Effects of tramadol hydrochloride on the thermal threshold in cats

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Objective—To determine the thermal antinociceptive effect of oral administration of tramadol hydrochloride at doses between 0.5 and 4 mg/kg in cats.

Animals—6 healthy adult domestic shorthair cats.

Procedures—Baseline (before drug administration; time 0) thermal threshold was determined by applying a thermal probe to the thorax of each cat. Tramadol (0.5, 1, 2, 3, or 4 mg/kg) or a placebo was then administered orally in accordance with a Latin square design. Thermal threshold was determined by an observer who was unaware of treatment at various times until thermal threshold returned to baseline values or 6 hours had elapsed. Plasma tramadol and O-desmethyl-tramadol concentrations were measured prior to drug administration and at 1-hour intervals thereafter. Effect-concentration data were fitted to effect maximum models.

Results—Highest plasma tramadol and O-desmethyl-tramadol concentrations increased with increasing tramadol dose. Significant effects of dose and time on thermal threshold were detected. Thermal threshold was significantly higher than the baseline value at 80 and 120 minutes for the 0.5 mg/kg dose, at 80 and from 120 to 360 minutes for the 2 mg/kg dose, from 40 to 360 minutes for the 3 mg/kg dose, and from 60 to 360 minutes for the 4 mg/kg dose.

Conclusions and Clinical Relevance—Tramadol induced thermal antinociception in cats. Doses of 2 to 4 mg/kg appeared necessary for induction of significant and sustained analgesic effects. Simulations predicted that 4 mg/kg every 6 hours would maintain analgesia close to the maximum effect of tramadol. (Am J Vet Res 2009;70:1465–1470)

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>EC50</td>
<td>Concentration that induces 50% of the maximum effect</td>
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<tr>
<td>Emax</td>
<td>Effect maximum</td>
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<tr>
<td>LC</td>
<td>Liquid chromatography</td>
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<tr>
<td>MAC</td>
<td>Minimum alveolar concentration</td>
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<td>MS</td>
<td>Mass spectrometry</td>
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<td>m/z</td>
<td>Mass-to-charge ratio</td>
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Tramadol hydrochloride is a centrally acting analgesic agent that interacts with opioid, adrenergic, and serotonin receptors.1–3 One of the metabolites of tramadol, O-desmethyl-tramadol, has an affinity for opioid receptors approximately 200 times as great as the affinity for the parent drug, and it has been postulated that this metabolite is important for analgesic effects of tramadol.3,5

Tramadol is efficacious and tolerated well in humans and is increasingly being used to treat pain in dogs and cats. However, to our knowledge, limited data are available on the analgesic efficacy of tramadol in cats. Investigators in 1 study6 reported that tramadol had little or no effect on thermal and mechanical thresholds in cats. However, only 1 dose (1 mg/kg) was used in that study. Moreover, tramadol was administered SC in that study,6 and tramadol currently is not available in an injectable form in the United States, which limits the clinical applicability of the findings. In particular, if O-demethyl-tramadol is responsible for part of the analgesic effects of tramadol, then it is possible that oral administration results in better analgesia than parenteral administration because the first-pass metabolism would be expected to result in faster production of the active metabolite. In another study,7 tramadol decreased the MAC of sevoflurane in cats. However, decreases in MAC (or a lack thereof) should not be taken as evidence for analgesic effect because some drugs that cause a decrease in the MAC of inhalation anesthetics do not appear to result in analgesia, and drugs that result in analgesia do not always cause a decrease in MAC.8–12 Moreover, in that study,7 time for equilibration between end-tidal and effect-site concentrations appears inadequate, which limits the interpretability of the reported decrease in MAC.
The purpose of the study reported here was to characterize the effects of oral administration of tramadol on the thermal threshold in cats and the dose-dependent nature of this effect and to examine the relationship between plasma tramadol and O-desmethyl-tramadol concentrations and antinociceptive effect. We hypothesized that oral administration of tramadol would increase the thermal threshold in a dose-dependent manner.

Materials and Methods

Animals—Six healthy adult domestic shorthair cats (3 spayed females and 3 neutered males; mean ± SD weight, 4.2 ± 0.5 kg) were used in the study. All cats were housed in a group. Each cat was used in the study 6 times, with a minimum of 2 weeks allowed between successive experiments. Cats were observed for behavioral changes and other visible drug effects for the duration of the study. The study was approved by the Institutional Animal Care and Use Committee at the University of California-Davis.

Instrumentation and drug administration—The day before an experiment, cats were anesthetized with isoflurane in oxygen delivered in an acrylic chamber. After anesthesia was induced, the trachea was intubated and anesthesia was maintained with isoflurane in oxygen delivered via a coaxial Mapleson F circuit. A 22-gauge, 10-cm catheter was aseptically placed in a jugular vein. The lateral aspect of the thorax was clipped, and cats were allowed to recover from anesthesia.

Tramadol hydrochloride was packaged into gelatin capsules in doses of approximately 0.5, 1, 2, 3, and 4 mg/kg. The day of an experiment (ie, at least 12 hours after anesthesia), tramadol (one of the aforementioned doses per day) or placebo (an empty gelatin capsule) was administered orally (time 0) in accordance with a Latin square design.

Determination of thermal threshold—Each cat was placed in a cage (80 × 80 × 65 cm) that had a transparent acrylic door and mirrors on each sidewall. Cats had been acclimated to the cage and placement of the thermal threshold probe prior to the study. Cats had unlimited access to food and water during the experiments. A probe containing a heater element and an adjacent temperature sensor, both of which were embedded in epoxy, was attached to a pressure cuff and held in place on the thoracic wall of the cat. The pressure cuff was inflated to 100 mm Hg to ensure good contact between the probe and skin. Before each measurement, the probe was connected to a control unit by a flexible cable; cats were allowed to move freely in the cage during testing.

Skin temperature was measured, and the heater was activated (rate of temperature increase, 0.6°C/s). The cat was observed until a reaction (eg, jumping, turning the head toward the probe, or licking or biting the probe area or cable) was detected or a maximal temperature (55°C) was reached. When a reaction was observed, the temperature was recorded and considered the thermal threshold and the heater was turned off. Thermal thresholds were always determined by the same investigator (BHP), who was unaware of treatment assignments. Before drug administration, at least 30 minutes was allowed for equilibration between probe and skin temperatures; then, baseline skin temperature and thermal threshold were determined in duplicate at 20-minute intervals. After drug administration, skin temperature and thermal threshold were determined every 20 minutes for 4 hours, then at 1-hour intervals for an additional 2 hours or until the thermal threshold had returned to the baseline value. A single thermal threshold determination was performed at each time point.

Thermal probes were calibrated weekly during the study. For calibration, each probe was securely attached to the top of a 9.0 × 9.0 × 0.5-cm aluminum plate. A thermocouple was placed in a previously drilled horizontal hole so that the tip was directly below the probe and was connected to a digital thermometer, the accuracy of which had been verified against a certified thermometer. The aluminum block was placed on a standard laboratory hotplate that was heated to approximately 85°C and then allowed to cool to 30°C. The measurements for the probe and thermocouple were recorded at each decrease of 5.0°C in hotplate temperature between 65° and 30°C. The probe response was recorded within that range (linear regression R² > 0.998). A calibration curve was constructed by use of linear regression, and observed temperatures were mathematically corrected by use of the most recent curve for that probe.

Measurement of plasma concentrations—A blood sample (2 mL) was collected from the catheter in the jugular vein before drug administration and at 1-hour intervals thereafter until completion of the experiment. Blood samples were transferred to tubes containing EDTA and immediately centrifuged for 10 minutes at 4°C; plasma was harvested and stored at −20°C until analyzed for tramadol and O-desmethyl-tramadol concentrations.

Tramadol was quantitated in feline plasma by LC-MS analysis of protein-precipitated samples. Calibration standards were prepared. Stock solutions were made by dissolving 10 mg of tramadol standard in 10 mL of acetonitrile. Working solutions were prepared by dilution of the tramadol stock solution with acetonitrile to achieve concentrations of 100 and 500 ng/mL. Plasma calibrators were prepared by dilution of the working tramadol solutions with drug-free feline plasma to achieve concentrations of 1, 5, 10, 50, 100, 200, 500, and 1,000 ng/mL. Calibration curves and negative control samples were prepared fresh for each quantitative assay. In addition, quality-control samples (plasma fortified with analytes at concentrations at the midpoint of the standard curve) were routinely included as an additional assessment of accuracy. The concentration of tramadol in each sample was determined with the internal standard (tramadol-3-C18 method by use of the peak area ratio and linear regression analysis. The limit of quantification was 1.0 ng/mL, and the limit of detection was 0.01 ng/mL.

Quantitative analyses were performed on a triple quadrupole mass spectrometer coupled with an LS system. Chromatography involved use of a 10-cm × 2.1-mm, 3-µm column and a linear gradient of acetonitrile in water with 0.2% formic acid at a flow rate of 0.3 mL/min. The acetonitrile concentration was held at 5% for 0.2 minutes and increased to 90% during a 5-minute period. Prior to analysis, plasma proteins,
control samples, and calibrators were extracted by precipitation with 0.5 mL of a solution consisting of 9:1 acetonitrile:acetic acid (1M), to which 10 ng of internal standard/mL had been added; tubes were vortex mixed for 2 minutes, refrigerated for 30 minutes, and then centrifuged (1,864 × g for 3 minutes). Injection volume was 20.0 μL.

Detection and quantification used full-scan LC-MS-MS transitions of initial product ions for tramadol (m/z, 264.2) and O-desmethyl-tramadol (m/z, 250.2). The response for the major product ion (m/z, 68.1, which was the same for both tramadol and O-desmethyl-tramadol) was plotted, and peaks at the proper retention time were integrated by use of a computer program. This software program was used to generate calibration curves and quantify the analyte concentrations in all samples.

Plasma tramadol and O-desmethyl-tramadol concentrations and the corresponding thermal thresholds were fitted to ordinary Emax and sigmoid-Emax models by use of nonlinear regression and a computer program. The appropriate model was selected by observation of the residuals plot and by use of Akaike’s information criterion.

Statistical analysis—Power analysis based on results of another thermal threshold study conducted by our laboratory group suggested that 6 cats were needed to detect an increase of 5°C in thermal threshold, with α = 0.05 and power of 0.8. The relationship between plasma tramadol and O-desmethyl-tramadol concentrations was examined by use of the Pearson product-moment correlation. Skin temperature and thermal threshold data were analyzed for time and treatment effects by use of a repeated-measures Latin square. The Dunnett test was used when appropriate to detect differences in thermal threshold, compared with the baseline value, within a dose and differences in thermal threshold between doses within a time point. Only data for the first 6 hours of each experiment were analyzed because the number of cats from which data was collected for each tramadol dose varied after the first 6 hours. Significance was set at a value of P < 0.05. Data were reported as mean ± SD unless otherwise indicated.

Results

None of the cats had an exaggerated response to the stimulus or had evidence of burns to the skin. Actual mean ± SD amount of tramadol in the placebo and 0.5, 1, 2, 3, and 4 mg/kg treatments was 0 ± 0 mg/kg, 0.50 ± 0 mg/kg, 0.99 ± 0.01 mg/kg, 2.00 ± 0.01 mg/kg, 3.00 ± 0.02 mg/kg, and 3.99 ± 0.02 mg/kg, respectively. Plasma tramadol concentrations were related to the dose administered and decreased over time (Figure 1). The highest plasma tramadol concentration was in the first sample (1 hour after tramadol administration) in all cats after administration of tramadol at 0.5 and 1 mg/kg. The highest plasma tramadol concentration was detected at 1 and 2 hours in 5 and 1 cats, respectively; after administration of tramadol at 2 mg/kg. The highest plasma tramadol concentration was measured at 1 and 2 hours in 4 and 2 cats, respectively, after administration of tramadol at 3 mg/kg. The highest plasma tramadol concentration was measured at 1, 2, and 3 hours in 3, 1, and 2 cats, respectively, after administration of tramadol at 4 mg/kg. Mean ± SD highest measured plasma tramadol concentrations were 23.8 ± 8.2 ng/mL, 90.3 ± 62.6 ng/mL, 165.7 ± 58.3 ng/mL, 367.5 ± 139.8 ng/mL, and 539.8 ± 134.1 ng/mL after administration of tramadol at 0.5, 1, 2, 3, and 4 mg/kg, respectively.

Plasma O-desmethyl-tramadol concentrations were related to tramadol dose and decreased over time (Figure 2). Plasma O-desmethyl-tramadol concentration was measured at 1, 2, and 3 hours in 3, 1, and 2 cats, respectively, after administration of tramadol at 4 mg/kg. Mean ± SD highest measured plasma O-desmethyl-tramadol concentrations were 94.5 ±
Skin temperature did not change significantly over time for any dose of tramadol (data not shown). Thermal threshold increased significantly after tramadol administration (Figure 3). No difference in baseline thermal threshold was detected among tramadol doses. Thermal threshold did not change significantly over time for the placebo treatment or the 1 mg/kg dose. Compared with their respective baseline values, thermal threshold increased significantly at 80 and 120 minutes for the 0.5 mg/kg dose, at 80 and from 120 to 360 minutes for the 1 mg/kg dose, from 40 to 360 minutes for the 3 mg/kg dose, and from 60 to 360 minutes for the 4 mg/kg dose. Thermal threshold was significantly higher for the 2, 3, and 4 mg/kg doses than for the placebo treatment at various time points. Thermal threshold returned to baseline values in < 6 to 11 hours, < 6 to 13 hours, 9 to 12 hours, and 10 to 16 hours for the 1 and 2 mg/kg doses, 0.5 mg/kg dose, 3 mg/kg dose, and 4 mg/kg dose, respectively.

Thermal threshold-plasma concentration data for both tramadol and O-desmethyl-tramadol best fitted an ordinary Emax model with baseline effect. This model was defined by use of the following equation:

\[ E = E_0 + \frac{(E_{max} - E_0) \times C}{EC_{50} + C} \]

where E is the effect (ie, thermal threshold) at a specific concentration (C), \( E_0 \) is the effect when the concentration is 0 (ie, baseline thermal threshold), and \( E_{max} \) is the maximum effect induced. Tramadol data for 2 cats and O-desmethyl-tramadol data for 3 cats did not fit the model because no maximum effect was reached. These cats were excluded from the analysis. Median values for \( E_0 \), \( E_{max} \), and \( EC_{50} \) were 44.9°C (range, 43.4° to 47.2°C), 53.5°C (range, 50.2° to 55.9°C), and 17.2 ng/mL (range, 5.3 to 104.9 ng/mL), respectively, for tramadol and 45.3°C (range, 43.3° to 47.1°C), 54.2°C (range, 52.5° to 59.4°C), and 112.9 ng/mL (range, 46 to 214 ng/mL), respectively, for O-desmethyl-tramadol.

### Discussion

In the study reported here, oral administration of tramadol resulted in thermal antinociception in cats in a dose-dependent manner. Doses of ≥ 2 mg/kg were necessary to yield a significant and sustained effect. This is in agreement with results of another study in which tramadol at 1 mg/kg, SC, had minimal or no effect on thermal and mechanical thresholds in cats.

Some of the behavioral and physical effects subjectively observed after administration of tramadol, such as euphoria and mydriasis, were similar to classical effects observed after administration of moderate doses of opioids in cats. Whether these effects were related to stimulation of opioid receptors cannot be determined from the data in the study reported here. No clinically important adverse effect was observed in the cats of this study for any of the tramadol doses; however, this study was not designed, nor did it have the necessary power, to assess the safety of tramadol in cats.

The analgesic effect of tramadol is thought to be related to interactions with opioid, \( \alpha_2 \)-adrenergic, and serotonin receptors. In addition, the effect on opioid receptors may be related to O-desmethyl-tramadol (a tramadol metabolite) rather than to tramadol. This is suggested by the facts that O-desmethyl-tramadol binds opioid receptors with a much higher affinity than does tramadol and that in humans deficient in CYP2D6, the O-desmethyl-tramadol concentration after tramadol administration is reduced, as is the analgesic efficacy of tramadol. It has also been reported that the analgesic effect of O-desmethyl-tramadol is abolished in \( \mu \)-opioid receptor knockout mice. It is unclear whether the effects detected in the study reported here were related to tramadol, O-desmethyl-tramadol, or the combination of these 2 compounds. Although the effect of tramadol and O-desmethyl-tramadol on thermal threshold could
be modeled for 4 and 3 cats, respectively, the relation to the plasma concentration of either compound was highly variable, as illustrated by the wide range of estimated \( EC_{50} \) values. Moreover, because plasma tramadol and O-desmethyl-tramadol are highly correlated, it is not possible from the data in this study to determine whether the effect on thermal threshold was primarily attributable to tramadol or its metabolite. This high correlation was expected because metabolism of tramadol was the only source of O-desmethyl-tramadol. This also implies that distinguishing the effects of these 2 compounds is somewhat clinically irrelevant in animals able to metabolize tramadol in a normal physiologic manner.

The \( EC_{50} \) predicted by the Emax model was highly variable among cats, and the Emax model did not adequately describe the data for some cats. High variability in effective tramadol and O-desmethyl-tramadol concentrations has been reported in people.16,17 In those studies, the effective tramadol concentrations were higher, whereas the effective O-desmethyl-tramadol concentrations were lower, than the \( EC_{50} \) estimated from our data (effective tramadol and O-desmethyl-tramadol concentrations for humans of approx 290 to 600 ng/mL and 36 to 84 ng/mL, respectively, compared with \( EC_{50} \) of 17 and 113 ng/mL for tramadol and O-desmethyl-tramadol, respectively, for the cats of the study reported here). The higher effective tramadol concentrations reported in the human studies may have been related to the fact that tramadol was used to treat post-surgical pain, which is likely to require higher analgesic concentrations than those needed to increase thermal nociceptive thresholds. Moreover, given that the 2 studies in humans used patient-controlled analgesia, it is likely that patients were maintaining concentrations higher than the \( EC_{50} \) in humans because they needed more than 50% of the analgesic effect of tramadol to adequately control their pain. The data in the study reported here suggested that to achieve 95% of the maximum thermal antinociceptive effect of tramadol, concentrations of approximately 350 ng/mL would have been required, which would be within the range of concentrations that provide postoperative analgesia in humans. The higher effective tramadol concentration than O-desmethyl-tramadol concentration in humans is the opposite of the results for the present study. This may have been attributable to a species difference, and it is possible that O-desmethyl-tramadol plays a minor role in thermal analgesia of cats after administration of tramadol.

Simulation based on pharmacokinetic parameters for tramadol in cats reported in another study18 revealed that administration of 4 mg/kg every 6 hours is expected to result in plasma tramadol concentrations of \( \geq 350 \) ng/mL for 89% of the time overall and \( > 90% \) of the time after the first 3 doses. The maximum concentration predicted by the model with this administration regimen is 2,303 ng/mL. As mentioned previously, concentrations of 350 ng/mL are predicted by the pharmacodynamic model to yield 93% of the maximum antinociceptive effect of tramadol. Nevertheless, plasma tramadol and O-desmethyl-tramadol concentrations detected after tramadol administrations were highly variable among cats, and it is therefore expected that optimal dosing would require adjustment for clinical patients as a function of the resulting effect.

In another study39 conducted by our laboratory group, the disposition of O-desmethyl-tramadol closely followed that of tramadol after oral administration of tramadol at a dose of 5 mg/kg. In the study reported here, similar results were detected after administration of tramadol at doses of 3 and 4 mg/kg. However, with lower tramadol doses, plasma O-desmethyl-tramadol concentrations were approximately 2 to 4 times as high as plasma tramadol concentrations, as illustrated by the highest measured concentrations. This may suggest that the biotransformation of tramadol in O-desmethyl-tramadol is a somewhat dose-dependent event and reaches saturation for doses of approximately 3 mg/kg. Alternatively, the pharmacokinetics of O-desmethyl-tramadol may not be linear but instead may be a dose-dependent event. Although the slope of the elimination portion of the O-desmethyl-tramadol concentration-versus-time data appears fairly similar for all tramadol doses, the study reported here was not designed to determine the pharmacokinetics of tramadol or its metabolite.

The magnitude of the effect of tramadol administration on thermal threshold, as well as its duration, was related to the dose. Doses of 3 and 4 mg/kg appeared to result in more intense analgesia than did lower doses. Thermal analgesia after administration of tramadol appears to be of a magnitude similar to that reported for opioids. The pharmacodynamic model predicts a median maximum thermal threshold of 53.5°C, which is similar to or higher than values observed in studies on the effect of butorphanol and buprenorphine40 or on the effects of hydromorphone, morphine, buprenorphine, and methadone.20–22 Investigators in most of these studies only examined 1 dose of each drug, and it is possible that other doses would have increased the effect; it is also possible that our pharmacodynamic model slightly underestimated the maximum possible thermal threshold. This is suggested by the fact that in some cats, no maximum effect was detected (ie, the response did not reach a plateau) and the relationship between thermal threshold and plasma tramadol or O-desmethyl-tramadol could not be modeled by use of the Emax model. Moreover, the maximum predicted effect for tramadol and O-desmethyl-tramadol (53.5°C and 54.2°C, respectively) was close to the resolution of this nociceptive model because a cutoff temperature of 55°C was used to prevent skin burns; therefore, maximum possible effect could not exceed that temperature.

The main limitation of the present study was related to use of a thermal threshold model. Thermal antinociception does not necessarily correlate with analgesia for other types of pain, which may be encountered more commonly in clinical patients. Nevertheless, the thermal threshold model has been widely used in cats, particularly to evaluate the analgesic effects of opioids, and is validated in that species.21

Tramadol induced thermal antinociception in cats. Doses of \( \geq 2 \) mg/kg appeared necessary to induce a significant and sustained effect. Simulation based on previously reported pharmacokinetics of tramadol in cats and on the pharmacodynamic model obtained from the data in this study suggests that a dose of 4 mg/kg ad-
administered every 6 hours will maintain analgesia close to the maximum effect of tramadol.

b. Tramadol hydrochloride, Akyma Pharmaceutical, Glasgow, Ky.
c. Tramadol, Cerrilliant, Round Rock, Tex.
d. HPLC-grade acetonitrile, Burdick and Jackson, Muskegon, Mich.
e. Cerrilliant, Round Rock, Tex.
f. TSQ Quantum Ultra, ThermoFisher Scientific, San Jose, Calif.
g. 1100 Series, Agilent Technologies, Palo Alto, Calif.
h. ACE 3 C18, Mac-Mod Analytical, Chadds Ford, Pa.
i. LCQuan Thermo, Fisher Scientific, San Jose, Calif.
j. WinNonLin Pro 5.2, Pharsight Corp, Mountain View, Calif.

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