Pharmacokinetics of repeated oral administration of tramadol hydrochloride in Hispaniolan Amazon parrots (Amazona ventralis)

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Objective—To determine the pharmacokinetics of tramadol hydrochloride (30 mg/kg) following twice-daily oral administration in Hispaniolan Amazon parrots (Amazona ventralis).

Animals—9 healthy adult Hispaniolan Amazon parrots.

Procedures—Tramadol hydrochloride was administered to each parrot at a dosage of 30 mg/kg, PO, every 12 hours for 5 days. Blood samples were collected just prior to dose 2 on the first day of administration (day 1) and 5 minutes before and 10, 20, 30, 60, 90, 180, 360, and 720 minutes after the morning dose was given on day 5. Plasma was harvested from blood samples and analyzed by high-performance liquid chromatography. Degree of sedation was evaluated in each parrot throughout the study.

Results—No changes in the parrots’ behavior were observed. Twelve hours after the first dose was administered, mean ± SD concentrations of tramadol and its only active metabolite M1 (O-desmethyltramadol) were 53 ± 57 ng/mL and 6 ± 6 ng/mL, respectively. At steady state following 4.5 days of twice-daily administration, the mean half-lives for plasma tramadol and M1 concentrations were 2.92 ± 0.78 hours and 2.14 ± 0.07 hours, respectively. On day 5 of tramadol administration, plasma concentrations remained in the therapeutic range for approximately 6 hours. Other tramadol metabolites (M2, M4, and M5) were also present.

Conclusions and Clinical Relevance—On the basis of these results and modeling of the data, tramadol at a dosage of 30 mg/kg, PO, will likely need to be administered every 6 to 8 hours to maintain therapeutic plasma concentrations in Hispaniolan Amazon parrots. (Am J Vet Res 2013;74:957–962)

The use of analgesics is now considered standard care in avian medicine. Studies of various analgesics in avian species have shown that, in many situations, the drugs must be administered frequently and by injection. However, for some analgesics, only the pharmacokinetics or pharmacodynamics of 1 dose has been examined and the efficacy and adverse effects of repeated administration are often unknown. The situation is unfortunate because a long-acting, orally administered analgesic that has little or no adverse effects when repeatedly given might improve the quality of life of birds with painful conditions.

Tramadol is a centrally acting, weak μ-receptor agonist, and its main metabolite M1 (O-desmethyltramadol) has greater activity at these receptors than the parent compound. Tramadol also acts by inhibiting the reuptake of serotonin and norepinephrine, leading to activation of the descending pain inhibitory system. Tramadol has numerous metabolites, but only M1 has been shown to have clinically important analgesic properties.

The pharmacokinetics of 1 dose of tramadol HCl has been examined in Hispaniolan Amazon parrots (Amazona ventralis), red-tailed hawks, bald eagles, and peafowl. Mean ± SD therapeutic plasma concentrations of tramadol and M1 in humans are reported to be 298 ± 171 ng/mL to 590 ± 410 ng/mL and 39.6 ± 29.5 ng/mL to 84 ± 34 ng/mL, respectively, but therapeutic concentrations in avian species are largely unknown. Oral administration of tramadol at a dose
of 30 mg/kg to Hispaniolan Amazon parrots can result in antinociceptive effects in response to a thermal noxious stimulus, with therapeutic plasma concentrations similar to those in humans. To date, no study has been conducted to evaluate the pharmacokinetics of repeated tramadol administration. It is possible that with repeated administration, tramadol and its metabolites could accumulate and lead to adverse effects such as sedation and a decrease in appetite. The purpose of the study reported here was to determine whether tramadol or its major metabolite, M1, accumulates in the bloodstream following oral, twice-daily administration to Hispaniolan Amazon parrots and to observe any behavioral changes indicating sedation.

Materials and Methods

Animals—Nine healthy adult Hispaniolan Amazon parrots with a mean ± SD body weight of 321 ± 28 g were used in this study. The parrots were members of a closed colony housed at the University of Tennessee College of Veterinary Medicine. Food was not withheld at any point during the study. All procedures were approved by the University of Tennessee Institutional Animal Care and Use Committee.

Experimental protocol—Tramadol HCl was compounded into a solution as described for administration with a gavage tube into the crop. The compounded drug was administered to each bird at a dosage of 30 mg/kg, PO, every 12 hours for 5 days. Prior to each administration, birds were evaluated for signs of sedation by the same observer (MJS; Appendix).

Blood sample collection and processing—A blood sample (0.25 mL) was collected from each parrot by means of venipuncture with a 26-gauge needle and insulin syringe to evaluate plasma concentrations of the morning dose was given on day 5. Samples were transferred into lithium heparin tubes and refrigerated at 4°C until centrifugation at 16,000 g for 10 minutes. The plasma was separated and kept frozen in 2 months after collection.

Measurement of plasma drug concentrations—Plasma samples were analyzed with a modified version of a published method for reverse-phase high-performance liquid chromatography. The system consisted of a 2695 separations module, 2475 fluorescence detector, and computer. Tramadol and M1 were extracted from plasma samples through liquid extraction. Briefly, previously frozen plasma samples were thawed and mixed with a vortex device, and a 100-µL aliquot was transferred to a clean test tube, followed by 30 µL of internal standard (butorphanol [5 µg/mL]). Seventy microliters of 29.7% ammonium hydroxide was added, followed by 1.5 mL of a mixture of ethyl acetate and hexane (40:60 [vol/vol]). Tube contents were mixed with a vortex device for 1 minute and then centrifuged for 20 minutes at 1,000 × g. The organic layer was harvested and transferred to a clean tube, then evaporated to dryness with nitrogen gas. Samples were reconstituted in 225 µL of mobile phase, and 100 µL of each sample was analyzed.

The compounds were separated on a C18 column with a C18 guard column. The mobile phase was a mixture (92.8 [vol/vol]) of 0.01M potassium phosphate buffer (pH, 2.9; with 0.1% triethylamine) and acetonitrile. The flow rate was 1.1 mL/min, and the column was kept at room temperature (22°C). Fluorescence was measured at an excitation wavelength of 202 nm and an emission wavelength of 296 nm.

Pharmacokinetic analysis—Values of pharmacokinetic parameters for tramadol and M1 were calculated by use of a noncompartmental approach and computer software. On the basis of the assumption that absorption of tramadol is a first-order process, values for the terminal semilogarithmic slope, t1/2, maximum plasma concentration, time to maximum plasma concentration, AUC0–∞, AUMC0–∞, and MRT were calculated from the noncompartmental analysis. The AUC and area under the first moment time curve were calculated following the linear and log-linear trapezoidal rules. The MRT was calculated as AUMC0–∞ /AUC0–∞. The compartmental pharmacokinetic model was a 1-compartmental model with first-order elimination. In this model, the assumption is that K01 is considerably greater than K10 or that there is no so-called flip-flop effect caused by slow absorption. The K01 assumes first-order absorption.

The initial parameters (volume of distribution, K01, and K10) required for the simulation were obtained. The best model included an absorption term and biexponential decay and was weighted 1/yhat and used to simulate the concentration-time profile for several doses. Parameters of this mean model were defined by mean ± SD volume of distribution (0.0586 ± 0.0150 mL/kg), K01 (1.7877 ± 0.4720 h–1), and K10 (0.2731 ± 0.0802 h–1) and were used to generate simulated dosing curves.

Data were analyzed through the use of different weighting schemes for the various models. After each analysis, values of the Akaike information criterion and Schwartz criterion as well as goodness of fit (residual and function plots) were inspected and used to identify the most appropriate model.

Pharmacokinetic parameters for a 1-time dose of tramadol HCl were determined through the use of data from another study that involved the same analyses of plasma with the same equipment as described for the present study; additionally, birds used in both studies were from the same colony housed at the University of Tennessee. Nine adult birds (3 males, 3 females, and 1 of unknown sex) were used in the previous study and had a mean body weight of 319 ± 30 g. Approximately
2 years had elapsed between data collection for the previous and present studies.

Statistical analysis—Pharmacokinetic parameter data were tested for normality of distribution and equal variance with the aid of commercially available software. When the data were normally distributed and had equal variances, a t test was performed to determine whether differences existed between pharmacokinetic parameters from 1-time and repeated oral administration of tramadol HCl. When the values were not normally distributed or did not have equal variances, the Mann-Whitney test was performed. Values of P < 0.05 were considered significant for all statistical tests.

Results

Animals—No adverse effects were observed in any of the 9 Hispaniolan Amazon parrots at any point during the study period. Degree of sedation was assessed a total of 81 times (9 time points for each of the 9 birds). Of these evaluations, 76 yielded a sedation score of 3 (ie, bird was tranquil and responded to moderate auditory stimuli or movement in front of the cage) and only 5 yielded a score of 2 (ie, bird alert and oriented with a brisk response to movement in front of the cage). The baseline score prior to drug administration for all 9 birds was 3. No signs of sedation were noticed in any of the birds at any point during the study.

Pharmacokinetic parameters—Calculated values of pharmacokinetic parameters were summarized (Table 1), and plasma concentrations of tramadol and M1 across time were graphically displayed (Figure 1). Twelve hours after the first dose was administered, mean ± SD concentrations of tramadol and its only active metabolite M1 were 53 ± 57 ng/mL and 6 ± 6 ng/mL, respectively. At steady state following 4.5 days of twice-daily administration, the mean half-lives for plasma tramadol and M1 concentrations were 2.92 ± 0.78 hours and 2.14 ± 2.1 hours, respectively. Comparison with findings of previous studies suggested that therapeutic plasma concentrations of tramadol (158 ± 47 ng/mL) and M1 (33 ± 30 ng/mL) were maintained for approximately 6 hours in the present study. Plasma concentrations of M2, M4, and M5 ranged from 8 to 563 ng/mL, 34 to 134 ng/mL, and 7 to 264 ng/mL, respectively.

Mathematical simulation showed that hypothetical administration of 30 mg of tramadol HCl/kg every 6 hours could result in tramadol concentrations that ranged from 150 to 450 ng/mL (Figure 2). Administration of the same dose every 8 hours could produce concentrations that ranged from 75 to 410 ng/mL (Figure 3). Both models resulted in concentrations in
the therapeutic range for most of the administration period. Significant differences between 1-time and repeated oral administration of tramadol were identified for $t_\frac{1}{2}$ ($P < 0.001$), elimination rate ($P = 0.002$), and MRT ($P < 0.001$) for tramadol and for $t_\frac{1}{2}$ ($P = 0.004$), $K_{10}$ ($P = 0.03$), and $\text{AUC}_0^\infty$ ($P = 0.35$) for $M1$ (Table 1).

Discussion

No evidence of sedation or accumulation of metabolites was evident when Hispaniolan Amazon parrots were given tramadol HCl at a dosage of 30 mg/kg, PO, every 12 hours for 5 days, despite therapeutic plasma concentrations being maintained for approximately 6 hours. In a study involving humans, the bioavailability of tramadol HCl administered PO increased with repeated administration; however, the frequency of administration was 4 times/d. The increase in bioavailability is believed to be caused by saturation of first-pass metabolism. In humans, cytochrome P450 2D6 is responsible for the metabolism of tramadol, but a large degree of variability exists in how quickly the drug is metabolized as well as in other species. Because of this variability, species-specific values of pharmacokinetic parameters must be determined.

The exact mechanisms of tramadol HCl metabolism are not known in parrots, but because many pharmacokinetic values did not increase with repeated drug administration and some values actually decreased in the present study, it is unlikely that twice-daily administration led to saturation of any metabolic pathways. It is possible that repeated oral administration led to induction of certain enzymes that metabolize tramadol, leading to a lower $t_\frac{1}{2}$, $K_{10}$, and MRT than would be attained with only 1 dose. More frequent administration than every 12 hours may lead to saturation of metabolic pathways, therefore altering pharmacokinetic values. Additionally, some differences in pharmacokinetic values were identified between the present study and the previous study, some of which might have been partly attributable to the 2 years that elapsed between the studies and aging processes in the birds, which might have altered how the drug was metabolized. The parrots of the present study had other metabolites of tramadol HCl ($M2$, $M4$, and $M5$) in their bloodstream, but it is unknown whether any of these metabolites have a therapeutic effect. No evidence is available to suggest that any metabolite other than $M1$ is therapeutic in other species. Additional studies are needed to examine the mechanisms of tramadol metabolism in parrots.

Previous studies involving a thermal noxious stimulus have shown that the mean therapeutic plasma concentration of tramadol in Hispaniolan Amazon parrots is similar to that in humans ($298 \pm 171 \text{ ng/mL}$).
to 590 ± 410 ng/mL), and concentrations in our study were within this range for up to approximately 6 hours after the final dose was administered. Additional studies in parrots may be needed to determine whether therapeutic plasma concentrations vary depending on the type of noxious stimulus. Because therapeutic tramadol concentrations were maintained for only about 6 hours after drug administration concluded in this study, additional analyses were performed to simulate tramadol administration at more frequent intervals than every 12 hours to determine a potential optimal dosing regimen for maintenance of therapeutic concentrations in parrots.

Initially, several 2-compartment models and weighting options were attempted but failed to generate parameters required for dose simulations. Therefore, a 1-compartment model was used. Although increasing the dose of tramadol HCl to 60 mg/kg would increase the period during which a therapeutic range could be attained, the likelihood of sedation or other adverse effects would increase because of higher maximum concentrations. A simulated dosing regimen of 30 mg/kg every 6 to 8 hours was predicted to maintain therapeutic concentrations of tramadol for an adequate duration without greatly increasing the maximum concentration and therefore increasing the incidence of adverse effects (Figures 2 and 3). Some limitations of our predictions include the assumption of linear pharmacokinetics within the range of simulated concentrations, the unknown effect of interindividual pharmacokinetic variability in the population of parrots, and the application of a human therapeutic range to parrots.

Studies have not yet been performed to determine whether pharmacokinetic values in Hispaniolan Amazon parrots would be different following repeated oral administration of tramadol (30 mg/kg) at a frequency of every 6 to 8 hours rather than every 12 hours, and it is unknown whether this increased frequency would lead to sedative effects in the birds. Analogic and adverse effects are likely to differ among avian species; therefore, responses to drug administration should be monitored in individual birds. A study9 in humans has shown that absorption increases when tramadol is administered with food, so doses may need to be adjusted in anorexic patients. Additionally, humans with renal insufficiency or hepatic impairment may have a slower than typical metabolism and clearance and require a lower dose to achieve a therapeutic concentration,9 which may also be true in veterinary patients. Finally, a more concentrated compounded solution of tramadol HCl (ie, > 10 mg/mL) may ease administration by bird owners to ensure more accurate dosing.

References

Appendix
Scoring system for evaluation of the degree of sedation in Hispaniolan Amazon parrots (*Amazona ventralis*).

<table>
<thead>
<tr>
<th>Score</th>
<th>Bird behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Signs of anxiety and agitation or restless, or both</td>
</tr>
<tr>
<td>2</td>
<td>Alert and orients with brisk response to movement in front of the cage</td>
</tr>
<tr>
<td>3</td>
<td>Tranquil and responds to moderate auditory stimuli or movement in front of the cage</td>
</tr>
<tr>
<td>4</td>
<td>Responds to moderate auditory stimuli only</td>
</tr>
<tr>
<td>5</td>
<td>Briskly responds to loud auditory stimulus</td>
</tr>
<tr>
<td>6</td>
<td>Sluggishly responds to loud auditory stimulus</td>
</tr>
<tr>
<td>7</td>
<td>Nonresponsive to loud auditory stimulus</td>
</tr>
<tr>
<td>8</td>
<td>Nonresponsive to any stimulus except direct physical contact</td>
</tr>
</tbody>
</table>