Pharmacokinetics and antinociceptive effects of oral tramadol hydrochloride administration in Greyhounds

Butch KuKanich, DVM, PhD, and Mark G. Papich, DVM, MS

Objective—To determine the pharmacokinetics of tramadol, the active metabolite O-desmethyltramadol, and the metabolites N-desmethyltramadol and N,O-didesmethyltramadol after oral tramadol administration and to determine the antinociceptive effects of the drug in Greyhounds.

Animals—6 healthy 2- to 3-year-old Greyhounds (3 male and 3 female), weighing 25.5 to 41.1 kg.

Procedures—A mean dose of 9.9 mg of tramadol HCl/kg was administered PO as whole tablets. Blood samples were obtained prior to and at various points after administration to measure plasma concentrations of tramadol and its metabolites via liquid chromatography with mass spectrometry. Antinociceptive effects were determined by measurement of pain-pressure thresholds with a von Frey device.

Results—Tramadol was well tolerated, and a significant increase in pain-pressure thresholds was evident 5 and 6 hours after administration. The mean maximum plasma concentrations of tramadol, O-desmethyltramadol, N-desmethyltramadol, and N,O-didesmethyltramadol were 215.7, 5.7, 379.1, and 237.2 ng/mL, respectively. The mean area-under-the-curve values for the compounds were 592, 16, 1,536, and 1,013 h•ng/mL, respectively. The terminal half-lives of the compounds were 1.1, 1.4, 2.3, and 3.6 hours, respectively. Tramadol was detected in urine 5 days, but not 7 days, after administration.

Conclusions and Clinical Relevance—Oral tramadol administration yielded antinociceptive effects in Greyhounds, but plasma concentrations of tramadol and O-desmethyltramadol were lower than expected. Compared with the approved dose (100 mg, PO) in humans, a mean dose of 9.9 mg/kg, PO resulted in similar tramadol but lower O-desmethyltramadol plasma concentrations in Greyhounds. (*Am J Vet Res* 2011;72:256–262)

Tramadol is a central-acting analgesic approved for use in humans for the treatment of moderate to moderately severe pain. The drug has low abuse potential and minimal cardiorespiratory and gastrointestinal effects.[1–3] With a complex mechanism of action, tramadol exists as chiral isomers, each of which may have different activity. Tramadol's activity also has been attributed to a metabolite of tramadol, O-desmethyltramadol, known as M1. There are many other metabolites (as many as 20), but pharmacological effects have only been confirmed with 1, O-desmethyltramadol, after routine tramadol administration. Tramadol acts as a

Abbreviations

AUC	Area under the plasma concentration curve from 0 to infinity
$AUC_{extrapolated}$	Percentage of the area under the plasma concentration curve extrapolated to infinity
AUMC	Area under the first-moment curve from 0 to infinity
C_{MAX}	Maximum plasma concentration
Cl/F	Clearance per fraction of the dose absorbed
CYP	Cytochrome P450
HPLC	High-performance liquid chromatography
λz	Terminal rate constant
LC-MS	Liquid chromatography–mass spectrometry
LLOQ	Lower limit of quantification
MRT	Mean residence time from 0 to infinity
m/z	Mass-to-charge ratio
PPT	Pain-pressure threshold
SPE	Solid-phase extraction
T½λz	Terminal half-life
T_{MAX}	Time to maximum plasma concentration
Vz/F	Volume of distribution (area method) per fraction of the dose absorbed

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From the Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506 (KuKanich); and the Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606 (Papich).
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Address correspondence to Dr. KuKanich (kukanich@ksu.edu).

low-affinity opiate μ-receptor agonist and a serotonin and norepinephrine reuptake inhibitor, whereas O-desmethyltramadol acts as a high-affinity opiate μ-receptor agonist (> 200 times as potent as tramadol) and also as a serotonin and norepinephrine reuptake inhibitor.[1,4–9] O-desmethyltramadol reportedly also binds to M1 muscarinic receptors, which may produce pharmacological effects.[10]

In humans, tramadol is transformed to the active metabolite O-desmethyltramadol by CYP2D6, which exists in various phenotypes. After 100 mg of tramadol is administered PO in humans, CYP ultrametabolizers produce the highest concentrations of O-desmethyltramadol (C_{MAX}, approx 132 ng/mL), extensive metabolizers produce an intermediate amount of O-desmethyltramadol (C_{MAX}, approx 106 ng/mL), and poor metabolizers produce the lowest amount of O-desmethyltramadol (C_{MAX}, approx 18 ng/mL).[11]

The specific CYP associated with tramadol metabolism to O-desmethyltramadol has not been identified in dogs; however, the pharmacokinetics of these compounds have been evaluated in dogs after various routes of administration.[12–16] Only 3 studies have been conducted to evaluate the pharmacokinetics of oral tramadol administration in dogs; 2 involved an immediate-release product,[12,16] and the third evaluated a sustained-release product.[15] In the first study,[12] a mean dose of 11.2 mg of tramadol HCl/kg was administered to 6 Beagles (3 male and 3 female) in immediate-release tablets and the plasma concentrations of tramadol and O-desmethyltramadol were determined via HPLC with fluorescence detection. The second study[16] involved similar detection methods to determine plasma drug and metabolite concentrations after oral administration of immediate-release tramadol HCl in dogs after administration of a 4 mg/kg dose to 8 male Beagles. Although tramadol concentrations in each of these studies were proportionately similar, there were distinct differences in the metabolite concentrations reported. In the study by Giorgi et al,[16] O-desmethyltramadol concentrations were much (approx 10 times) lower than in the study[12] by the present authors and O-desmethyltramadol was considered a minor metabolite. In addition, the other research group reported that another metabolite, N-desmethyltramadol (also referred to as M2), was a major metabolite that exceeded concentrations of tramadol and other metabolites. The presence of N-desmethyltramadol was not measured in our previous study[12] because a reference standard was not available.

The aforementioned studies[12,16] differed in design and analysis techniques (chromatographic conditions for HPLC were not the same), dog characteristics (both sexes vs all males), and potential for interdog variability. Large intersubject variability existed in both. The primary purpose of the study reported here was to evaluate the pharmacokinetics and antinociceptive effects of tramadol administered PO to healthy Greyhounds at a targeted dose of 10 mg of tramadol HCl/kg by use of a sensitive and specific LC-MS method. The secondary purpose of the study was to determine the period during which urine concentrations of tramadol were detectable after PO administration.

Materials and Methods

Animals—Six healthy Greyhounds (3 neutered males and 3 sexually intact females) weighing 25.5 to 41.1 kg and aged 2 to 3 years were included. Food was withheld from dogs 12 hours before the study began. The Institutional Animal Care and Use Committee at Kansas State University approved the study protocol.

Experimental protocol—Tramadol HCl[a] was administered to each dog at a targeted dose of 10 mg/kg to the nearest whole tablet. Whole blood samples (9 mL each) were collected via an aseptically placed jugular catheter[b] prior to drug administration and 10, 20, 30, and 45 minutes and 1, 2, 3, 4, 5, 6, 8, and 12 hours after drug administration. Samples were immediately placed into tubes containing lithium heparin, mixed, and placed on ice until plasma separation. Plasma was separated after centrifugation (20 minutes at 3,000 × *g*) and stored frozen at −70°C until analyzed. Urine was collected by means of midstream catch via natural voiding or surface collection at targeted times of 12, 24, 48, 72, 120, and 168 hours after drug administration.

Pain-pressure thresholds were measured by use of a modified von Frey device[c] in accordance with methods described elsewhere.[17,18] The von Frey device included a 0.5-mm solid plastic tip, and the pressure was quantified on an electronic load cell calibrated from 100 to 1,000 g. Measurements of PPT were obtained prior to drug administration (time 0; baseline) and 1, 2, 3, 4, 5, 6, 8, and 12 hours after drug administration. Each measurement point consisted of 3 measurements/forefoot (left and right) for a total of 6 measurements/dog/h. The PPTs were converted to percentage change from the time 0 mean measurement for each forefoot for each dog.

Plasma and urine drug analysis—Plasma and urine concentrations of tramadol[d] (m/z, 264.1→58.0; 264.1 qualifying ion to 58.0 product ion), O-desmethyltramadol[e] (m/z, 250.1→58.0), N-desmethyltramadol[e] (m/z, 250.1→44.1), and N,O-didesmethyltramadol[f] (m/z, 236.2→44.1) were determined by use of LC-MS.[g,h] Ketamine[e] (m/z, 238.1→124.9) was the internal standard for O-desmethyltramadol and N,O-didesmethyltramadol, and *cis*-C13-tramadol-d3[e] (m/z, 268.1→58) was the internal standard for tramadol and N-desmethyltramadol. The standard curves were linear from 1 to 1,000 ng/mL for tramadol and O-desmethyltramadol, and those for N-desmethyltramadol and N,O-didesmethyltramadol were linear from 5 to 1,000 ng/mL. The accuracy and coefficient of variation of the analytic method for tramadol were 100 ± 5% of the actual concentration and 5%, respectively. The accuracy and coefficient of variation of the analytic method for O-desmethyltramadol were 99 ± 11% of the actual concentration and 12%, respectively. The accuracy and coefficient of variation of the analytic method for N-desmethyltramadol were 98 ± 10% of the actual concentration and 11%, respectively. The accuracy and coefficient of variation of the analytic method for N,O-didesmethyltramadol were 100 ± 6% of the actual concentration and 5%, respectively.

Drug was extracted from plasma samples with SPE cartridges. Briefly, 0.5 mL of plasma was added to 0.1 mL of internal standard (500 ng of ketamine/mL and 500 ng of *cis*-C13-tramadol-d3/mL), followed by 0.5 mL of 0.1M borate buffer. The SPE cartridges[i] were conditioned with 1 mL of methanol followed by 1 mL of deionized water, the plasma mixture was added, the SPE cartridges were rinsed with 1 mL of 5% methanol, and the drug was eluted with 1 mL of methanol. The eluate was evaporated to dryness at 40°C under a stream of air followed by reconstitution with 0.2 mL of 50% methanol. The reconstituted sample was centrifuged for 10 minutes at 10,000 × *g* to sediment particulate, and the supernatant was transferred to an injection vial with 25 µL injected. Drug was extracted from urine in a similar manner, except 1 mL of urine and 1 mL of 0.1M borate buffer were used. Drug from extracted urine was reconstituted with 0.2 mL of 15% methanol, and 50 µL was the injection volume. The LLOQ of tramadol in urine was 5 ng/mL.

Separation was achieved with a C18 column[j] maintained at 40°C. The mobile phase consisted of acetonitrile (A) and 10mM ammonium formate (pH, 5.0; B) with a flow rate of 0.4 mL/min. The mobile phase gradient started at 100% B from 0 to 0.5 minutes, was changed to a linear gradient to 70% B from 0.5 to 2 minutes, was held at 70% B until 3.5 minutes, and then was changed to a linear gradient to 100% B at 4.5 minutes, for a total run duration of 6 minutes.

Pharmacokinetic analysis—Pharmacokinetic analyses were performed with computer software.[k] The calculated noncompartmental pharmacokinetic parameters included AUC calculated with the linear trapezoidal method, $AUC_{extrapolated}$, AUMC, Cl/F, T½λz, λz, MRT, and Vz/F. The C_{MAX} and T_{MAX} were determined directly from the plasma concentrations.

Statistical analysis—Statistical analysis of the PPTs was performed with computer software.[l] Because the data were not normally distributed, the Mann-Whitney rank sum test was used to compare PPTs at time 0 with PPTs at later times.[17,18] Values of $P < 0.05$ were considered significant.

Results

Animals—Tramadol appeared to be well tolerated after oral administration to the 6 healthy Greyhounds. All dogs remained alert and responsive throughout the study. No vomiting, diarrhea, or agitation was observed in any of the dogs during the study.

Pharmacokinetics—The mean ± SD actual dose of tramadol HCl administered was 9.9 ± 0.4 mg/kg, which was administered as whole tablets. Tramadol, O-desmethyltramadol, N-desmethyltramadol, and N,O-didesmethyltramadol were rapidly eliminated after tramadol administration with geometric mean T½λzs of 1.1, 1.4, 2.3, and 3.6 hours (**Figures 1–5; Tables 1–4**). The mean (range) C_{MAX} values for tramadol, O-desmethyltramadol, N-desmethyltramadol, and N,O-didesmethyltramadol were 215.7 (85.4 to 454.0), 5.7 (2.8 to 13.8), 379.1 (161.0 to 659.0), and 237.2 (142.0 to 306.0) ng/mL, respectively, but large intersubject variability was present for each analyte

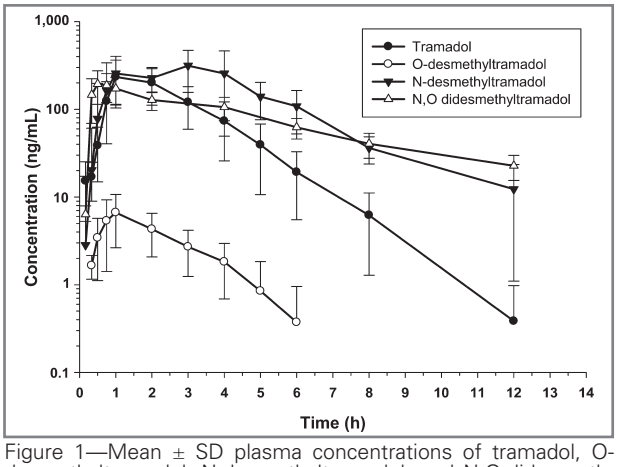

Figure 1—Mean ± SD plasma concentrations of tramadol, O-desmethyltramadol, N-desmethyltramadol, and N,O-didesmethyltramadol in 6 healthy Greyhounds after a mean ± SD dose of 9.9 ± 0.4 mg of tramadol HCl/kg was administered PO. For the determination of the mean and SD, values less than the LLOQ of the assay were entered as 0.

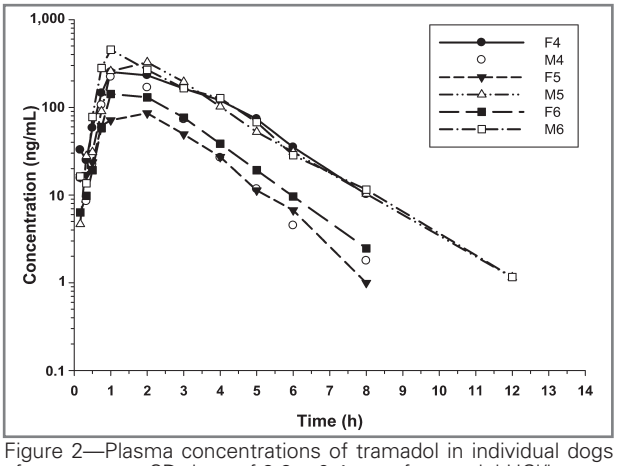

Figure 2—Plasma concentrations of tramadol in individual dogs after a mean ± SD dose of 9.9 ± 0.4 mg of tramadol HCl/kg was administered PO to 6 healthy Greyhounds. F = Female dog. M = Neutered male dog. The number following the F or M designation represents the unique animal number.

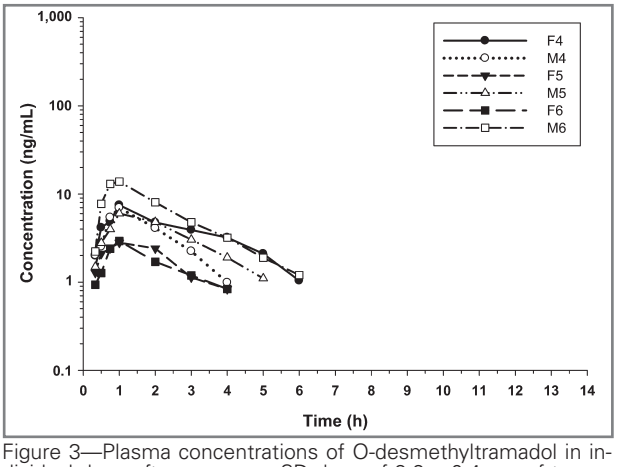

Figure 3—Plasma concentrations of O-desmethyltramadol in individual dogs after a mean ± SD dose of 9.9 ± 0.4 mg of tramadol HCl/kg was administered PO to 6 healthy Greyhounds. *See* Figure 2 for key.

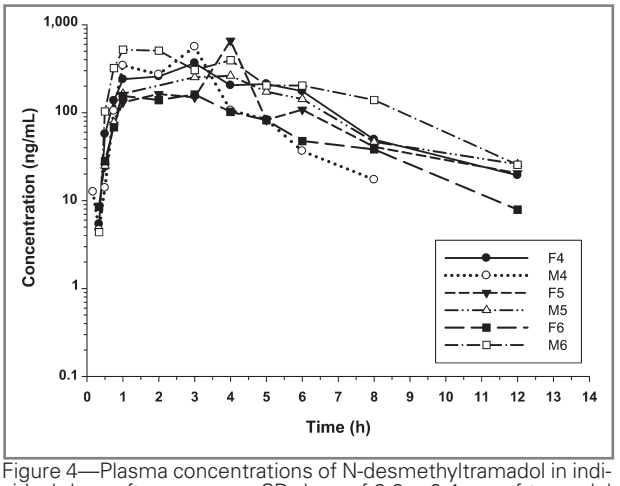

Figure 4—Plasma concentrations of N-desmethyltramadol in individual dogs after a mean ± SD dose of 9.9 ± 0.4 mg of tramadol HCl/kg was administered PO to 6 healthy Greyhounds. *See* Figure 2 for key.

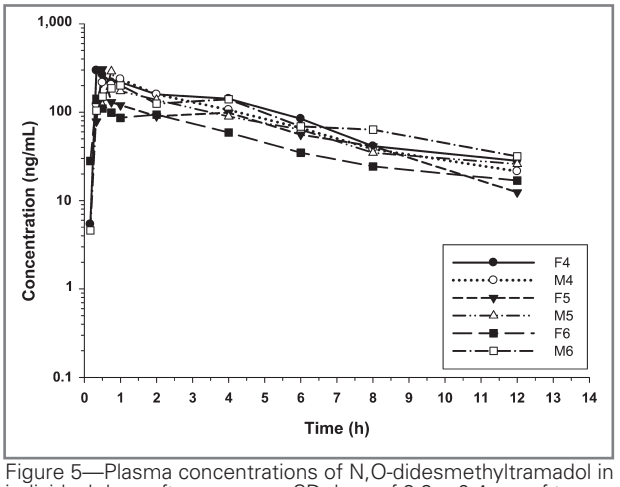

Figure 5—Plasma concentrations of N,O-didesmethyltramadol in individual dogs after a mean ± SD dose of 9.9 ± 0.4 mg of tramadol HCl/kg was administered PO to 6 healthy Greyhounds. See Figure 2 for key.

Table 1—Values of tramadol pharmacokinetic parameters for 6 healthy Greyhounds after a mean ± SD dose of 9.9 ± 0.4 mg of tramadol HCl/kg was administered PO.

Parameter	Geometric mean	Minimum	Median	Maximum
$AUC_{extrapolated}$ (%)	0.52	0.19	0.54	1.83
AUC (h•ng/mL)	592	256	675	1,083
AUMC (h•h•ng/mL)	1,498	624	1766	2,809
Cl/F (mL/min/kg)	245.0	143.9	234.4	543.7
C_{MAX} (ng/mL)	215.7	85.4	237.5	454.0
T½λz (h)	1.1	0.9	1.1	1.3
λz (1/h)	0.640	0.553	0.631	0.820
MRT (h)	2.5	2.1	2.5	2.9
T_{MAX} (h)	1.3	1.0	1.0	2.0
Vz/F (L/kg)	23.0	15.4	22.8	39.8

as indicated by the large range in values. The mean T_{MAX} values for tramadol, O-desmethyltramadol, N-desmethyltramadol, and N,O-didesmethyltramadol were 1.3, 1.0, 2.8, and 0.6 hours, respectively. The mean AUCs for tramadol, O-desmethyltramadol, N-desmethyltramadol, and N,O-didesmethyltramadol were 592, 16, 1,536, and 1,013 h•ng/mL, respectively, and were also highly variable.

Table 2—Values of O-desmethyltramadol pharmacokinetic parameters for 6 healthy Greyhounds after a mean ± SD dose of 9.9 ± 0.4 mg of tramadol HCl/kg was administered PO.

Parameter	Geometric mean	Minimum	Median	Maximum
$AUC_{extrapolated}$ (%)	12.5	7.2	10.9	25.3
AUC (h•ng/mL)	16	8	17	35
AUMC (h•h•ng/mL)	42	23	40	89
Cl/F (mL/min/kg)	9,057.9	4,448.6	8,718.7	17,314.6
C_{MAX} (ng/mL)	5.7	2.8	6.5	13.8
T½λz (h)	1.4	1.0	1.4	1.8
λz (1/h)	0.497	0.396	0.483	0.684
MRT (h)	2.6	2.0	2.7	3.0
T_{MAX} (h)	1.0	1.0	1.0	1.0
Vz/F (L/kg)	1,094.0	558.6	894.5	2,623.9

Table 3—Values of N-desmethyltramadol pharmacokinetic parameters for 6 healthy Greyhounds after a mean ± SD dose of 9.9 ± 0.4 mg of tramadol HCl/kg was administered PO.

Parameter	Geometric mean	Minimum	Median	Maximum
$AUC_{extrapolated}$ (%)	3.8	2.7	3.2	6.6
AUC (h•ng/mL)	1,536	832	1,560	2,684
AUMC (h•h•ng/mL)	6,659	3,588	7,880	11,827
Cl/F (mL/min/kg)	94.4	58.1	91.0	173.7
C_{MAX} (ng/mL)	379.1	161.0	442.5	659.0
T½λz (h)	2.3	1.5	2.2	3.2
λz (1/h)	0.309	0.214	0.320	0.459
MRT (h)	4.3	3.0	4.5	5.2
T_{MAX} (h)	2.8	1.0	3.0	4.0
Vz/F (L/kg)	18.4	10.3	19.0	31.8

Table 4—Values of N,O-didesmethyltramadol pharmacokinetic parameters for 6 healthy Greyhounds after a mean ± SD dose of 9.9 ± 0.4 mg of tramadol HCl/kg was administered PO.

Parameter	Geometric mean	Minimum	Median	Maximum
$AUC_{extrapolated}$ (%)	11.0	5.9	12.3	15.6
AUC (h•ng/mL)	1,013	661	1,082	1,312
AUMC (h•h•ng/mL)	5,569	3,927	5,721	8,433
Cl/F (mL/min/kg)	143.1	109.4	132.7	218.6
C_{MAX} (ng/mL)	237.2	142.0	263.5	306.0
T½λz (h)	3.6	2.7	3.7	4.4
λz (1/h)	0.195	0.159	0.189	0.255
MRT (h)	5.5	4.7	5.5	6.6
T_{MAX} (h)	0.6	0.3	0.6	1.0
Vz/F (L/kg)	44.1	33.3	42.5	75.6

Significant ($P < 0.05$) increases in PPTs were evident 5 and 6 hours after tramadol administration (**Figure 6**) but not at other measurement points. The PPTs at 8 and 12 hours were similar to baseline (time 0) measurements. A hysteresis plot suggested that a counterclockwise hysteresis loop existed after tramadol administration (**Figure 7**), assuming the O-desmethyltramadol concentrations were too low to elicit an antinociceptive effect.

Tramadol was present in the urine at concentrations exceeding 5 ng/mL in 1 of 1 dog at 3 hours, 1 of 1 dog at 8 hours, 2 of 2 dogs at 12 hours, 6 of 6 dogs at 24 hours, 3 of 5 dogs at 48 hours, 1 of 6 dogs at 72 hours,

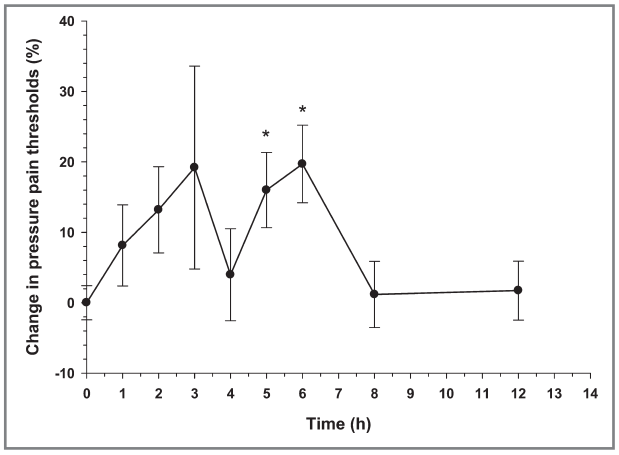

Figure 6—Mean ± SEM von Frey PPT values in 6 healthy Greyhounds after a mean ± SD dose of 9.9 ± 0.4 mg of tramadol HCl/kg was administered PO. *Indicated value differs significantly ($P < 0.05$) from time 0 (baseline) values.

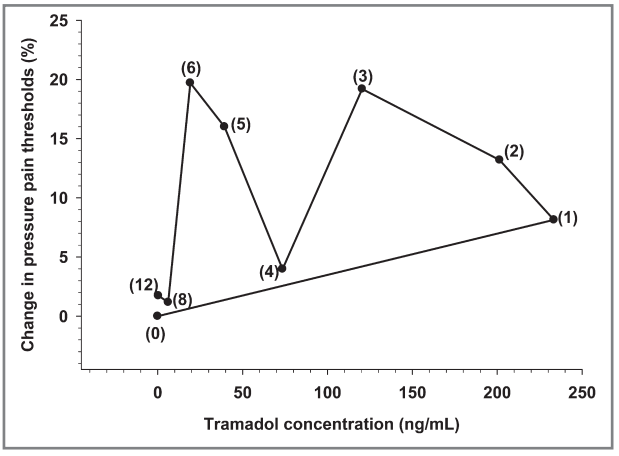

Figure 7—Hysteresis plot of mean tramadol concentration versus mean von Frey PPT value for 6 healthy Greyhounds after a mean ± SD dose of 9.9 ± 0.4 mg of tramadol HCl/kg was administered PO. The time after tramadol administration (in hours) that each measurement was obtained is included in parentheses on the plot.

2 of 6 dogs at 120 hours, and 0 of 6 dogs at 168 hours after administration.

Discussion

In the study reported here, the pharmacokinetics of tramadol and its metabolites and the antinociceptive effects of tramadol HCl were evaluated in healthy Greyhounds. Tramadol was well tolerated after a mean dose of 9.9 mg/kg was administered PO to the 6 dogs. The drug was rapidly absorbed and eliminated, with mean plasma N-desmethyltramadol concentrations exceeding tramadol concentrations at most measurement points after administration. The active metabolite O-desmethyltramadol was present in all dogs but at low concentrations, never exceeding 13.8 ng/mL in any dog. Despite low concentrations of O-desmethyltramadol, antinociceptive effects were significant at 5 and 6 hours after tramadol administration as determined through measurement of PPTs.

In humans, those who poorly metabolize tramadol to O-desmethyltramadol have lower concentrations of O-desmethyltramadol after tramadol administration than extensive metabolizers or ultrametabolizers.[11] Poor metabolizers have a significantly higher nonresponder rate to tramadol analgesia after surgery (46.7% vs 21.6% in others), but analgesic effects not attributable to O-desmethyltramadol may still exist because analgesia has been detected in some poor metabolizers.[19]

Significant antinociceptive effects were evident in the Greyhounds of the present study, despite the low plasma concentrations of O-desmethyltramadol. The peak antinociceptive effects were evident at 6 hours after tramadol administration, suggesting an indirect response between plasma concentrations of tramadol and PPTs. The plasma concentrations of O-desmethyltramadol 6 hours after drug administration, at which time PPTs were significantly increased, were < 1 ng/mL in most dogs. Concentrations of O-desmethyltramadol this low have not been shown to be analgesic. These data suggested that the antinociceptive effects were independent of O-desmethyltramadol concentrations or that a long lag to antinociceptive effect existed at very low concentrations. In these dogs, the mean C_{MAX} of O-desmethyltramadol was only 5.7 ng/mL, but when tramadol administration in human studies[20,21] resulted in analgesia, the mean O-desmethyltramadol concentrations were 39 to 84 ng/mL and mean tramadol concentrations ranged from 298 to 590 ng/mL. We believe that these data support our conclusion that antinociceptive effects in the study Greyhounds may have been independent of plasma O-desmethyltramadol concentration.

If the antinociceptive effects are due to tramadol alone and not O-desmethyltramadol, then the effects may be independent of opioid receptor activity. Tramadol is a norepinephrine reuptake inhibitor with a reported affinity constant of 205 ng/mL,[1] which is within the range of plasma tramadol concentrations achieved in the study Greyhounds. The α^2-adrenoceceptor antagonist yohimbine partially reverses the antinociceptive effects of tramadol,[22] further supporting the role of α^2-adrenoceceptors in the antinociceptive effects of tramadol. The affinity constant of tramadol as a serotonin reuptake inhibitor is 260 ng/mL, which is also in the range of plasma tramadol concentrations achieved in our study. The serotonin (5HT2a) receptor antagonist ketanserin will also partially reverse the antinociceptive effects of tramadol.[23] Additional studies are needed to assess the effects of yohimbine, ketanserin, and naloxone on the antinociceptive effects of tramadol in dogs.

Significant antinociceptive effects, as inferred from PPTs, were present at 5 and 6 hours after tramadol administration in the present study. However, the correlation of changes in PPTs and clinical analgesia has not been reported to the authors' knowledge. Therefore, conclusions regarding the clinical analgesic effects of tramadol cannot be made. The PPTs also unexpectedly decreased 4 hours after tramadol administration, compared with values at 3 and 5 hours. The reason for this decrease is unknown, but this finding demonstrates a limitation of determining the antinociceptive properties of drugs in dogs through use of PPTs. Behavioral

effects, such as the sounds of animal caretakers, food bowls, or equipment outside of the room, or even variability of the investigative team (eg, talking or bearing the scent of food or another animal) could have affected the behavioral response (withdrawal) upon stimulation with the von Frey device.

The C_{MAX} and AUC for tramadol and O-desmethyltramadol were lower than our previous published values for Beagles[12] but agree with the low concentrations reported by Giorgi et al.[16] Likewise, the present study found high plasma concentrations of N-desmethyltramadol in the Greyhounds, consistent with the metabolic pattern reported by Giorgi et al.[16] In addition, the mean (range) C_{MAX} of tramadol (215.7 ng/mL [85.4 to 454.0 ng/mL]) was markedly lower when compared with mean ± SD values (1,402.8 ± 695.5 ng/mL) in Beagles administered a similar dose (11.2 mg/kg)[12] and was lower than the concentrations reported by Giorgi et al[16] when normalized for dose.

The lower tramadol AUC and C_{MAX} in Greyhounds, compared with the AUC and C_{MAX} in Beagles,[12,16] may indicate the drug is less bioavailable in Greyhounds. Tramadol was not administered IV to Greyhounds; therefore, the bioavailability could not be determined. The mean $T_{½\lambda z}$ of tramadol in Greyhounds (1.1 hours) was shorter than those reported for Beagles (1.7 hours[12] and 2.2 hours[16]). Therefore, if a more rapid elimination is the consequence of faster clearance, then Greyhounds may have more rapid clearance than Beagles, thus reducing the oral systemic availability. The cause of this difference between breeds is uncertain. It may reflect a true difference between dog breeds in absorption and clearance, a difference between techniques used in each of the studies cited, or random differences. Each study involved a different assay technique, and the present study involved LC-MS detection, whereas the other studies involved fluorescence detection. The 2 studies in which fluorescence detection was used also involved different chromatographic conditions which may have affected the order of elution of parent drug and metabolites from the column. Our first study[12] was not designed to detect N-desmethyltramadol, N,O-didesmethyltramadol or the other metabolites, and the possibility that metabolites were coeluted, producing a falsely high concentration of O-desmethyltramadol, cannot be ruled out. The LC-MS method is more specific for the analytes, and there is less of a potential for misinterpreting coeluting chromatographic peaks.

Over 20 tramadol metabolites have been identified in dogs,[24] and most are not commercially available as pure chemical to assess for coelution via HPLC. Therefore, concentrations of tramadol or O-desmethyltramadol could be overestimated by HPLC when multiple metabolites elute concurrently with tramadol or O-desmethyltramadol. In our first study,[12] only tramadol and O-desmethyltramadol were assessed, whereas in the study by Giorgi et al,[16] N-desmethyltramadol and N-,O-didesmethyltramadol were also measured. Therefore, 16 to 18 metabolites could be present and could coelute, resulting in an overestimation of plasma drug concentrations of tramadol and specific metabolites. Additionally, the LC-MS method is more sensitive than HPLC, with a lower LLOQ (1 ng/mL) in plasma, compared with 20[12] and 5 ng/mL[16] for HPLC. The lower LLOQ may allow for better estimation of the pharmacokinetic parameters because less extrapolation is required, which could explain some of the apparent pharmacokinetic differences in Greyhounds and Beagles.

Tramadol was detected in urine samples with an LLOQ of 5 ng/mL in some Greyhounds up to 5 days (120 hours) after administration but was not detected 7 days (168 hours) afterward. It is possible more sensitive methods or instruments could still detect tramadol (or metabolites) in the urine 7 days (or longer) after drug administration.

The amount of pharmacokinetic data on oral administration of the immediate-release formulation of tramadol in dogs is fairly sparse, including only 6 Beagles,[12] 8 Beagles,[16] and 6 Greyhounds (present study). Each of the studies revealed large intersubject variability in the pharmacokinetics of immediate-release tramadol HCl in dogs, and there was a large interstudy variability. Therefore, more studies of oral tramadol HCl administration, including studies with larger numbers of dogs and multiple doses, are needed. More comparisons among dog breeds are recommended that use the same analytic technique to fully characterize the metabolic pattern of tramadol.

a. Tramadol hydrochloride, 50 mg, Amneal, Pharmaceuticals LLC, Patterson, NJ.
b. Venocath-16, Abbott Ireland, Sligo, Republic of Ireland.
c. Model 2390-5, IITC Life Sciences, Woodland Hills, Calif.
d. Spectrum Chemical, Gardena, Calif.
e. Cerilliant, Round Rock, Tex.
f. LGC Standards, East Greenwich, RI.
g. Shimadzu Prominence, Shimadzu Scientific Instruments, Columbia, Md.
h. API 2000, Applied Biosystems, Foster City, Calif.
i. Bond Elut C18, Varian, Palo Alto, Calif.
j. Supelco Discovery, 50 mm × 2.1 mm × 5 µm, Sigma-Aldrich, St Louis, Mo.
k. WinNonlin, version 5.2, Pharsight Corp, Mountain View, Calif.
l. SigmaStat, version 3.11, SyStat Software Inc, Point Richmond, Calif.

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