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Evaluation of the analgesic effects of oral and subcutaneous tramadol administration in red-eared slider turtles

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Abstract

Objective—To determine the dose- and time-dependent changes in analgesia and respiration caused by tramadol administration in red-eared slider turtles (*Trachemys scripta*).

Design—Crossover study.

Animals—30 adult male and female red-eared slider turtles.

Procedures—11 turtles received tramadol at various doses (1, 5, 10, or 25 mg/kg [0.45, 2.27, 4.54, or 11.36 mg/lb], PO; 10 or 25 mg/kg, SC) or a control treatment administered similarly. Degree of analgesia was assessed through measurement of hind limb thermal withdrawal latencies (TWDLs) at 0, 3, 6, 12, 24, 48, 72, and 96 hours after tramadol administration. Nineteen other freely swimming turtles received tramadol PO (5, 10, or 25 mg/kg), and ventilation (V_E), breath frequency, tidal volume (V_T) and expiratory breath duration were measured.

Results—The highest tramadol doses (10 and 25 mg/kg, PO) yielded greater mean TWDLs 6 to 96 hours after administration than the control treatment did, whereas tramadol administered at 5 mg/kg, PO, yielded greater mean TWDLs at 12 and 24 hours. The lowest tramadol dose (1 mg/kg, PO) failed to result in analgesia. Tramadol administered SC resulted in lower TWDLs, slower onset, and shorter duration of action, compared with PO administration. Tramadol at 10 and 25 mg/kg, PO, reduced the V_E at 12 hours by 51% and 67%, respectively, and at 24 through 72 hours by 55% to 62% and 61% to 70%, respectively. However, tramadol at 5 mg/kg, PO, had no effect on the V_E .

Conclusions and Clinical Relevance—Tramadol administered PO at 5 to 10 mg/kg provided thermal analgesia with less respiratory depression than that reported for morphine in red-eared slider turtles.

Several obstacles limit successful analgesic use in animals, such as subjectivity in pain assessment, inadequate knowledge of analgesic efficacy, and the unknown relationship between risks and benefits for specific drugs.¹⁻⁷ These limitations are particularly true for nondomestic species, for which information on analgesic use is often unreliably extrapolated from established protocols for domestic animals. To optimize patient care across taxonomic groups, studies are needed to evaluate the efficacy and adverse effects of analgesics and identify appropriate doses, routes of administration, and durations of action.^{1-5,8,9}

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In reptiles, opioid drug administration has yielded unexpected results with respect to analgesia. Butorphanol (κ -opioid receptor agonist and partial μ -opioid receptor agonist-antagonist), which is the most commonly used analgesic in reptile medicine,⁵ reportedly does not change thermal withdrawal latencies (interval to withdrawal of limb from a thermal stimulus) in redeared slider turtles⁸ and bearded dragons⁹ or thermal thresholds in green iguanas.¹⁰ Morphine, a μ -opioid receptor agonist, increases thermal withdrawal latencies in turtles⁸ and bearded dragons⁹ at doses ranging between 1.5 and 20 mg/kg (0.68 and 9.09 mg/lb) but is ineffective at doses up to 40 mg/kg (18.18 mg/lb) in corn snakes.⁹ Similar to findings in mammals, however, morphine administration results in profound respiratory depression in red-eared slider turtles.⁸ Thus, a need exists for identifying an efficacious analgesic in reptiles that causes minimal or no respiratory depression.

Tramadol is a candidate drug for use in reptiles because it is a noncontrolled, commonly used analgesic in small animal practice. The drug and its metabolite, O-desmethyl-tramadol (M1), cause analgesia in mammals by activating μ -opioid receptors but also by inhibiting serotonin and norepinephrine reuptake in the CNS.¹¹⁻¹⁵ The M1 metabolite, which is formed in the liver by a cytochrome P450-driven reaction,^{16,17} is a mixture of (+) and (–)-enantiomers. The (+)-enantiomer preferentially activates μ -opioid receptors, inhibits serotonin reuptake, and facilitates serotonin release, whereas the (–)-enantiomer primarily inhibits norepinephrine reuptake.¹⁵ The parent drug tramadol has μ -opioid receptor activity, but M1 has up to 200 times as great an affinity for μ -opioid receptors.^{18,19} Overall, tramadol binds μ -opioid receptors with 6,000 times less affinity than morphine,^{19,20} thus having the potential for producing fewer μ -opioid-induced adverse effects. In fact, tramadol administration does not appear to alter breathing in humans²¹⁻²³ and causes significantly less respiratory depression than morphine in cats and dogs.^{24,25} Thus, we hypothesized that tramadol administration in reptiles would increase thermal withdrawal latencies with less respiratory depression than morphine administration. The purpose of the study reported here was to determine the dose- and time-dependent changes in analgesia and respiration caused by tramadol administration in red-eared slider turtles.

Materials and Methods

Animals

Thirty adult red-eared slider turtles (*Trachemys scripta*) with a mean $\pm$ SD body weight of 812.5 ± 100.2 g (1.79 ± 0.22 lb) were obtained from a commercial supplier^a and kept in 1,800-L open tanks (5 to 20 turtles/tank) with free access to dechlorinated water for swimming and dry areas for basking. Room temperature was maintained near their optimal body temperature²⁶ at 27° to 28°C (80.6° to 82.4°F), with light provided 14 h/d. Turtles were fed floating food sticks^b 3 to 4 times/wk. All procedures were approved by the Animal Care and Use Committee at the University of Wisconsin, Madison, School of Veterinary Medicine.

Study design

A crossover experimental design was used to evaluate tramadol- and morphine-dependent changes in thermal analgesia (11 turtles; 6 males and 5 females) and respiration (19 turtles; 9 males and 10 females). All turtles received all treatments, and each turtle was allowed a minimum of 2 weeks between treatments. The observer in the analgesia experiments was blinded to treatments received.

^aNiles Biological Supply, Sacramento, Calif.

^bReptoMin, Tetra, Blacksburg, Va.

Thermal analgesia experiments

Analgesia experiments were conducted by applying infrared thermal stimuli to the plantar surface of turtle hind limbs by use of a standard apparatus^c and established methods.^{8,27} Turtles were placed into clear, ventilated plastic boxes (17 × 13 × 14 cm) that were elevated on a clear acrylic surface and contained dividers to prevent visual contact with other turtles. An infrared radiation source was activated directly below the surface upon which the turtle rested the plantar surface of either hind limb. Hind limb thermal withdrawal latencies were measured by a motion-sensitive timer, which stopped automatically when the hind limb was removed from the noxious stimulus. A maximum exposure duration of 32.5 seconds was allowed to prevent tissue damage. At each time point, 2 thermal withdrawal latencies were measured. When the difference between the 2 thermal withdrawal latencies was > 2 seconds, a third measurement was obtained. All latency measurements were separated by at least 5 minutes to avoid a conditioning response to stimuli. In addition, the tested hind limb was alternated for each treatment to avoid plantar lesions, which were observed as skin ulcers and blisters among turtles in preliminary studies when the same hind limb was tested repeatedly (these turtles were not used in subsequent experiments).

After 1 day of training, 11 turtles received either water PO (equivalent to the tramadol PO volume; control treatment) or tramadol PO at doses of 1, 5, 10, and 25 mg/kg (0.45, 2.27, 4.54, and 11.36 mg/lb). The doses of tramadol were dissolved in 0.1 mL of water, and food coloring was added to detect regurgitation. If drug was regurgitated, turtles were not tested and were allowed 2 weeks before retesting. To evaluate whether parenteral tramadol administration would result in effects similar to those of the orally administered drug, the same 11 turtles were given either physiologic saline (0.9% NaCl) solution SC (equivalent to tramadol SC volume; control treatment) or tramadol SC at 10 and 25 mg/kg dissolved in 0.1 mL of saline solution. Thermal withdrawal latencies were measured before drug administration (baseline) and at 7 time points after administration: 3, 6, 12, 24, 48, 72, and 96 hours.

Respiratory experiments

Ventilation (in mL/min/kg) was measured in conscious, freely swimming turtles by use of established methods.^{28,29} Individual turtles were placed into respiratory tanks consisting of clear plastic containers (16 × 42 × 42 cm) filled to the top with water at 23°C (Figure 1). A circular breathing chamber (diameter, 8 cm; volume, 250 mL) was sealed into the top, providing the only location within the respiratory tank for the turtle to breathe. Flow meters maintained a constant flow (approx 500 mL/min) of room air through the breathing chamber. Airflow changes were converted to electronic signals with a pneumotachometer^d that was connected to a computer data-acquisition system.^e Signals were analyzed off-line with commercially available software.^f The pneumotachometer was calibrated according to published methods.³⁰ One end of plastic tubing (inner diameter, 0.3 mm; length, approx 40 cm) was inserted into the breathing chamber, and the other end was connected to a motor-driven 25-mL glass syringe. The syringe was set at various volumes (range, 2.5 to 16.5 mL) and rhythmically moved back and forth at cycle periods of 1.5 to 4.5 seconds (similar to the duration of 1 turtle expiratory-inspiratory cycle).

For a given syringe volume, expiratory trace areas were averaged at various frequencies and plotted versus the logarithm of the syringe frequency. These plots revealed that expiratory

^cPlantar analgesia instrument (Hargreaves's apparatus), model 37370, Ugo Basile Co, Comerio, Italy.

^dGodart, Gould Electronics, Eastlake, Ohio.

^eLabPro, Vernier Software & Technology, Beaverton, Ore.

^fClampfit, version 10.0, Axon Instruments Inc, Union City, Calif.

area measurements were relatively insensitive to frequency, with a maximum of 10% error in V_E and V_T measurements observed only at the highest frequencies and V_T in turtles. Thus, the impact of systematic pneumotachometer errors was deemed minimal with respect to the major findings.

Turtles were conditioned to the respiratory tank for at least 3 hours prior to entering a trial. On the first day of a trial, turtles were placed in the respiratory tank for 3 hours prior to drug administration. The first hour of data was discarded to allow for acclimation to the respiratory tank; the next 2 hours represented baseline breathing. Turtles were given either water PO (equivalent to tramadol PO volume; control treatment) or tramadol PO at 5, 10, and 25 mg/kg dissolved in water (similar to the drug administration methods described for the analgesia experiments). For short-term trials, turtles were returned to the respiratory tank for another 12 continuous hours. In separate long-term trials, turtles were not returned to the respiratory tank immediately following drug administration. Instead, at 24, 48, and 72 hours after drug administration, turtles were placed into the respiratory tank for 3 hours.

Statistical analysis

For each turtle, all thermal withdrawal latencies measured at a given time point were averaged together. These mean thermal withdrawal latencies were then averaged for all turtles given the same treatment. The V_E was calculated by summing of the area under individual expiratory traces within a 60-minute period and conversion of the value to volume by use of the pneumotachometer calibration data. Breath frequency was defined as the mean number of breaths per minute. The V_T was calculated by division of V_E by breath frequency. All respiratory data were averaged into 2- (short-term) or 3-hour (long-term) values. Two-way, repeated-measures ANOVAs were performed with the aid of commercially available computer software.^g If the normality assumption was not satisfied, data were ranked, and the ANOVA was performed again on the ranked data. Post hoc comparisons were made by use of the Student-Newman-Keul test. All data are expressed as mean $\pm$ SEM. Values of $P < 0.05$ were considered significant.

Results

Thermal analgesia experiments

When the 11 turtles received water PO (control treatment), mean baseline thermal withdrawal latencies started at 12.6 ± 0.6 seconds, decreased to a minimum of 10.9 ± 0.8 seconds at 6 hours, and increased to a maximum of 15.5 ± 0.8 seconds at 72 hours after water administration, but no significant changes were evident ($P > 0.05$ for all time points; Figure 2). When raw thermal withdrawal latency data were graphed for the same turtles administered tramadol PO, mean baseline thermal withdrawal latencies for the 1, 5, 10, and 25 mg/kg doses were similar at 16.6 ± 1.0 seconds, 15.3 ± 1.1 seconds, 13.5 ± 0.6 seconds, and 15.3 ± 0.8 seconds, respectively ($P > 0.05$). The mean withdrawal latencies when turtles received 1 mg of tramadol/kg, PO, were significantly ($P = 0.025$ for drug effect) increased at 12 hours after tramadol administration, compared with values when they received water PO. Likewise, when turtles received 5 mg of tramadol/kg, PO, mean withdrawal latencies were significantly ($P = 0.002$ for drug effect) increased at 6, 12, and 24 hours after tramadol administration, compared with control values. When turtles received tramadol PO at 10 or 25 mg/kg, withdrawal latencies increased significantly with respect to control and baseline values starting at 6 hours after administration. Twelve to 96 hours after tramadol administration, mean withdrawal latencies were significantly ($P < 0.001$ for drug effects) greater than baseline values from 7.7 to 10.0 seconds and 7.9 to 12.7 seconds for the 10 and

^gSigma Stat, version 2.03, Jandel Scientific Software, San Rafael, Calif.

25 mg/kg doses, respectively. With tramadol administered at 25 mg/kg, withdrawal latency data were greater than those after administration of 10 mg/kg at the 6-, 12-, and 24-hour time points ($P < 0.05$). However, when these data were normalized to their respective baseline values (ie, graphed as the change in withdrawal latency), the significant drug effects for the 1 and 5 mg/kg tramadol doses were no longer significant ($P = 0.23$ and $P = 0.29$, respectively). However, tramadol at 5 mg/kg increased withdrawal latencies at 12 and 24 hours after drug administration, compared with water administered PO ($P < 0.05$). In contrast, when change in withdrawal latency was evaluated for the 10 and 25 mg/kg tramadol data, the significant ($P < 0.001$) drug effects remained. Additionally, the withdrawal latencies for 25 mg of tramadol/kg were only greater than the 10 mg/kg withdrawal latencies at the 24-hour time point ($P = 0.021$).

With respect to SC tramadol administration, when turtles received saline solution, baseline withdrawal latencies started at 14.9 ± 0.5 seconds and remained near baseline values for the 3- to 96-hour time points ($P > 0.05$; Figure 3). Similar to PO tramadol administration, tramadol at 10 and 25 mg/kg, SC, yielded significant drug effects, compared with the control treatment ($P = 0.002$ and $P < 0.001$, respectively).

No difference in withdrawal latencies or drug effects was found between the PO and SC routes of tramadol administration at the 25 mg/kg dose ($P > 0.05$). However, differences between the PO and SC routes of administration were of the magnitude and time course of withdrawal latency changes for the 10 mg/kg data. When graphed as the change in withdrawal latency, the withdrawal latencies for tramadol administration at 10 mg/kg, SC, were not greater than baseline values at 6, 72, or 96 hours after drug administration ($P > 0.05$), nor were they greater than control values at the 96-hour time point ($P = 0.265$). Also, in contrast to the data for tramadol administered PO, a drug effect ($P = 0.016$) was evident between the 10 and 25 mg/kg, SC, doses of tramadol, with significant differences at the 12-, 24-, 72-, and 96-hour time points ($P < 0.05$). Withdrawal latencies for the 10 mg/kg, SC, dose of tramadol were decreased relative to withdrawal latencies for the 10 mg/kg, PO, dose at 48 to 96 hours after drug administration ($P < 0.05$). Finally, when the 11 turtles received 25 mg of tramadol/kg (PO or SC), flaccid limbs and necks were observed in 4.

Respiratory experiments

In the short-term respiratory trials involving PO administration of treatments (Figure 4), the mean baseline V_E s when the 19 turtles received water and tramadol (5, 10, 25 mg/kg) were similar at 17.6 ± 4.0 mL/min/kg, 16.6 ± 2.9 mL/min/kg, 18.2 ± 4.3 mL/min/kg, and 16.1 ± 2.8 mL/min/kg, respectively ($P > 0.05$). No significant ($P = 0.612$ for drug effect) change from baseline was detected for water ($P > 0.05$) or the 5 mg/kg dose of tramadol at the 3-, 6-, 9-, or 12-hour time points. When the same turtles received the 10 mg/kg dose of tramadol, mean V_E was significantly ($P < 0.001$) decreased at 6 through 12 hours when compared with baseline values, but only decreased at the 6- and 9-hour time points when compared with control values ($P < 0.05$). Overall, a significant ($P = 0.064$) drug effect was not detected for the 10 mg/kg dose of tramadol. On the other hand, the mean V_E for the 25 mg/kg dose was significantly decreased at 3 through 12 hours when compared with baseline ($P < 0.05$) and water ($P < 0.002$) values.

The mean baseline breath frequency was also similar among treatment groups at 2.5 to 2.8 breaths/min ($P > 0.05$). For turtles administered water PO, no significant ($P > 0.05$) change in breath frequency from baseline occurred. However, a drug effect was evident for the 5, 10, and 25 mg/kg doses of tramadol ($P = 0.009$, $P = 0.001$, and $P < 0.001$, respectively). Compared with values for water, the mean breath frequency when turtles received the 5 mg/kg dose decreased at 3, 6, and 12 hours after administration ($P < 0.05$), whereas the 10 and

25 mg/kg doses resulted in a decreased mean frequency at 3 through 12 hours after tramadol administration ($P < 0.05$ and $P < 0.001$, respectively).

For V_T , a drug effect was evident only for the 5 and 25 mg/kg doses of tramadol ($P = 0.041$ and $P = 0.037$, respectively). However, the statistical power for drug effects associated with V_T was 50%, suggesting low confidence in these results.

In the long-term respiratory trials for PO administration of treatments, the mean baseline V_E s when turtles were given water and tramadol (5, 10, 25 mg/kg) were similar at 14.3 ± 3.3 mL/min/kg, 18.0 ± 5.2 mL/min/kg, 13.0 ± 3.5 mL/min/kg, and 13.4 ± 3.5 mL/min/kg, respectively ($P > 0.05$; Figure 5). A drug effect associated with V_E was detected when turtles received the 10 and 25 mg/kg doses of tramadol ($P = 0.013$ and $P = 0.010$, respectively), but not when turtles received 5 mg/kg ($P = 0.249$). The mean breath frequency when the same turtles received water PO started at 2.2 ± 0.3 breaths/min and remained near baseline frequencies at the 24-through 72-hour time points ($P > 0.05$). For the 5, 10, and 25 mg/kg doses of tramadol, mean baseline frequencies were similar to control values ($P > 0.05$), but mean frequencies were decreased at 24 through 72 hours after tramadol administration for all doses when compared with their respective baseline values ($P < 0.001$). Compared with control values, mean breath frequencies at the 24- and 48-hour time points after the 5 mg/kg dose of tramadol was administered were decreased ($P < 0.002$), whereas when the same turtles received the 10 and 25 mg/kg doses, the mean frequencies were decreased at 24 through 72 hours ($P < 0.05$ and $P < 0.001$, respectively).

The mean baseline V_T was similar when turtles received water and tramadol (5 through 25 mg/kg) at 0.10 to 0.11 mL/breath/kg ($P > 0.05$). Although the mean V_T remained near baseline values when turtles received water ($P > 0.05$), a drug effect was evident for the 5, 10, and 25 mg/kg dose of tramadol ($P = 0.034$, $P = 0.024$, and $P < 0.001$, respectively). The 10 and 25 mg/kg dose increased the mean V_T at 24 through 72 hours after tramadol administration with respect to both baseline and water values ($P < 0.05$ and $P = 0.001$, respectively). Nonetheless, the 5 mg/kg dose also increased the mean V_T at the 24- and 48-hour time points relative to control values ($P < 0.05$).

Discussion

In the study reported here, tramadol administration resulted in analgesia with mild respiratory depression (compared with the effects of morphine¹⁰) in redeared slider turtles.⁸ As demonstrated by the systematic dose- and time-dependent analgesia and respiratory experiments, 5 to 10 mg of tramadol/kg, PO, appeared to be the dose range for providing pain relief with the least respiratory depression. The highest dose (25 mg/kg, PO) was associated with flaccid limbs and necks, as well as severe respiratory depression, whereas the lowest dose (1 mg/kg, PO) failed to yield sufficient analgesia. Oral tramadol administration was more effective for analgesia because SC administration resulted in lower withdrawal latencies, slower onset, and decreased duration of action. When given tramadol (5 to 25 mg/kg, PO), all turtles had a decrease in breath frequency, but a decreased V_E was only evident when turtles received the higher tramadol doses (10 and 25 mg/kg, PO). Tramadol-treated (5 to 25 mg/kg, PO) turtles also had a long-term compensatory increase in V_T . To our knowledge, this is the first study in which the analgesic and respiratory effects of tramadol were investigated in a nonmammalian species.

The use of noxious thermal stimuli to assess nociception in turtles is advantageous because turtles are unrestrained, thus preventing a stress-induced decrease in nociception.³⁰ Furthermore, turtles have a quantifiable hind limb withdrawal latency, which is a reflex enabling immediate escape from the painful stimulus.⁸ Limitations of this experimental

approach include the steep heating slope of infrared stimuli that may preferentially activate A- δ fibers rather than C fibers, which respond with greater sensitivity to μ -opioid receptor agonists.³¹ Also, repeated daily and bimonthly exposure to thermal stimuli may cause skin lesions or decreased withdrawal latencies in control groups because of induction of hyperalgesia.³² Finally, heat avoidance in red-eared slider turtles is consistent and predictable, but the physiologic impact of noxious thermal stimuli in this species is not well understood. Despite these limitations, thermal withdrawal latencies provide an unambiguous and reproducible measure of complex nociceptive behavior.

In veterinary medicine, tramadol is primarily used in dogs and cats at doses that typically do not exceed 4.0 mg/kg (18.18 mg/lb).³³ Larger tramadol doses ranging between 11 and 80 mg/kg (5.0 and 36.36 mg/lb) were used safely for studies in cats and rodents.^{13,34} Because of limited clinical experience with tramadol in reptiles, a wide range of doses (1 to 25 mg/kg) were used in the present study to test antinociception in turtles. In mammals, the analgesic effects of tramadol typically begin within 30 minutes and last for 6 hours.³⁵ In contrast, tramadol (5 mg/kg, PO) administered to turtles significantly increased withdrawal latencies for 12 to 24 hours after drug administration, and higher doses (10 or 25 mg/kg, PO) had effects lasting 6 to 96 hours after administration. We speculate that tramadol acted on μ -opioid receptors because μ -opioid receptor activation increases withdrawal latencies in turtles.³⁶ Given the lack of pharmacokinetic studies with tramadol in turtles, it is unknown whether formation of M1 occurs in redeared slider turtles. Nonetheless, increased withdrawal latencies may also be due to centrally released serotonin and norepinephrine because the (+)-enantiomer and (–)-enantiomer of M1 inhibit serotonin and norepinephrine reuptake, respectively.¹⁴ In mammals, increased serotonin and norepinephrine release in the CNS contributes to descending inhibition in pain pathways via multiple mechanisms.³⁷

The most striking observation in the present study was the long-lasting tramadol-induced analgesia, particularly after oral administration. The reasons for the long-lasting effects may be slower pharmacokinetics (eg, gut absorption, liver metabolism, tissue distribution, and renal excretion) caused by a lower body temperature in turtles relative to mammals. Plasma M1 concentrations peak later than parent tramadol concentrations in mammals,³⁵ thus a relatively low body temperature may also cause greater temporal separation in the plasma concentrations of M1 and its parent drug. Finally, the long-lasting analgesia could be due to neuroplasticity in central nociceptive centers caused by μ -opioid receptor activation or an increase in central serotonin and norepinephrine concentrations.³⁷

Pharmacodynamic differences were evident between the PO and SC routes of tramadol administration in the turtles of this report. Specifically, tramadol administration SC was associated with lower withdrawal latencies, slower onset, and decreased duration of action, compared with tramadol administration PO. In mammals, first-pass metabolism in the liver is an important component in the analgesic effect of tramadol.^{16,17} Orally administered tramadol has the advantage of being absorbed in the intestinal tract and transported to the liver before reaching systemic circulation. On the other hand, a dose administered SC, particularly in the forelimb, is absorbed directly into systemic circulation, and a considerable portion of drug may be excreted by the kidneys before reaching the liver.³⁸ Hind limbs in turtles drain blood more directly to the liver via the renal-portal system,³⁹ compared with the forelimbs, and may be a more suitable injection site in turtles for drugs that require bioactivation in the liver to attain complete pharmacological effect. Oral administration of tramadol, however, imparts the clinical challenge of regurgitation and, thus, loss of an unknown amount of drug. Unfortunately, an injectable tramadol formulation is not commercially available. In our study, propping turtles up vertically and restricting water access for 5 minutes after tramadol administration mostly eliminated the risk of

regurgitation. Nonetheless, use of tramadol tablets rather than a water-based formulation could be beneficial for retrieving and readministering the exact amount of drug regurgitated.

Tramadol is a clinically useful drug because it provides analgesia with no respiratory depression in humans²¹⁻²³ and less respiratory depression than morphine in cats and dogs.^{24,25} In red-eared slider turtles, tramadol caused clinically important ventilatory depression at the 10 and 25 mg/kg doses, but not at the 5 mg/kg dose. The largest decrease in V_E was 70%, which occurred with the 25 mg/kg dose of tramadol at 24 hours after administration, whereas the 10 mg/kg dose resulted in a maximum 63% decrease in V_E at 48 hours after administration. In comparison, at 3 hours after morphine injection in red-eared slider turtles, V_E decreased by 83%, which is significantly greater than was evident for 5 and 10 mg/kg tramadol.⁸ Although the biological importance of respiratory depression in hypoxia-resistant species is unknown, anecdotal observations are that even low-dose opioid administration can cause adverse effects in red-eared slider turtles. Tramadol-induced respiratory depression in turtles is not surprising because injection of a specific μ -opioid agonist in awake turtles decreases V_E by decreasing breath frequency.⁴⁰ Lack of a compensatory increase in V_T suggests that μ -opioid receptor activation blunts CO_2 chemosensitivity.⁴⁰ Because tramadol and M1 act as μ -opioid receptor agonists, similar results in turtle respiration were evident with decreased V_E and decreased breath frequency. In contrast, V_T increased during tramadol-induced respiratory depression, suggesting that tramadol and M1 do not inhibit CO_2 chemosensitivity. If true, this is striking because tramadol causes no significant changes to CO_2 sensitivity in humans⁴¹ and causes a decrease in CO_2 sensitivity in cats.²⁴ A possible mechanism for the V_T increase in turtles is augmentation of motor output to respiratory-related muscles via serotonin release onto spinal respiratory motoneurons.⁴²

Respiratory depression is the major limiting factor for the clinical use of μ -opioid agonists.² Despite its excellent analgesic effect, tramadol administered PO at 25 mg/kg was the only dose associated with flaccid limbs and necks as well as respiratory depression within the first 12 hours after administration. Thus, this dose is not clinically safe for red-eared slider turtles. In terms of thermal analgesic effect and minimal respiratory depression, we concluded that the most clinically useful and safest dose range for red-eared slider turtles is 5 to 10 mg of tramadol/kg, PO. Although tramadol causes less respiratory depression than morphine, analgesics with μ -opioid agonist action should be used cautiously in turtles with respiratory disease to prevent further respiratory compromise. Future research should focus on the analgesic efficacy of tramadol (5 to 10 mg/kg, PO) in a surgical setting as well as alternative analgesics to μ -opioid receptor agonists for the relief of moderate and severe pain in reptiles.

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Abbreviations

V_E	Ventilation
V_T	Tidal volume

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Figure 1. Schematic diagram of a freely swimming turtle in a respiratory tank that allows for breathing in a circular chamber attached to a pneumotachometer (A) and representative voltage trace from a pneumotachograph (B). Upward deflections in the voltage trace represent expirations (exp), and downward deflections represent inspirations (insp). The bar indicates 2 seconds.

Figure 2.

Mean $\pm$ SEM hind limb withdrawal latency values as elicited by a noxious thermal stimulus (A) and change in withdrawal latency from the baseline (0 hours, before treatment) value (B) in 11 conscious red-eared slider turtles at 3, 6, 12, 24, 48, 72, and 96 hours after PO administration of single doses of tramadol and an equivalent volume of water (small circles) in a complete crossover study. Tramadol was administered at doses of 1, 5, 10, and 25 mg/kg (0.45, 2.27, 4.54, and 11.36 mg/lb; squares, triangles, diamonds, and large circles, respectively). Data symbols filled with black are significantly ($P < 0.05$) different from their respective baseline value. *Withdrawal latency value at this time point is significantly ($P < 0.05$) different from the value for water. †A significant ($P < 0.05$) drug effect existed for the indicated treatment when compared with the effect of water treatment.

Figure 3.

Mean $\pm$ SEM hind limb withdrawal latency values as elicited by a noxious thermal stimulus (A) and change in withdrawal latency from baseline (0 hours, before treatment) value (B) in 11 conscious red-eared slider turtles at 3, 6, 12, 24, 48, 72, and 96 hours after SC administration of single doses of tramadol and an equivalent volume of saline (0.9% NaCl) solution (small circles) in a complete crossover study. Tramadol was administered at doses of 10 and 25 mg/kg (diamonds and large circles, respectively). Data symbols filled with black are significantly ($P < 0.05$) different from their respective baseline value at 0 hours. *Withdrawal latency value at this time point is significantly ($P < 0.05$) different from the value for saline solution. †Significant ($P < 0.05$) drug effect exists for the indicated treatment when compared with the effect of saline solution.

Figure 4.

Mean $\pm$ SEM V_E (A), breath frequency (B), and V_T (C) in 11 conscious red-eared slider turtles at baseline (0 hours, before treatment) and 3, 6, 9, and 12 hours after PO administration of single doses of tramadol and an equivalent volume of water (small circles) in a complete crossover study. Tramadol was administered at doses of 5, 10, and 25 mg/kg (triangles, diamonds, and large circles, respectively). Data symbols filled in with black are significantly ($P < 0.05$) different from their respective baseline value at 0 hours. See Figure 2 for remainder of key.

Figure 5.

Mean $\pm$ SEM V_E (A), breath frequency (B), and V_T (C) in 8 conscious red-eared slider turtles at baseline (0 hours, before treatment) and 24, 48, and 72 hours after PO administration of single doses of tramadol and an equivalent volume of water (small circles) in a complete crossover study. Tramadol was administered at doses of 5, 10, and 25 mg/kg (triangles, diamonds, and large circles, respectively). See Figure 2 for remainder of key.