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

Allison J. Stewart, BVSc, MS; Dawn M. Boothe, DVM, PhD; Crisanta Cruz-Espindola, BS; Emily J. Mitchum, DVM, MS; Jenny Springfield, DVM

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 of 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)

Abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;0–∞&lt;/sub&gt;</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>C&lt;sub&gt;0&lt;/sub&gt;</td>
<td>Extrapolated serum drug concentration at time zero</td>
</tr>
<tr>
<td>C&lt;sub&gt;L&lt;/sub&gt;</td>
<td>Total body clearance</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximal observed concentration</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>MRT</td>
<td>Mean residence time</td>
</tr>
<tr>
<td>NDT</td>
<td>N-desmethyltramadol</td>
</tr>
<tr>
<td>NODT</td>
<td>N,O-desmethyltramadol</td>
</tr>
<tr>
<td>ODT</td>
<td>O-desmethyltramadol</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt;</td>
<td>Half-life</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Time to maximum plasma drug concentration</td>
</tr>
</tbody>
</table>

Received October 31, 2009.
Accepted April 9, 2010.
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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).<sup>1</sup> It has been used for many years to treat painful conditions in humans.<sup>2</sup> Tramadol is considered to have a low addictive abuse potential and, unlike opiates, is not a controlled substance in most states.<sup>3</sup> Tramadol has little effect on gastrointestinal motility, has no clinically relevant cardiovascular or respiratory effects, and lacks pharmacodynamic tolerance in humans.<sup>4,5</sup> 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.<sup>1,6</sup>

The use of tramadol has become common in canine medicine. It is as effective as morphine for the control...
of early postoperative pain in dogs following ovario-
hysterectomy.7–9 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 de-
methylation reaction that produces ODT.14 It appears
that ODT is only minimally produced in horses, but the
percentage has not been reported.13,16 The major metab-
olite produced by horses appears to be NDT, which is
not considered an active metabolite in other species.

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 attrac-
tive drug to investigate for future use in critically ill
adult horses or those in which NSAIDs are contrain-
dicated. The recent emergence of generic preparations
has resulted in a substantial reduction of the cost of tra-
madol, making it a potentially cost-effective analgesic
for use in horses.

There have been a limited number of studies13,16 of
tramadol in horses, and results from pharmacokinetic
studies, especially reported bioavailability, are contra-
dictory. The percentage of ODT produced by horses has
not been reported.13,16 The purpose of the study report-
ed 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 Ani-
mal Care and Use Committee. Twelve healthy univer-
sity-owned horses (6 mares and 6 geldings) were stud-
ied. 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 Ten-
nessee Walking Horse (1). All horses were deemed to
be healthy on the basis of history, physical examina-
tion findings, and results of CBC, fibrinogen concentra-
tion 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
grasn hay and water were offered ad libitum.

Tramadol—Tramadol was administered IV and
orally in a randomized crossover design. Half the hors-
es 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 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 dissolved in
sterile water. Injectable tramadol was passed through a
22-µM filter into a sterile vial, then stored in the refig-
erator and protected from light until IV injection. One
1.0-mL aliquot was frozen immediately after recon-
stitution, and a second 1.0-mL aliquot was frozen im-
immediately prior to injection for strength measurement.
Results of stability studies15 indicate that injection solu-
tion that is stored in refrigeration and protected from
light retains > 95% of its potency for up to 92 days af-
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 ad-
ministration, 2 mL of 2% lidocaine was administered
SC for local anesthesia and an IV catheter was asepti-
ically placed in 1 jugular vein for collection of blood
samples. On the days of IV tramadol administration, a
second IV catheter was aseptically placed in the oppo-
site 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 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 adminis-
tration 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 sam-
ple, the catheter was flushed with 5 mL of saline solu-
tion, 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 Pharmacol-
ogy Laboratory by use of reverse-phase HPLC with flu-
orescence detection on the basis of published methods
with minor modifications.13,17,18 The HPLC system con-
sisted of a controller,1 autosampler,6 and fluorescence
detector.9 At the time of analysis, serum samples were
thawed and vortexed. Serum samples were extracted
by use of solid-phase extraction cartridges.1 Cartriges
were conditioned with 1 mL of methanol followed by
1 mL of water, after which 200 µL of the serum sam-
ple was added; then the cartrige 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 un-
der 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 X 3.9-mm internal diameter) preceded by a guard column (125A; 10 µm; 20 X 3.9-mm internal diameter). 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.

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 0.9984. 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 0.9994, 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. Parameters determined included Cl, 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\textsubscript{0–\textinfty} was calculated to infinity (by use of the log-linear trapezoidal rule). Systemic bioavailability of tramadol (absolute bioavailability) or percentage metabolite AUC\textsubscript{0–\textinfty} to tramadol AUC\textsubscript{0–\textinfty} (relative bioavailability) \( \text{IV} \) was calculated from noncompartmental parameters by use of the following equation:

\[
F = \frac{(\text{AUC}_{0–\text{\textinfty}})(\text{oral}) \times \text{dose}_{\text{IV}}) / (\text{AUC}_{0–\text{\textinfty}})(\text{IV}) \times \text{dose}_{\text{oral}} \]

where F is oral bioavailability, AUC\textsubscript{0–\text{\textinfty}}(oral) is the AUC\textsubscript{0–\text{\textinfty}} after oral administration, \( \text{dose}_{\text{IV}} \) is the dose administered IV, AUC\textsubscript{0–\text{\textinfty}}(IV) is the AUC\textsubscript{0–\text{\textinfty}} after IV administration, and \( \text{dose}_{\text{oral}} \) 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 ±4.7 ± 0.05 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 analysis.

### 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Tramadol (IV)</th>
<th>Tramadol (PO)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \lambda_1 ) (h\textsuperscript{-1})</td>
<td>0.45 ± 0.066 (0.32–0.58)</td>
<td>0.47 ± 0.07 (0.33–0.61)</td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>2.55 ± 0.85 (0.82–4.27)</td>
<td>2.14 ± 0.50 (1.16–3.12)</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>2.81 ± 1.28 (0.30–5.32)</td>
<td>3.34 ± 0.68 (2.01–4.67)</td>
</tr>
<tr>
<td>C\textsubscript{0} (mM/h)</td>
<td>30.1 ± 2.56 (25.1–35.1)</td>
<td>NA</td>
</tr>
<tr>
<td>V\textsubscript{ss} (L/kg)</td>
<td>4.02 ± 1.38 (1.37–6.87)</td>
<td>NA</td>
</tr>
<tr>
<td>V\textsubscript{area} (L/kg)</td>
<td>5.63 ± 1.36 (2.96–5.30)</td>
<td>NA</td>
</tr>
<tr>
<td>AUC\textsubscript{0–\textinfty} (ng/mL)</td>
<td>2,701 ± 70.5 (2,162–3,240)</td>
<td>503.7 ± 50 (365–642)</td>
</tr>
<tr>
<td>AUMC\textsubscript{0–\textinfty} (ng/h)</td>
<td>10,305 ± 41.3 (8,276–12,240)</td>
<td>94,839 ± 8,707 (58,313–131,366)</td>
</tr>
<tr>
<td>C\textsubscript{0} (ng/mL)</td>
<td>5,027 ± 368 (3,776–6,277)</td>
<td>NA</td>
</tr>
<tr>
<td>C\textsubscript{max} (ng/mL)</td>
<td>NA</td>
<td>238 ± 41.3 (157–319)</td>
</tr>
<tr>
<td>T\textsubscript{max} (h)</td>
<td>NA</td>
<td>0.91 ± 0.29 (0.34–1.48)</td>
</tr>
<tr>
<td>Oral bioavailability (%)</td>
<td>NA</td>
<td>9.50 ± 1.28 (7.0–12.0)</td>
</tr>
</tbody>
</table>

*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.

\( \lambda_1 \) = Slope (elimination rate constant), NA = Not applicable, V\textsubscript{area} = Volume of distribution of the area, V\textsubscript{ss} = Volume of distribution at steady state.
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 oral 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) 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.

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 AUCCLUS 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 AUCCLUS 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 AUCCLUS 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 AUCCLUS for oral vs IV administration was 0.9 ± 0.01). A comparison of pharmacokinetic parameters among studies in humans, dogs, and horses was made (Tables 3 and 4).

![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.](image)

Table 3—Comparison of pharmacokinetic parameters as reported in humans, dogs, and horses after IV administration of tramadol.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Present study (5 mg/kg)</th>
<th>Human1 (50 mg)</th>
<th>Canine13 (3.9 mg/kg)</th>
<th>Equine15 (2 mg/kg)</th>
<th>Equine16 (5 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t1/2 (h)</td>
<td>2.55 ± 0.88</td>
<td>5.5</td>
<td>0.90 ± 0.12</td>
<td>1.37 ± 0.17</td>
<td>0.69 ± 0.10</td>
</tr>
<tr>
<td>Cl (mL/min/kg)</td>
<td>3.81 ± 1.38</td>
<td>—</td>
<td>0.93 ± 0.12</td>
<td>1.38 ± 0.17</td>
<td>—</td>
</tr>
<tr>
<td>Vdss (L/kg)</td>
<td>4.02 ± 1.35</td>
<td>267</td>
<td>1.01 ± 0.45</td>
<td>2.17 ± 0.52</td>
<td>1.42 ± 0.30</td>
</tr>
<tr>
<td>AUCclus (h·ng/mL)</td>
<td>2.701 ± 275</td>
<td>1,556</td>
<td>1,203 ± 181</td>
<td>1,313 ± 196</td>
<td>4,470 ± 910</td>
</tr>
<tr>
<td>AUCmax (h·ng/mL)</td>
<td>613.9 ± 62.5</td>
<td>2,161</td>
<td>273.4 ± 41</td>
<td>656.5 ± 98</td>
<td>894 ± 182</td>
</tr>
<tr>
<td>C0 (ng/mL)</td>
<td>5,027 ± 638</td>
<td>347.4</td>
<td>1,707 ± 399</td>
<td>—</td>
<td>3,590 ± 200</td>
</tr>
<tr>
<td>C0/dose (mg/mL)</td>
<td>1,142 ± 145</td>
<td>482.5</td>
<td>388 ± 90.7</td>
<td>—</td>
<td>718 ± 40.0</td>
</tr>
</tbody>
</table>

* 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, dogs, and horses after oral administration of tramadol.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Present study (10 mg/kg)</th>
<th>Human1 (100 mg)</th>
<th>Canine13 (11.2 mg/kg)</th>
<th>Equine15 (2 mg/kg)</th>
<th>Equine16 (5 mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time food was withheld (h)</td>
<td>0</td>
<td>—</td>
<td>12</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>2.14 ± 0.50</td>
<td>5.64</td>
<td>1.71 ± 0.12</td>
<td>—</td>
<td>1.54 ± 0.23</td>
</tr>
<tr>
<td>Cl (mL/min/kg)</td>
<td>3.34 ± 0.68</td>
<td>—</td>
<td>3.00 ± 0.44</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>AUCclus (h·ng/mL)</td>
<td>503.7 ± 70.5</td>
<td>2,649</td>
<td>3,866 ± 2,218</td>
<td>43 ± 21</td>
<td>2,890 ± 350</td>
</tr>
<tr>
<td>AUCmax (h·ng/mL)</td>
<td>503.7 ± 70.5</td>
<td>—</td>
<td>345 ± 198</td>
<td>21.5 ± 10.5</td>
<td>578 ± 70</td>
</tr>
<tr>
<td>Cmax (ng/mL)</td>
<td>23.8 ± 4.13</td>
<td>308</td>
<td>1,462 ± 895</td>
<td>33 ± 13</td>
<td>1,770 ± 220</td>
</tr>
<tr>
<td>Cmax/dose (mg/mL)</td>
<td>23.8 ± 4.13</td>
<td>125.2 ± 62.0</td>
<td>16.8 ± 9.5</td>
<td>354 ± 44</td>
<td>722 ± 100</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.91 ± 0.29</td>
<td>1.6</td>
<td>1.04 ± 0.51</td>
<td>0.83 ± 0.3</td>
<td>0.42 ± 0.08</td>
</tr>
<tr>
<td>Oral bioavailability (%)</td>
<td>9.50 ± 1.28</td>
<td>68</td>
<td>65 ± 35</td>
<td>3 ± 2</td>
<td>64.5 ± 0.36</td>
</tr>
</tbody>
</table>

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 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 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. For ODT, the lowest and highest concentrations reported in humans are 39.6 ± 29.5 ng/mL and 84 ± 34 ng/mL, respectively. 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. In dogs, the lowest therapeutic concentration of tramadol used for pharmacokinetic and pharmacodynamic integration is 100 ng/mL. 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, dogs, and horses is made (Table 3). Horses had elimination t1/2 after oral tramadol administration that is intermediate between that observed in humans and dogs. However, 2 other studies in horses found a slightly longer t1/2, 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. The Tmax was longer in humans than in horses and dogs. The Cmax and AUC∞ 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∞ (divided by the administered dose) in 2 previous studies in horses were markedly different, with one study having values 20 to 25 times those of the other study and the present study.

Bioavailability in horses in the present study (9.50 ± 1.28%) was much lower than in humans or dogs. 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 and 84.6 ± 18.3% when 5 mg/kg was administered to 6 horses. 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).

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 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.

Rectal administration of tramadol is also reported to be as effective as oral administration in humans. 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∞ were much higher in the horses of the present study than in dogs administered 3.9 mg of tramadol/kg, IV. In the study 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 AUC0–∞ after normalizing for dose, was higher in 2 other studies 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 in horses but 26 times those in a second study in horses. In dogs, values were 1.8 times those in the horses reported here.
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 study13 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 studies15,16 in horses and 1 study17 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∞_∞ 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∞_∞ 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∞_∞ 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 t1/2, low AUC∞∞ 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.17 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 bioavailability.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-di demethyltramadol, 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 CYP2B6.3 Animals may have different expression of CYP enzymes, compared with humans. In a study28 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, 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.9 Epidural administration does not allow for metabolism to various metabolites; therefore, the analgesic effects can be attributed solely to tramadol.9 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 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) 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. Continuous rate infusion of 10 mg of tramadol/h is also efficacious. 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.

References