Pharmacokinetics of tramadol and metabolites O-desmethyltramadol and N-desmethyltramadol in adult horses

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Objective—To determine the pharmacokinetics of tramadol and its metabolites O-desmethyltramadol (ODT) and N-desmethyltramadol (NDT) in adult horses.

Animals—12 mixed-breed horses.

Procedures—Horses received tramadol IV (5 mg/kg, over 3 minutes) and orally (10 mg/kg) with a 6-day washout period in a randomized crossover design. Serum samples were collected over 48 hours. Serum tramadol, ODT, and NDT concentrations were measured via high-performance liquid chromatography and analyzed via noncompartmental analysis.

Results—Maximum mean ± SEM serum concentrations after IV administration for tramadol, ODT, and NDT were 5,027 ± 638 ng/mL, 0 ng/mL, and 73.7 ± 12.9 ng/mL, respectively. For tramadol, half-life, volume of distribution, area under the curve, and total body clearance after IV administration were 2.55 ± 0.88 hours, 4.02 ± 1.35 L/kg, 2,701 ± 275 h•ng/mL, and 30.1 ± 2.56 mL/min/kg, respectively. Maximal serum concentrations after oral administration for tramadol, ODT, and NDT were 238 ± 41.3 ng/mL, 86.8 ± 17.8 ng/mL, and 159 ± 20.4 ng/mL, respectively. After oral administration, half-life for tramadol, ODT, and NDT was 2.14 ± 0.50 hours, 1.01 ± 0.15 hours, and 2.62 ± 0.49 hours, respectively. Bioavailability of tramadol was 9.50 ± 1.28%. After oral administration, concentrations achieved minimum therapeutic ranges for humans for tramadol (> 100 ng/mL) and ODT (> 10 ng/mL) for 2.2 ± 0.46 hours and 2.04 ± 0.30 hours, respectively.

Conclusions and Clinical Relevance—Duration of analgesia after oral administration of tramadol might be < 3 hours in horses, with ODT and the parent compound contributing equally. (*Am J Vet Res* 2011;72:967–974)

Tramadol is a synthetic centrally acting analgesic drug with 2 synergistic mechanisms of action. It acts as a weak opioid receptor agonist with selectivity for the μ-receptor and a weak inhibitor of the reuptake of monoamine neurotransmitters norepinephrine and serotonin (5-hydroxytryptamine).[1] It has been used for many years to treat painful conditions in humans.[2] Tramadol is considered to have a low addictive abuse potential and, unlike opiates, is not a controlled substance in most states.[3] Tramadol has little effect on gastrointestinal motility, has no clinically relevant cardiovascular or respiratory effects, and lacks pharmacodynamic tolerance in humans.[1,4–6] In human medicine, tramadol represents a first choice for moderate to severe pain in pediatric, adult, and elderly patients, including those with poor cardiopulmonary function. The incidence of adverse effects is low.[1,6]

The use of tramadol has become common in canine medicine. It is as effective as morphine for the control

Abbreviations

$AUC_{0-\infty}$	Area under the curve
C0	Extrapolated serum drug concentration at time zero
Cl_T	Total body clearance
Cmax	Maximal observed concentration
CYP	Cytochrome P450
HPLC	High-performance liquid chromatography
MRT	Mean residence time
NDT	N-desmethyltramadol
NODT	N,O-desmethyltramadol
ODT	O-desmethyltramadol
$t_{1/2}$	Half-life
Tmax	Time to maximum plasma drug concentration

Received October 31, 2009.
Accepted April 9, 2010.
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Supported by the Grayson Jockey Club and the Auburn University College of Veterinary Medicine Research Grant funded by Animal Health & Disease Research Funds.
Presented in abstract form at the 26th American College of Veterinary Internal Medicine Conference, San Antonio, Tex, June 2008.
The authors thank Chris Schreiber, Lane Adams, Ashley MacIntosh, and Charles Smith for technical assistance.
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of early postoperative pain in dogs following ovariohysterectomy.[7] Tramadol has similar analgesic effects to epidurally administered morphine in the management of postoperative pain in humans and experimentally in horses.[7–9] Analgesic effects of tramadol are thought to be attributable to effects on opiate, adrenergic, and serotonin receptor systems.[1,10,11] The active metabolite ODT is predominantly responsible for the analgesic properties in mice and rats.[1] Compared with tramadol, ODT has 2 to 4 times the analgesic potency and 4 to 200 times the affinity for the μ-receptor.[12] The percentage of tramadol demethylated to ODT is 16% in humans and 5% in dogs.[13] The isoenzyme CYP2D6 facilitates the demethylation reaction that produces ODT.[14] It appears that ODT is only minimally produced in horses, but the percentage has not been reported.[15,16] The major metabolite produced by horses appears to be NDT, which is not considered an active metabolite in other species.[1,15]

Expense, potential adverse effects, and problems associated with the use of controlled substances often limit effective pain management in equine practice. The apparent efficacy, low incidence of adverse effects, and high therapeutic safety index make tramadol an attractive drug to investigate for future use in critically ill adult horses or those in which NSAIDs are contraindicated. The recent emergence of generic preparations has resulted in a substantial reduction of the cost of tramadol, making it a potentially cost-effective analgesic for use in horses.

There have been a limited number of studies[15,16] of tramadol in horses, and results from pharmacokinetic studies, especially reported bioavailability, are contradictory. The percentage of ODT produced by horses has not been reported.[15,16] The purpose of the study reported here was to evaluate the pharmacokinetic profile of tramadol and its metabolites ODT and NDT following IV and oral administration in horses.

Materials and Methods

Animals—All aspects of this study were approved by the Auburn University Institutional Laboratory Animal Care and Use Committee. Twelve healthy university-owned horses (6 mares and 6 geldings) were studied. Mean ± SD age was 7.4 ± 3.5 years (range, 2 to 19 years) and body weight was 493 ± 67 kg (range, 405 to 598 kg). Breeds represented included Quarter Horse (n = 7), Thoroughbred (3), Saddlebred (1), and Tennessee Walking Horse (1). All horses were deemed to be healthy on the basis of history, physical examination findings, and results of CBC, fibrinogen concentration determination, and serum biochemical profile. No horse had received any medication within the previous month. The day prior to the study, horses were moved from small paddocks to box stalls. Coastal Bermuda grass hay and water were offered ad libitum.

Tramadol—Tramadol was administered IV and orally in a randomized crossover design. Half the horses initially received tramadol orally at a dose of 10 mg/kg, and half were given tramadol IV at a dose of 4.4 mg/kg. After a 6-day washout period, each group received tramadol via the other route (IV or PO). Each horse was administered tramadol HCl[a] orally. The tramadol dose for oral administration was rounded to the nearest half of a 50-mg tablet. Tramadol tablets were dissolved in 400 mL of water and administered via nasogastric intubation. The tramadol solution for IV injection was prepared the evening prior to administration as a 10 mg/mL preparation of tramadol powder[b] diluted in sterile water. Injectable tramadol was passed through a 22-μM filter into a sterile vial, then stored in the refrigerator and protected from light until IV injection. One 1.0-mL aliquot was frozen immediately after reconstitution, and a second 1.0-mL aliquot was frozen immediately prior to injection for strength measurement. Results of stability studies[13] indicate that injection solution that is stored in refrigeration and protected from light retains > 95% of its potency for up to 92 days after reconstitution. For the present study, tramadol was used within 1 day after reconstitution. Tramadol was injected over a 3-minute period into the temporary jugular catheter and flushed with 10 mL of saline (0.9% NaCl) solution prior to catheter removal.

Blood sampling—On the morning of tramadol administration, 2 mL of 2% lidocaine was administered SC for local anesthesia and an IV catheter[c] was aseptically placed in 1 jugular vein for collection of blood samples. On the days of IV tramadol administration, a second IV catheter[d] was aseptically placed in the opposite jugular vein for administration of tramadol IV. This catheter was removed immediately after the tramadol had been administered IV.

Blood samples were collected from the jugular catheter into plain evacuated serum clot tubes[e] prior to tramadol administration and at 5, 10, 20, 30, 45, 60, and 90 minutes and 2, 3, 4, 6, 8, 12, 18, 24, and 48 hours after IV or oral administration of tramadol. Blood samples were also collected 3 minutes after IV administration of tramadol. Blood samples were allowed to clot for 30 minutes at room temperature (21°C) and then were centrifuged; serum was harvested and frozen at –20°C until analysis of serum tramadol and metabolite concentrations. Prior to collection of each blood sample, the catheter was flushed with 5 mL of saline solution, then 5 mL of blood was withdrawn and discarded. After sample collection, the catheter was flushed with 5 mL of saline solution containing 10 U of heparin/mL. All samples were frozen at –20°C until analysis.

Sample measurements—Serum tramadol, ODT, and NDT concentrations were measured from frozen samples at the Auburn University Clinical Pharmacology Laboratory by use of reverse-phase HPLC with fluorescence detection on the basis of published methods with minor modifications.[13,17,18] The HPLC system consisted of a controller,[f] autosampler,[g] and fluorescence detector.[h] At the time of analysis, serum samples were thawed and vortexed. Serum samples were extracted by use of solid-phase extraction cartridges.[i] Cartridges were conditioned with 1 mL of methanol followed by 1 mL of water, after which 200 μL of the serum sample was added; then the cartridge was washed with 1 mL of 0.1N hydrochloric acid followed by 1.0 mL of methanol. The analyte was eluted with a mixture of methanol and ammonium hydroxide (95:5). The eluent was dried under nitrogen evaporation and reconstituted with 200 μL

of mobile phase. The mobile phase was a mixture of 0.01M potassium phosphate buffer with 0.1% triethylamine and acetonitrile (90:10 vol/vol) adjusted to a pH of 4.0 with phosphoric acid. The flow rate was 1.5 mL/min. The HPLC was performed by use of a column (10 µm; 300 × 3.9-mm internal diameter)[j] preceded by a guard column (125A; 10 µm; 20 × 3.9-mm internal diameter).[k] The column was heated to 40°C. Drug signal was detected by use of a fluorescence detector with excitation at 275 nm and emission at 300 nm. Unknown concentrations were calculated by comparing signal with standard concentrations made with known amounts of tramadol and each metabolite.[b,l]

The limit of quantification for tramadol, ODT, and NDT was 15 ng/mL, and the limit of detection was 5 ng/mL. The linear regression analysis for tramadol was made by plotting the peak area (y-axis) versus analyte concentration (x-axis). In the concentration range from 50 to 4,000 ng/mL, r^2 was 0.9997, and from 5 to 100 ng/mL, r^2 was 0.9988. For ODT, in the concentration range from 20 to 1,000 ng/mL, r^2 was 0.9997, and from 5 to 100 ng/mL, r^2 was 0.9995. For NDT, in the concentration range from 20 to 1,000 ng/mL, r^2 was 0.9940, and from 5 to 100 ng/mL, r^2 was 0.9984.

For tramadol concentrations from 50 to 1,000 ng/mL, the HPLC assay accuracy deviated from the measured concentration from –0.83% to 0.52%, with a precision of 1.84% to 11.43%. For ODT concentrations from 20 to 400 ng/mL, there was a deviation from the measured concentration of ODT from –6.96% to –0.51%, with a precision of 1.55% to 11.02%. For NDT concentrations from 20 to 400 ng/mL, there was a deviation from the measured concentration of NDT from –10.01% to 1.12%, with a precision of 2.65% to 11.75%.

Concentration verification—The aliquot of tramadol solution (10 mg/mL) prepared for each individual horse the evening prior to administration was analyzed to confirm concentration of the administered solution from aliquots frozen after reconstitution. All methods were the same with the exception of the standard curve, which was determined in saline solution.

Pharmacokinetic and statistical analysis—Pharmacokinetic variables for tramadol, ODT, and NDT following oral and IV administration of tramadol were calculated via noncompartmental analysis by use of a commercial computer software program[m] and reported as mean ± SEM values. Parameters determined included Cl_T, volume of distribution at steady state, volume of distribution of the area, $t_{1/2}$, MRT, C0, Cmax, Tmax, and oral bioavailability. For oral and IV administration of tramadol, $AUC_{0-\infty}$ was calculated to infinity (by use of the log-linear trapezoidal rule). Systemic bioavailability of tramadol (absolute bioavailability) or percentage metabolite $AUC_{0-\infty}$ to tramadol $AUC_{0-\infty}$ (relative bioavailability)[19] was calculated from noncompartmental parameters by use of the following equation:

$$F = (AUC_{0-\infty(oral)} \times dose_{IV})/(AUC_{0-\infty(IV)} \times dose_{oral})$$

where F is oral bioavailability, $AUC_{0-\infty(oral)}$ is the $AUC_{0-\infty}$ after oral administration, $dose_{IV}$ is the dose administered IV, $AUC_{0-\infty(IV)}$ is the $AUC_{0-\infty}$ after IV administration, and $dose_{oral}$ is the dose administered orally.

Results

No adverse effects were observed after oral administration of tramadol. Mild to moderate muscle fasciculations were observed after IV administration of tramadol over 3 minutes in 7 of 12 horses. Fasciculations resolved completely within 14 to 68 minutes after administration. During IV administration, 1 horse became agitated and raised its head, prompting discontinuation of the IV administration. This horse received 94% of its calculated dose, with the administered dose used in the pharmacokinetic analysis.

The calculated IV dose received by each horse, after taking into account concentrations of the aliquots prepared and frozen for each individual horse, as measured by use of HPLC, was 4.47 ± 0.47 mg/kg base (equivalent to 5.1 ± 0.53 mg/kg). The oral dose of 10.0 ± 0.05 mg/kg was equivalent to 8.7 ± 0.05 mg/kg base. Individual doses were used in the pharmacokinetic analy-

Table 1—Pharmacokinetic parameters (mean ± SEM [95% confidence interval]) of tramadol after IV (5 mg/kg) and oral (10 mg/kg) administration of tramadol HCl to 12 horses.

Parameter	Tramadol (IV)	Tramadol (PO)*
λ_z (1/h)	0.45 ± 0.066 (0.32–0.58)	0.47 ± 0.07 (0.33–0.61)
$t_{1/2}$ (h)	2.55 ± 0.88 (0.82–4.27)	2.14 ± 0.50 (1.16–3.12)
MRT (h)	2.81 ± 1.28 (0.30–5.32)	3.34 ± 0.68 (2.01–4.67)
Cl_T (mL/min/kg)	30.1 ± 2.56 (25.1–35.1)	NA
$V_{d(ss)}$ (L/kg)	4.02 ± 1.35 (1.37–6.67)	NA
$V_{d(area)}$ (L/kg)	5.63 ± 1.36 (2.96–8.30)	NA
$AUC_{0-\infty}$ (h•ng/mL)	2,701 ± 275 (2,162–3,240)	503.7 ± 70.5 (365–642)
$AUMC_{0-\infty}$ (h•h•ng/mL)	10,305 ± 6,181 (0–22,420)	94,839 ± 18,636 (58,313–131,366)
C0 (ng/mL)	5,027 ± 638 (3,776–6,277)	NA
Cmax (ng/mL)	NA	238 ± 41.3 (157–319)
Tmax (h)	NA	0.91 ± 0.29 (0.34–1.48)
Oral bioavailability (%)	NA	9.50 ± 1.28 (7.0–12.0)

*Data for 11 horses were analyzed because data from 1 horse could not be analyzed by use of noncompartmental analysis. Values were computed after removal of data points associated with a second peak in 5 horses that occurred between 30 and 48 hours.

λ_z = Slope (elimination rate constant). NA = Not applicable. $V_{d(area)}$ = Volume of distribution of the area. $V_{d(ss)}$ = Volume of distribution at steady state.

Table 2—Pharmacokinetic parameters of NDT and ODT after IV (5 mg/kg) and oral (10 mg/kg) administration of tramadol to 12 horses.

Parameter	ODT after oral administration		NDT after IV administration		NDT after oral administration	
	Mean ± SEM (95% confidence interval)	No. of horses	Mean ± SEM (95% confidence interval)	No. of horses	Mean ± SEM (95% confidence interval)	No. of horses
λ_z (1/h)	0.81 ± 0.12 (0.57–1.04)	8	0.3 ± 0.04 (0.22–0.38)	11	0.35 ± 0.05 (0.25–0.45)	12
$t_{1/2}$ (h)	1.01 ± 0.15 (0.72–1.3)	8	2.73 ± 0.32 (2.1–3.36)	11	2.62 ± 0.49 (1.7–3.6)	12
MRT (h)	1.90 ± 0.36 (1.2–2.6)	8	4.08 ± 0.47 (3.16–5.0)	11	4.19 ± 0.66 (2.9–5.5)	12
$AUC_{0-\infty}$ (h•ng/mL)	169 ± 45.4 (80.0–258)	8	270 ± 25.6 (220–320)	11	650 ± 113 (428–871)	12
$AUMC_{0-\infty}$ (h•h•ng/mL)	23,149 ± 11,024 (1,542–44,756)	8	65,485 ± 9,542 (46,783–84,187)	11	189,439 ± 54,557 (82,507–296,371)	12
Cmax (ng/mL)	86.8 ± 17.8 (51.9–122)	12	73.7 ± 12.9 (48.4–99.0)	11	159 ± 20.4 (119–199)	12
Tmax (h)	0.69 ± 0.11 (0.47–0.91)	12	0.71 ± 0.12 (0.48–0.94)	11	1 ± 0.15 (0.71–1.3)	12
$AUC_{0-\infty}$ of tramadol (%)	41.6 ± 9.72 (22.5–60.6)	8	10.3 ± 1.62 (7.13–13.5)	11	139.9 ± 3.32 (133–146)	11

Values computed after removal of data points associated with a second peak in 5 horses that occurred between 30 and 48 hours.

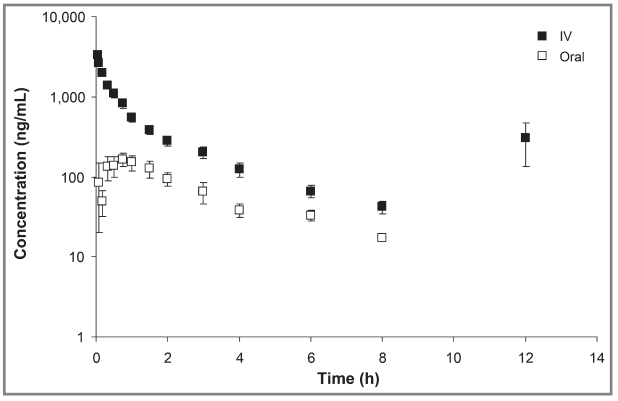

Figure 1—Semilogarithmic plot of mean ± SEM serum concentrations of tramadol after administration of tramadol HCl (5 mg/kg, IV, and 10 mg/kg, PO) to 12 horses. A second peak (not shown) was observed in 3 horses after oral administration and 3 horses after IV administration between 30 and 48 hours.

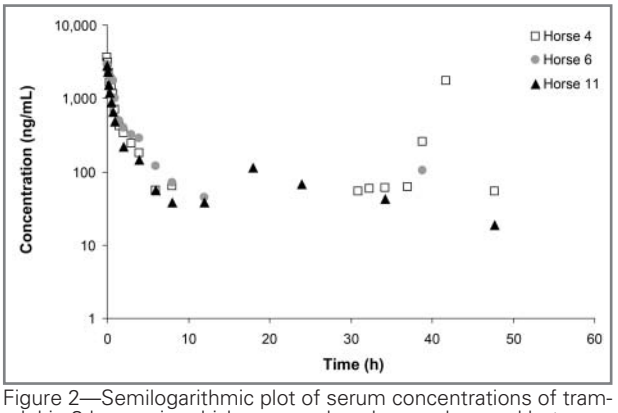

Figure 2—Semilogarithmic plot of serum concentrations of tramadol in 3 horses in which a second peak was observed between 30 and 48 hours after IV administration of tramadol HCl (5 mg/kg).

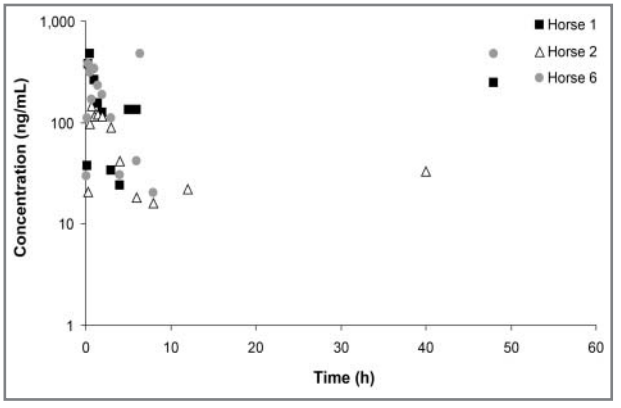

Figure 3—Semilogarithmic plot of serum concentrations of tramadol in 3 horses in which a second peak was observed between 40 and 48 hours after oral administration of tramadol HCl (10 mg/kg).

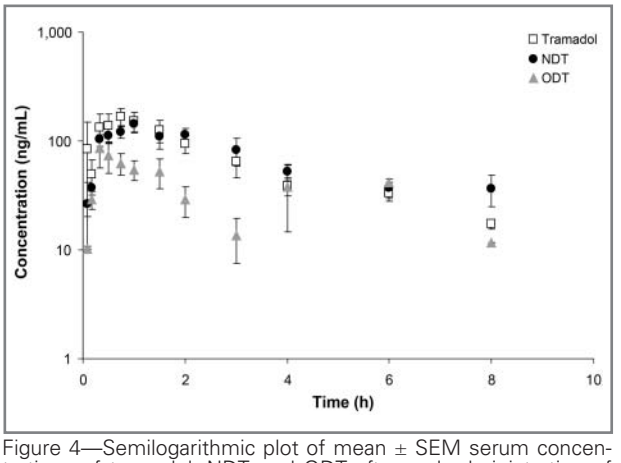

Figure 4—Semilogarithmic plot of mean ± SEM serum concentrations of tramadol, NDT, and ODT after oral administration of tramadol HCl (10 mg/kg) to 12 horses. A second peak (not shown) was observed in the tramadol concentration in 3 horses between 40 and 48 hours.

sis for each horse. For some horses, concentrations of tramadol (especially after oral administration) or metabolites were either nondetectable or nonquantifiable at multiple time points. Without a sufficient number of valid time points, pharmacokinetic analysis (including noncompartmental analysis) was not possible. The number of horses for which each pharmacokinetic parameter was derived was summarized (**Tables 1 and 2**).

After IV administration, serum tramadol concentrations were less than the limit of detection by 12 hours in 11 of 12 horses (**Figure 1**); in 1 horse, detectable serum concentrations persisted for 48 hours (**Figure 2**). A second peak in tramadol concentrations occurred between 30 to 48 hours in 2 other horses. After IV administration, serum tramadol concentrations exceeded the lowest therapeutic concentration reported for humans (100 ng/mL)[20] for 4.2 ± 0.33 hours (range, 3 to 6.25 hours). After oral administration, serum tramadol concentrations exceeded the lowest therapeutic concentration in 10 of 12 horses for 2.2 ± 0.46 hours

(range, 0.17 to 3.4 hours); in 2 horses, serum tramadol concentrations persisted longer because of a second peak (**Figure 3**).

The serum concentration versus time profiles of tramadol, ODT, and NDT after oral administration (**Figure 4**) and tramadol and NDT after IV administration were determined (**Figure 5**). After oral administration, serum ODT concentrations exceeded the lowest therapeutic concentration reported for humans (10 ng/mL)[21,22] in 11 of 12 horses for 2.04 ± 0.30 hours (range, 0.67 to 7.5 hours). Serum concentrations of ODT were not detected after IV administration of tramadol in any horse.

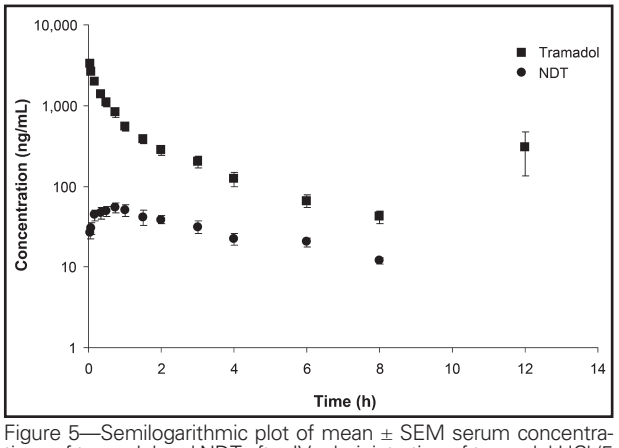

Figure 5—Semilogarithmic plot of mean ± SEM serum concentrations of tramadol and NDT after IV administration of tramadol HCl (5 mg/kg) to 12 horses. A second peak (not shown) was observed in the tramadol concentration in 3 horses between 30 and 48 hours.

The data points associated with the second peaks in 3 horses after oral administration of tramadol and 2 horses after IV administration (Figures 2 and 3) were insufficient to be modeled with noncompartmental analysis. Thus, time points from the second curve could not be included in the pharmacokinetic analysis. The pharmacokinetic parameters (mean ± SEM) for tramadol after IV and oral administration (Table 1) and for NDT after IV administration and ODT and NDT after oral administration (Table 2) were determined. After normalizing for dose, the Cmax for NDT was 16.8 ± 2.93 ng/mL after IV administration and 15.9 ± 2.04 ng/mL after oral administration. Likewise, the $AUC_{0-\infty}$ for tramadol and NDT was 613.9 ± 62.5 h•ng/mL and 14.9 ± 2.92 h•ng/mL, respectively, after IV administration and 50.4 ± 7.1 h•ng/mL and 18.9 ± 5.6 h•ng/mL, respectively, after oral administration. The ratio of $AUC_{0-\infty}$ for NDT to that for tramadol was 0.10 ± 0.02 after IV administration and 1.4 ± 0.03 after oral administration. The ratio of $AUC_{0-\infty}$ for ODT to that for tramadol after oral tramadol administration was 0.41 ± 0.01. The relative bioavailability of NDT after oral administration was 92.4 ± 10.9% (ie, the ratio of $AUC_{0-\infty}$ for oral vs IV administration was 0.9 ± 0.01). A comparison of pharmacokinetic parameters among studies in humans,[1] dogs,[13] and horses[15,16] was made (**Tables 3 and 4**).

Table 3—Comparison of pharmacokinetic parameters as reported in humans,[1] dogs,[13] and horses,[15,16] after IV administration of tramadol.

		Other studies			
Parameter	**Present study (5 mg/kg)**	**Human[1] (50 mg)**	**Canine[13] (3.9 mg/kg)**	**Equine[16] (2 mg/kg)**	**Equine[15] (5 mg/kg)**
$t_{1/2}$ (h)	2.55 ± 0.88	5.5	0.80 ± 0.12	1.37 ± 0.17	0.69 ± 0.10
MRT (h)	2.81 ± 1.28	—	0.93 ± 0.12	1.38 ± 0.17	—
Cl_T (mL/min/kg)	30.1 ± 2.56	34.8*	54.6 ± 8.19	26 ± 3	1.16 ± 0.10
$Vd_{(ss)}$ (L/kg)	4.02 ± 1.35	262†	3.01 ± 0.45	2.17 ± 0.52	1.42 ± 30.08
$AUC_{0-\infty}$ (h.ng/mL)	2,701 ± 275	1,556	1,203 ± 181	1,313 ± 196	4,470 ± 910
$AUC_{0-\infty}$/dose (h/mL)	613.9 ± 62.5	2,161	273.4 ± 41	656.5 ± 98	894 ± 182
C0 (ng/mL)	5,027 ± 638	347.4	1,707 ± 399	—	3,590 ± 200
C0/dose (mg/mL)	1,142 ± 145	482.5	388 ± 90.7	—	718 ± 40.0

*Total body clearance measured in liters per hour. †Volume of distribution at steady state measured in units of liters.
— = Not determined.

Table 4—Comparison of pharmacokinetic parameters as reported in humans,[1] dogs,[13] and horses,[15,16] after oral administration of tramadol.

		Other studies				
Parameter	**Present study (10 mg/kg)**	**Human[1] (100 mg)**	**Canine[13] (11.2 mg/kg)**	**Equine[16] (2 mg/kg)**	**Equine[15] (5 mg/kg)**	**Equine[15] (5 mg/kg)**
Time food was withheld (h)	0	—	12	12	10	0
$t_{1/2}$ (h)	2.14 ± 0.50	5.64	1.71 ± 0.12	—	1.54 ± 30.23	1.92 ± 0.27
MRT (h)	3.34 ± 0.68	—	3.00 ± 0.44	—	—	—
$AUC_{0-\infty}$ (h•ng/mL)	503.7 ± 70.5	2,649	3,866 ± 2,218	43 ± 21	2,890 ± 350	3,780 ± 820
$AUC_{0-\infty}$ dose (h/mL)	50.3 ± 7.05	—	345 ± 198	21.5 ± 10.5	578 ± 70	756 ± 164
Cmax (ng/mL)	238 ± 41.3	308	1,402 ± 695	33 ± 19	1,770 ± 220	3,610 ± 500
Cmax/dose (mg/mL)	23.8 ± 4.13	—	125.2 ± 62.0	16.8 ± 9.5	354 ± 44	722 ± 100
Tmax (h)	0.91 ± 0.29	1.6	1.04 ± 0.51	0.83 ± 0.3	0.42 ± 0.08	0.33 ± 0.11
Oral bioavailability (%)	9.50 ± 1.28	68	65 ± 35	3 ± 2	64.5 ± 8.36	84.6 ± 18.35

See Table 3 for key.

Discussion

The results reported here for IV and oral tramadol administration provide pharmacokinetic information for the design of further studies of pharmacodynamics, analgesic efficacy, and assessment of safety after repeated administration in adult horses. The pharmacokinetic information reported here details the contribution of ODT, the active metabolite associated with analgesia in humans, and NDT, the major metabolite produced by horses. This study builds on 2 previous reports[15,16] of pharmacokinetics of tramadol and metabolites in horses and is the first to describe pharmacokinetic parameters for ODT in horses, to the authors' knowledge.

The authors chose 100 ng/mL of tramadol as the minimum effective concentration. This choice was based on studies[21,22] in humans that revealed marked variability in therapeutic concentrations. For tramadol, the lowest and highest effective concentrations in humans are reported as 298 ± 171 ng/mL and 590 ± 410 ng/mL, respectively.[21,22] For ODT, the lowest and highest concentrations reported in humans are 39.6 ± 29.5 ng/mL and 84 ± 34 ng/mL, respectively.[21,22] Although there is a large variation in therapeutic concentrations reported in the literature, the therapeutic range suggested in human clinical medicine is 100 to 300 ng/mL for tramadol and > 10 ng/mL for ODT.[20–23] In dogs, the lowest therapeutic concentration of tramadol used for pharmacokinetic and pharmacodynamic integration is 100 ng/mL.[13] Accordingly, we chose 100 ng/mL for tramadol as the minimum effective concentration in horses. However, pharmacodynamic response studies are warranted in horses for tramadol and its active metabolites.

Differences exist in the disposition of tramadol among species so a comparison of pharmacokinetic parameters among studies in humans,[1] dogs,[13] and horses[15,16] was made (Table 3). Horses had elimination $t_{1/2}$ after oral tramadol administration that is intermediate between that observed in humans and dogs.[1,13] However, 2 other studies[15,16] in horses found a slightly shorter $t_{1/2}$, compared with that found in the present study. The Tmax in the horses in the present study was similar to that of dogs from which food was withheld and slightly longer than that in previous studies of fed horses and horses from which food was withheld.[13,15,16] The Tmax was longer in humans than in horses and dogs.[1,13,15,16] The Cmax and $AUC_{0-\infty}$ were much lower in the horses in the present study, compared with those of dogs orally administered a similar dose of tramadol. The Cmax and $AUC_{0-\infty}$ (divided by the administered dose) in 2 previous studies[15,16] in horses were markedly different, with one study[15] having values 20 to 25 times those of the other study[16] and the present study.

Bioavailability in horses in the present study (9.50 ± 1.28%) was much lower than in humans or dogs.[1,13] In 2 previous studies, reported bioavailability of tramadol in horses was dissimilar, with oral bioavailability of 3 ± 2% when 2 mg/kg was administered to 7 horses[16] and 84.6 ± 18.35% when 5 mg/kg was administered to 6 horses.[15]

Five of the horses of the present study had a second peak in serum tramadol concentrations that occurred 30 to 48 hours after IV administration and 40 to 48 hours after oral administration. After oral administration, this second peak was high in 2 horses and was equal to the Cmax in 1 horse. After IV tramadol administration, the second peak in 2 of the horses was 3 and 17 times that of the lowest therapeutic concentration reported for humans (100 ng/mL).[20]

In 4 of 12 horses, a second peak identified as tramadol prolonged the duration of time that serum tramadol concentration exceeded the minimum therapeutic range, after a drug-free period. Appearance of this secondary peak in a subset of horses may warrant further investigation with collection of a larger number of samples from 30 to 48 hours. In previous pharmacokinetic studies[15,16] of tramadol in horses, serum samples were only collected for shorter durations after lower doses (for 24 hours in 1 study after a dose of 5 mg/kg, PO, and for 48 hours after a dose of 2 mg/kg, PO, in a second study), with neither study reporting a second peak in serum tramadol concentrations. The identification of second peaks in the present study could be attributable to the larger dose (2 to 5 times that of the previous studies), which allowed for a longer period of detection. Alternatively, it is possible that tramadol secreted from the blood into the gastrointestinal lumen underwent enteric recycling in 5 of 12 horses. Further, reformation of tramadol by enterocytes from metabolized compounds could have contributed to the second tramadol peak. Enterohepatic recycling might also play a role, as might trapping of the tramadol in food and subsequent release with digestion. The presence of various absorption windows along the gastrointestinal tract has also been reported as an explanation of secondary peaks.[24-25] Rectal administration of tramadol is also reported to be as effective as oral administration in humans.[26] Finally, tramadol has many metabolites beyond those measured in the present study. It is possible that another metabolite coeluted with tramadol and was falsely identified as tramadol in the later peaks. This could possibly be resolved with the use of a more specific assay method, such as liquid chromatography–mass spectrometry. Analysis of urine concentrations of tramadol and metabolites may also help determine the elimination and recycling of tramadol and metabolites in horses. Studies of repeated administration of tramadol in horses may be warranted to determine whether therapeutic concentrations can be maintained throughout a 24-hour period in horses that have a second peak in serum concentrations.

After IV administration of tramadol, the C0 and $AUC_{0-\infty}$ were much higher in the horses of the present study than in dogs administered 3.9 mg of tramadol/kg, IV.[13] In the study[13] in dogs, the first jugular blood sample was collected after 10 minutes; however, samples were collected at 3 and 5 minutes in the present study. The earlier sampling times and the slightly higher dose may account for the higher C0 concentrations calculated in the present study. The $AUC_{0-\infty}$, after normalizing for dose, was higher in 2 other studies[15,16] performed in horses than in the present study. After IV administration, the clearance in the horses reported here was similar to that reported in a study[16] in horses but 26 times those in a second study in horses;[15] in dogs, values were 1.8 times those in the horses reported here.[13]

Because of the muscle fasciculations that were observed after IV administration over a 3-minute period in many of the horses in the present study, IV administration over a much longer time period should be recommended. Clinical signs such as confusion, agitation, tremor, and tachycardia of variable intensity were reported in another study[15] in which all 6 horses were administered 5 mg of tramadol/kg IV as a bolus, with adverse effects beginning within 3 to 5 minutes after administration and maximum effects noticed between 15 and 20 minutes after administration and all effects resolving by the end of the second hour. The adverse clinical signs that we observed had all dissipated within 14 to 68 minutes and varied from mild to moderate fasciculations in most horses to severe agitation in 1 horse that occurred 2.7 minutes into the 3-minute IV infusion of tramadol. Muscle fasciculations were also observed in 2 horses administered tramadol at 2 mg/kg IV over 5 to 6 minutes, but fasciculations did not occur when tramadol was administered over a 10-minute period to the 5 remaining horses in that study.[16]

After administration of 5 mg of tramadol/kg IV in our study, serum tramadol concentrations exceeded 100 ng/mL, which is the lowest therapeutic concentration reported for humans,[20] for 3 to 6 hours. Similar results were observed in the 2 other studies[15,16] in horses and 1 study[13] in dogs. It was interesting that no ODT was detectable in the serum of the horses in the present study after IV administration. The markedly higher percentage of tramadol converted to NDT after oral administration, compared with the percentage after IV administration, indicated that the rate of tramadol metabolism was higher than the rate of tramadol absorption. After normalizing for dose, the $AUC_{0-\infty}$ for tramadol was approximately 12 times higher after IV administration, compared with oral administration, which was reflected in the poor bioavailability. However, the Cmax and $AUC_{0-\infty}$ values for NDT after oral and IV administration were similar. This suggested that there may be enteric as well as hepatic conversion of tramadol to NDT with oral administration. There is also a possibility that tramadol was metabolized into ODT by the enterocytes, thus accounting for the presence of ODT after oral administration and lack of detectable ODT after IV administration. Therefore, although the absolute bioavailability of tramadol is low in horses, when it is absorbed, the amount of ODT formed from tramadol appears to be important, which is supported by the high ratio of $AUC_{0-\infty}$ ODT to tramadol after oral administration. Interestingly, horses in this study did not form ODT from tramadol after IV administration, perhaps suggesting a role of gastrointestinal metabolism in the formation of ODT after oral but not IV administration.

Our findings were similar to those of other investigators. After administration of 5 mg of tramadol/kg via IV, oral (in fed horses and horses from which food was withheld), and sustained release routes to horses, NDT was the main metabolite produced and ODT and NODT were only marginally produced.[15] The concentrations of ODT and NODT were assessed by those authors as similar, low, and variable, with their Cmax approximately half that for NDT (10 ng/mL), which also approached the limit of quantification of the HPLC assay.[15] A second investigation, based on sensitive liquid chromatography–mass spectrometry, reported ODT concentrations of 0 to 11 ng/mL after administration of 2 mg of tramadol/kg by either IV, IM, oral, or sustained release routes to horses. No other pharmacokinetic data were reported.[16] Pharmacokinetic parameters have not previously been reported for ODT in horses after tramadol administration. The present study used a much higher oral dose of tramadol than those used in other studies in horses, and ODT was rapidly formed in horses with a short Tmax and $t_{1/2}$, low $AUC_{0-\infty}$ and Cmax, and rapid clearance. It has been suggested that a rate of conversion of tramadol to ODT slower than the rate of elimination of ODT (ie, a flip-flop effect) may occur in horses and dogs.[15] However, on the basis of our data, the disappearance rate constant of NDT (ODT was not detectable after IV administration) was similar after either oral or IV administration. As such, we do not have evidence of a flip-flop rate of metabolite formation.

In humans, ODT is a major metabolite, but in dogs, ODT is considered a minor metabolite with only 5% conversion from tramadol. Regardless of this low conversion rate of tramadol to ODT in dogs, tramadol is still an effective analgesic in dogs because of high bioavailablity.[13] Studies on the analgesic efficacy of tramadol need to be performed in horses.

Tramadol undergoes extensive first-pass metabolism in the liver, with approximately 10% to 30% of an orally administered dose excreted unchanged in healthy human volunteers.[1] Tramadol and its metabolites are primarily excreted via the kidneys (90%).[1] In humans, the major metabolites are ODT and NDT, with smaller amounts of N-N-didesmethyltramadol, N,N,O-tridesmethyltramadol, and NODT.[27] The O-demethylation of tramadol to ODT is catalyzed by CYP2D6, and N-demethylation to NDT is catalyzed by CYP3A4 and CYP2B6.[5] Animals may have different expression of CYP enzymes, compared with humans. In a study[28] of hepatic CYP expression in horses, the amount of CYP3D6 was low, compared with that of CYP2B. Genetic polymorphism of CYP2D6 exists in humans and can partly explain the variation in analgesic potential of tramadol in humans.[14] The degree of genetic polymorphism for CYP enzymes has not been studied in horses.

The relative analgesic activities of tramadol and its metabolites have not been investigated in animals. Despite the low concentrations of ODT found in the present study and in other studies[15,16] in horses, the analgesic efficacy of the parent compound tramadol and its individual metabolites requires further investigation in horses. Tramadol has excellent analgesic activity when administered epidurally to horses.[8] Epidural administration does not allow for metabolism to various metabolites; therefore, the analgesic effects can be attributed solely to tramadol.[8] Thus, although the amount of ODT produced by horses is low, the potential use of tramadol as an analgesic in horses should not be ruled out.

In humans, peak analgesic effect occurs 1 to 4 hours after drug administration, with analgesia persisting for 3 to 6 hours.[1] Tramadol is unlikely to be as effective an analgesic in horses as in humans because of the low bioavailability found in 2 studies[15,16] in horses and low final concentrations of ODT produced. In the present

study, therapeutic concentrations (considered so on the basis of pharmacodynamic studies in humans[20]) could be maintained for approximately 2 to 2.5 hours after oral administration and for 4.2 hours after IV administration. Similar to dogs, frequent administration would therefore be required in horses. Because of the low bioavailability of tramadol in dogs, simulated oral administration regimens at 5 mg/kg every 6 hours or 2.5 mg/kg every 4 hours were predicted to result in tramadol and ODT concentrations consistent with analgesia in humans. In humans, the usual dosage is 50 to 100 mg, PO, every 4 to 6 hours as required.[1] Continuous rate infusion of 10 mg of tramadol/h is also efficacious.[1] Intravenous administration of tramadol to horses should ideally be performed as a constant rate infusion or at least over a period > 10 minutes to decrease the risk of muscle fasciculations. The safety of repeated oral and IV administration of tramadol in horses needs to be evaluated in addition to pharmacodynamic studies before the use of tramadol can be routinely recommended in equine practice.

a. Ultram (tramadol hydrochloride), 50-mg tablets, Ortho-McNeil Pharmaceutical, Titusville, NJ.
b. Tramadol hydrochloride powder, Spectrum Chemical Manufacturing Corp, Gardena, Calif.
c. 14-gauge, 5.25-inch PEP polymer, Angiocath, Becton Dickinson Infusion Therapy Systems Inc, Sandy, Utah.
d. 18-gauge, 3-inch PEP polymer, Angiocath, Becton Dickinson Infusion Therapy Systems Inc, Sandy, Utah.
e. No anticoagulant Vacutainer, 10-mL, 1.5-inch glass tube, Beckton, Dickinson & Co, Franklin Lakes, NJ.
f. Waters 600 controller, Waters Corp, Milford, Mass.
g. Waters 717 autosampler, Waters Corp, Milford, Mass.
h. Waters 474 fluorescence detector, Waters Corp, Milford, Mass.
i. Strata X-C 33-µm polymeric strong cation, 30 mg/mL SPE cartridges, Phenomenex, Torrance, Calif.
j. Bondaclone C18 column, Phenomenex, Torrance, Calif.
k. u Bondapak C18 guard column, Phenomenex, Torrance, Calif.
l. (-)-O-Desmethyltramadol and (-)-N-Desmethyltramadol, Toronto Research Chemicals Inc, North York, ON, Canada.
m. WinNonlin, version 4.0, Pharsight Corp, Mountain View, Calif.

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