variations in cytochrome P450 metabolism impact the efficacy of postoperative tramadol analgesia (Stamer et al. 2003). Such a horse-specific variation in cytochrome P450 metabolism may explain the lack of analgesic effect of tramadol. In most species, metabolism of tramadol results in production of *O*-desmethyl tramadol, which has greater affinity than tramadol for the mu-opioid receptor and therefore has analgesic properties. Investigations indicate that *O*-desmethyl tramadol is only a minor metabolite in horses (Giorgi et al. 2007; Shilo et al. 2008).

Following cumulative IV dosing of 3.1 mg kg^{-1} tramadol, the peak serum concentration was 619.5 (60.2) ng mL^{-1} and the elimination half-life was 114.3 (19.7) minutes, which was almost identical to that measured after the single dose of 2 mg kg^{-1}. A simple two-compartment model described tramadol disposition after IV administration (2 mg kg^{-1}) to horses. The elimination half-life of 2.1 ± 0.9 hours was longer than that published previously (1.4 hours: Shilo et al. 2008; 0.69 hours: Giorgi et al. 2007). This elimination half-life was similar to that reported in dogs (KuKanich & Papich 2004) but considerably shorter than that in humans (Murthy et al. 2000). Apparent volume of distribution in the present study was 2.48 ± 0.74 L kg^{-1}, which compared favorably to that reported previously at 2.17 ± 0.52 L kg^{-1} (Shilo et al. 2008). Clearance of tramadol in the present study was 20 ± 6 mL kg^{-1} $minute^{-1}$, similar to that reported following a 2 mg kg^{-1} dose (26 ± 3 mL kg^{-1} $minute^{-1}$; Shilo et al. 2008).

The minimum effective concentration (MEC) of tramadol in human patients determined after major orthopedic or gynecological procedures is large ranging from 20 to 2169 ng mL^{-1} (Grond & Sablotzki 2004) and some of this variation is due to differences among individuals in tramadol metabolism by cytochrome P450 (Stamer et al. 2003). Despite approaching the highest MEC values in our horses during the first assessments of analgesic effect, no antinociceptive effect was evident. The use of pre-selected horses may have impacted the results of this study. Initially, horses were evaluated for their response to the thermal stimulus, and horses were chosen to participate in the study if their baseline HWRL and STRL were less than 6 seconds. This was done to prevent tissue damage as a result of the hypothesized prolongation of the HWRL and STRL after administration of tramadol. Pre-selecting the horses may have resulted in the selection of other factors that may have influenced their responses in measured variables.

In summary, IV administration of tramadol (2 mg kg^{-1}) produces few unwanted effects in horses but unfortunately has no antinociceptive effect in a model of thermal pain. Further investigations may be warranted to determine whether it is absence of the *O*-desmethyl tramadol metabolite that limits pain control in horses even though the target serum levels of tramadol in people are being exceeded.

Acknowledgements

This study was supported by a grant from the Freeman Fund at Michigan State University College of Veterinary Medicine. We thank Dr. Thomas Tobin and Charles Hughes for providing the tramadol and heat lamp, and for performing the tramadol serum assays.

References

Boscan P, Van Hoogmoed LM, Farver TB et al. (2006) Evaluation of the effects of the opioid agonist morphine on gastrointestinal tract function in horses. Am J Vet Res 67, 992–997.

Carregaro AB, Neto FJ, Beier SL et al. (2006) Cardiopulmonary effects of buprenorphine in horses. Am J Vet Res 67, 1675–1680.

Carregaro AB, Luna SP, Mataqueiro MI et al. (2007) Effects of buprenorphine on nociception and spontaneous locomotor activity in horses. Am J Vet Res 68, 246–250.

Combie J, Shults T, Nugent EC et al. (1981) Pharmacology of narcotic analgesics in the horse: selective blockade of narcotic-induced locomotor activity. Am J Vet Res 42, 716–721.

Derksen FJ, Olszewski MA, Robinson NE et al. (1999) Aerosolized albuterol sulfate used as a bronchodilator in horses with recurrent airway obstruction. Am J Vet Res 60, 689–693.

Gibaldi M, Perrier D (1975) Pharmacokinetics. In: Pharmacokinetics. Marcel Decker, New York. p. 281.

Gibaldi M, Perrier D (1982) Pharmacokinetics 2, 2nd edn. Marcel Dekker, NY, USA. pp. 409–447.

Giorgi M, Soldani G, Maera C et al. (2007) Pharmacokinetics of tramadol and its metabolites M1,M2, and M5 in horses following intravenous, immediate release (fasted/fed) and sustained release single dose administration. J Eq Vet Sci 27, 481–487.

Grond S, Sablotzki A (2004) Clinical pharmacology of tramadol. Clin Pharmacokinet 43, 879–923.

Jurna I, Heinz G (1979) Differential effects of morphine and opioid analgesics on A and C fibre-evoked activity in ascending axons of the rat spinal cord. Brain 171, 573–576.

Kamerling SG, Cravens WM, Bagwell CA (1988) Objective assessment of detomidine-induced analgesia and sedation in the horse. Eur J Pharmacol 151, 1–8.

KuKanich B, Papich MG (2004) Pharmacokinetics of tramadol and the metabolite O-desmethyltramadol in dogs. J Vet Pharmacol Ther 27, 239–246.

LeBars D, Gozariu M, Cadden SW (2001) Animal models of nociception. Pharmacol Rev 53, 597–652.

Lewis KS, Han NH (1997) Tramadol: a new centrally acting analgesic. Am J Health Syst Pharm 54, 643–652.

Martin M, Matifas A, Maldonado R et al. (2003) Acute antinociceptive responses in single and combinatorial opioid receptor knockout mice: distinct mu, delta and kappa tones. Eur J Neurosci 17, 701–708.

Martinez M (1998a) Noncompartmental methods of drug characterization: statistical moment theory. J Am Vet Med Assoc 213, 974–980.

Martinez M (1998b) Volume, clearance, and half-life. J Eq Vet Sci 213, 1122–1127.

Mastrocinque S, Fantoni DT (2003) A comparison of preoperative tramadol and morphine for the control of early postoperative pain in canine ovariohysterectomy. Vet Anaesth Analg 30, 220–228.

Muir WW, Robertson JT (1985) Visceral analgesia: effects of xylazine, butorphanol, meperidine, and pentazocine in horses. Am J Vet Res 46, 2081–2084.

Murthy BV, Pandya KS, Booker PD et al. (2000) Pharmacokinetics of tramadol in children after i.v. or caudal epidural administration. Br J Anaesth 84, 346–349.

Natalini CC, Robinson EP (2000) Evaluation of the analgesic effects of epidurally administered morphine, alfentanil, butorphanol, tramadol, and U50488H in horses. Am J Vet Res 61, 1579–1586.

Pippi NL, Lumb WV (1979) Objective tests of analgesic drugs in ponies. Am J Vet Res 40, 1082–1086.

Raffa RB, Friderichs E, Reimann W et al. (1992) Opioid and nonopioid components independently contribute to the mechanism of action of tramadol, an 'atypical' opioid analgesic. J Pharmacol Exp Ther 260, 275–285.

Russo C, Wynne P (2001) Tramadol: metabolism and excretion in the horse. 13th International Conference of Racing Analysts and Veterinarians, Cambridge, UK, pp. 453–457.

Scott LJ, Perry CM (2000) Tramadol: a review of its use in perioperative pain. Drugs 60, 139–176.

Sellon DC, Monroe VL, Roberts MC et al. (2001) Pharmacokinetics and adverse effects of butorphanol administered by single intravenous injection or continuous intravenous infusion in horses. Am J Vet Res 62, 183–189.

Shilo Y, Britzi M, Eytan B et al. (2008) Pharmacokinetics of tramadol in horses after intravenous, intramuscular and oral administration. J Vet Pharmacol Ther 31, 60–65.

Stamer UM, Lehnen K, Hothker F et al. (2003) Impact of CYP2D6 genotype on postoperative tramadol analgesia. Pain 105, 231–238.

Tobin T, Dhanjal JK, Wilson DV et al. (2009) Tramadol in the horse: a preliminary report on its detection, pharmacokinetics, and pharmacodynamic responses. Proceedings of the 17th International Conference of Racing Analysts and Veterinarians, Antalya, Turkey.

Yeomans DC, Proudfit HK (1996) Nociceptive responses to high and low rates of noxious cutaneous heating are mediated by different nociceptors in the rat: electrophysiological evidence. Pain 68, 141–150.

Received 24 July 2008; accepted 31 December 2008.

