Plasma concentrations of praziquantel after oral administration of single and multiple doses in loggerhead sea turtles (Caretta caretta)

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Objective—To determine the pharmacokinetics of praziquantel following single and multiple oral dosing in loggerhead sea turtles.

Animals—12 healthy juvenile loggerhead sea turtles.

Procedure—Praziquantel was administered orally as a single dose (25 and 50 mg/kg) to 2 groups of turtles; a multiple-dose study was then performed in which 6 turtles received 3 doses of praziquantel (25 mg/kg, PO) at 3-hour intervals. Blood samples were collected from all turtles before and at intervals after drug administration for assessment of plasma praziquantel concentrations. Pharmacokinetic analyses included maximum observed plasma concentration (C\text{max}), time to maximum concentration (T\text{max}), area under the plasma praziquantel concentration-time curve, and mean residence time (MRT).

Results—Large interanimal variability in plasma praziquantel concentrations was observed for all dosages. One turtle that received 50 mg of praziquantel/kg developed skin lesions within 48 hours of administration. After administration of 25 or 50 mg of praziquantel/kg, mean plasma concentrations were below the limit of quantification after 24 hours. In the multiple-dose group of turtles, mean plasma concentration was 90 ng/mL at the last sampling time-point (48 hours after the first of 3 doses). In the single-dose study, mean C\text{max} and T\text{max} with dose were not significantly different between doses. After administration of multiple doses of praziquantel, only MRT was significantly increased, compared with values after administration of a single 25-mg dose.

Conclusions and Clinical Relevance—Oral administration of 25 mg of praziquantel/kg 3 times at 3-hour intervals may be appropriate for treatment of loggerhead sea turtles with spirorchidiasis. (Am J Vet Res 2003;64:304–309)

The family Spirorchiidae contains digenetic trematodes with life cycles that include fresh water and sea turtles as the final or definitive host. There are at least 8 genera and 20 described species of spirorchiids, which are known to infect loggerhead (Caretta caretta), green (Chelonia mydas), and hawksbill (Eretmochelys imbricata) sea turtles. While all members of the family Spirorchiidae require an intermediate host to complete their life cycle, no specific intermediate host has been identified for any of the sea turtle spirorchiids. Thus, the complete life cycle for sea turtle spirorchiids remains to be elucidated.

Spirorchiids are considered the most pathogenic of all the parasites known to infect sea turtles.2 Spirorchiids are parasites of the vascular system, preferentially affecting the heart and arterial system of their turtle hosts.1 Adult spirorchiids may cause endocarditis, arteritis, and thrombosis of vessels.4 In addition to direct effects of the adult parasite, eggs that are released within the vascular system may be transported to peripheral areas of the turtle’s body where they lodge in small vessels, often initiating a mild to severe granulomatous inflammatory response. The eggs can also migrate through the walls of vessels and cause tissue damage and inflammation in adjacent tissues. Parasitism with spirorchiids may also predispose turtles to secondary gram-negative bacterial infections.5

In the period from October 2000 to February 2001, juvenile loggerhead sea turtles with signs of CNS disease were seen in waters off southern Florida. Necropsies of turtles that died during this epidemic revealed neuropsorchiadiasis.6 Adult trematodes were found in the meninges of the brain, and eggs of these parasites were seen in the meninges and neuropil of the brain and spinal cord.

Humans in third world tropical nations are susceptible to infection with a related group of cardiovascular trematodes called schistosomes7; these parasites are responsible for considerable illness and death.8 An estimated 300,000 to 500,000 deaths from schistosome infection occur each year.9 In addition to visceral lesions, involvement of the CNS is known to occur in humans.10 Lesions in humans with neuroschistosomiasis show many similarities to the lesions of loggerhead sea turtles with neuropsorchiadiasis.

The broad-spectrum anthelmintic drug praziquantel is the drug of choice when treating humans with schistosomiasis infections.11,12 While treatment regimens for schistosomiasis have been extrapolated from humans to green turtles with spontaneous spirorchiid infection,11 the dose recommended in 1 study was subsequently judged to be too high.4

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The purpose of the study reported here was to determine the pharmacokinetics of praziquantel after oral administration of single and multiple doses in loggerhead sea turtles and to propose dosing recommendations for treatment of sea turtles infected with spirochiid trematodes.

**Materials and Methods**

Turtles—Twelve juvenile loggerhead sea turtles were used in this study. This project was approved by the University of Florida Institutional Animal Care and Use Committee. All samples were collected under the authorization of Florida Fish and Wildlife Conservation Commission Marine Turtle Permit No. 086.

**Single-dose study**—Twelve turtles were available for participation in the single-dose study. Data from 1 turtle were not included in the results of the single-dose study, because it received an incorrect dose of praziquantel. Eleven loggerhead sea turtles (2 females and 9 turtles of undetermined sex) that weighed between 7.3 and 81 kg were allocated to 1 of 2 groups (group 1, n = 6; group 2, n = 5). Each group had turtles with approximately the same range of weights. Turtles were maintained in indoor pools with water temperatures between 27 and 30°C. Their diet consisted of locally purchased squid (Opalesgus loligo). To administer praziquantel at the appropriate dose to each turtle, tablets were combined with a meal of squid. After removal of the tentacles and viscera from a squid, the required number of praziquantel tablets was inserted into the body cavity; to enclose the drug, the ends of the squid were overlapped and sutured with 3-0 absorbable suture material. The praziquantel-containing squid was offered to the designated turtle. Turtles in groups 1 and 2 received single doses of praziquantel orally (25 and 50 mg/kg, respectively). From each turtle, blood samples were collected prior to each dose of praziquantel; 1.5 hours after administration of each of the 3 doses; and 3, 6, 18, and 42 times the dose point. The AUCt and MRTt were calculated for individual turtles only if ≥ 3 plasma praziquantel concentrations exceeded the LOQ of the assay.

**Multiple-dose study**—On the basis of results from the single-dose study, a multiple-dose administration study was performed. Six turtles (2 females and 4 turtles of undetermined sex) weighing between 4.6 and 93.4 kg received 3 doses (25 mg/kg each) of praziquantel orally at 3-hour intervals. All turtles had previously received a single dose of praziquantel at a rate of 25 mg/kg (n = 2), 50 mg/kg (3), or 100 mg/kg (1 turtle was dosed in error); data from 5 of these turtles were included in the single-dose study. Because of this previous praziquantel treatment, a period of 4 to 5 months was allowed to elapse prior to the start of the multiple-dose study. Turtles were selected to represent the same weight range as in the single-dose groups. Blood samples were collected prior to each dose of praziquantel; 1.5 hours after administration of each of the 3 doses; and 3, 6, 18, and 42 hours after administration of the third dose. Blood was collected and processed as described. Plasma was transferred to cryotubes and frozen in a container of liquid nitrogen.

**Plasma assays**—All plasma samples were transported on dry ice to Midwest Research Institute, Kansas City, Mo. On arrival, plasma samples were stored for ≤ 3 weeks in a freezer (−10 to −20°C), until assays were performed. Plasma samples were analyzed in 5 sets. Set 1 included plasma samples from 4 turtles in the single-dose (23 mg/kg) group. Set 2 included plasma samples from each of 2 turtles in the single-dose (25 and 30 mg/kg) groups. Set 3 included plasma samples from 3 turtles in the single-dose (50 mg/kg) group. Set 4 included plasma samples from 4 turtles in the multiple-dose group. Set 5 included plasma samples from 2 turtles in the multiple-dose group. Plasma concentrations of praziquantel were determined by use of high-performance liquid chromatography (HPLC) coupled with UV detection. The technique is a modification of a similar assay reported for praziquantel measurements in plasma of dogs. A carbon-18 disposable column was conditioned with 5 mL of methanol, 5 mL of water, and 1 mL of 0.01M potassium hydroxide solution; care was taken to prevent drying of the column. Aliquots of plasma (500 µL) were mixed with an equal amount of water and transferred to the conditioned carbon-18 column. Vacuum pressure (approx 5 mm Hg) was applied to force the sample through the column. The column was rinsed twice with 5 mL of 10% methanol solution. Praziquantel was eluted into a clean tube with 2 rinses of 5 mL of 100% ethyl acetate solution. The sample was evaporated to dryness under nitrogen at 37 ± 5°C. The residue was dissolved in 500 µL of acetonitrile, and the vial was sonicated for 5 minutes. Water (500 µL) was added to the vial, mixed, and 50 µL of the resultant solution were injected onto the HPLC-UV system. While a percentage recovery experiment was not performed for loggerhead sea turtles, calibration curves and quality control samples (consisting of a solution of praziquantel-naïve loggerhead sea turtle plasma with praziquantel added to known concentrations) were analyzed with each set of samples. Calibration curves had a linear coefficient of determination (r²) of at least 0.99 for each analysis. For 3 sets of samples, the limit of quantitation (LOQ) was 12.5 ng/mL, whereas for 2 sets of samples, the LOQ was 25.0 ng/mL because the 12.5 ng/mL standard was outside the range of specificity. Samples with concentrations < LOQ were assigned values of zero for calculation purposes. The overall accuracy for this assay was 95% with a standard deviation of 10%. All samples were assayed once.

**Pharmacokinetic analysis**—Plasma praziquantel concentrations determined in each turtle were analyzed by use of statistical moment theory. Maximum observed plasma concentration (Cmax) and time to maximum concentration (Tmax) were determined directly from the data. The area under the plasma praziquantel concentration-time curve (AUCt) was calculated with the standard method of summing trapezoids from time 0 to the last sampling point. However, AUCt was not extrapolated to infinity because a distinct decay phase was not detected in most turtles. Mean residence time (MRTt) was similarly calculated to the last sampling point. The AUCt and MRTt were calculated for individual turtles only if ≥ 3 plasma praziquantel concentrations exceeded the LOQ of the assay.

**Statistical analyses**—Because of the small sample size, a nonparametric Kruskal-Wallis 1-way ANOVA on ranks was used to compare the values of Cmax, ηmax, AUCt, and MRTt, that were obtained after single doses of praziquantel (25 and 50 mg/kg) or after multiple doses. If a significant (P ≤ 0.05) difference among groups was found, the Dunn multiple-comparison test was used to determine which factors were significantly different.

**Results**

**Single-dose study**—After a single orally administered dose of 25 or 50 mg of praziquantel/kg, large interindividual variability in plasma praziquantel concentrations was observed (Fig 1 and 2). Of the turtles that received a single dose of 25 mg of praziquantel/kg, 2 had plasma praziquantel concentrations that exceeded the assay’s LOQ at 2 time points, 3 had plasma praziquantel concentrations that exceeded the assay's LOQ at 2 time points, 3 had plasma praziquantel concentrations that exceeded the assay's LOQ at 2 time points, 3 had plasma praziquantel concentrations that exceeded the assay's LOQ at 2 time points.
iquantel concentrations that exceeded the LOQ at 4 time points, and 1 had plasma praziquantel concentrations that exceeded the LOQ at 6 time points. Mean ± SD $C_{\text{max}}$ was 109 ± 50 ng/mL, mean $T_{\text{max}}$ was 10 ± 8 hours, mean $AUC_t$ was 1,331 ± 688 ng·mL$^{-1}$·h, and MRT$_t$ was 9 ± 3 hours. Of the turtles that were given a single dose of 50 mg/kg, 2 had plasma praziquantel concentrations that exceeded the LOQ at 2 time points, 1 had plasma praziquantel concentrations that exceed-

Figure 1—Plasma praziquantel concentration versus time after oral administration of praziquantel as a single dose of 25 mg/kg in 6 loggerhead sea turtles. *Forty-eight hours after drug administration, plasma praziquantel concentration was < 25 ng/mL in all turtles.

Figure 2—Plasma praziquantel concentration versus time after oral administration of praziquantel as a single dose of 50 mg/kg in 5 loggerhead sea turtles. See Figure 1 for key.

Table 1 Pharmacokinetic variables for praziquantel in loggerhead sea turtles after oral administration of a single dose at a rate of 25 mg/kg ($n = 6$) or 50 mg/kg ($n = 5$) and after oral administration of 3 doses at a rate of 25 mg/kg every 3 hours ($n = 6$)

<table>
<thead>
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<th>Variable</th>
<th>Single dose</th>
<th>Multiple doses</th>
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<tr>
<td></td>
<td>25 mg/kg</td>
<td>50 mg/kg</td>
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<td>$C_{\text{max}}$ (ng/mL)</td>
<td>109* ± 50</td>
<td>342* ± 341</td>
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<td>111 (53–188)</td>
<td>165 (51–873)</td>
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<td>$T_{\text{max}}$ (h)</td>
<td>10* ± 8</td>
<td>14* ± 10</td>
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<td>9 (1–24)</td>
<td>12 (3–24)</td>
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<td>$AUC_t$ (ng·mL$^{-1}$·h)</td>
<td>1,331* ± 888</td>
<td>4,853* ± 2,932</td>
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<td>1,074 (839–2,338)</td>
<td>6,433 (1,470–6,656)</td>
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<tr>
<td>MRT$_t$ (h)</td>
<td>9* ± 3</td>
<td>11* ± 5</td>
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<td></td>
<td>9 (6–12)</td>
<td>10 (7–16)</td>
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Data are presented as mean ± SD and median (range). *Within a row, values with different superscripts are significantly (P ≤ 0.05) different. 'n = 4, 'n = 3, 'n = 5.

$C_{\text{max}}$ = Maximum observed plasma concentration. $T_{\text{max}}$ = Time of maximum plasma concentration. $AUC_t$ = Area under the plasma praziquantel concentration-time curve from zero to the last sampling time. MRT$_t$ = Mean residence time from zero to the last sampling time.

Figure 3—Dermatologic response after single oral administration of praziquantel (50 mg/kg) in a loggerhead sea turtle. A—Photograph of ventral aspect of head and neck of the affected sea turtle. Notice raised necrotizing skin lesions. B—Photomicrograph of a section of dermis from the affected sea turtle. Notice an area of epidermal necrosis (arrow), with accumulation of necrotic debris on the skin surface (arrowhead) and coagulation necrosis (CN) of the dermis beneath the basement membrane. H&E stain; bar = 100 µm.
ed the LOQ at 4 time points, 1 had plasma praziquantel concentrations that exceeded the LOQ at 5 time points, and 1 had plasma praziquantel concentrations that exceeded the LOQ at 6 time points. Forty-eight hours after dosing, plasma praziquantel concentrations in all turtles were < LOQ. Mean ± SD C max was 342 ± 341 ng/mL, mean T max was 14 ± 10 hours, mean AUC i was 4,853 ± 2,932 ng·mL⁻¹·h, and MRT i was 11 ± 5 hours. Mean values of C max and AUC i increased with dose, whereas MRT i and T max were similar after either dose (Table 1).

One of 3 loggerhead sea turtles that received 50 mg of praziquantel/kg developed raised necrotizing skin lesions within 48 hours (Fig 3). None of the turtles that received praziquantel at a dose of 25 mg/kg developed these lesions. Biopsy specimens were collected, and light microscopy revealed necrosis of the epidermis and the dermis beneath the basement membrane (Fig 3). The lesions healed within 11 days.

Multiple-dose study—Considerable interindividual variability in praziquantel plasma concentrations was also observed after oral administration of 3 doses of praziquantel at 25 mg/kg of body weight (Fig 4). After multiple doses of praziquantel, 1 turtle had plasma praziquantel concentrations that exceeded the LOQ at 2 time points, 1 turtle had plasma praziquantel concentrations that exceeded the LOQ at 3 time points, 1 turtle had plasma praziquantel concentrations that exceeded the LOQ at 5 time points, 1 turtle had plasma praziquantel concentrations that exceeded the LOQ at 6 time points, and 2 turtles had plasma praziquantel concentrations that exceeded the LOQ at 7 time points. Plasma praziquantel concentrations in 5 of the 6 turtles remained greater than the assay's LOQ 48 hours after the administration of the first dose. Mean ± SD C max was 221 ± 274 ng/mL, mean T max was 28 ± 17 hours, mean AUC i was 5,047 ± 3,760 ng·mL⁻¹·h, and mean MRT i was 26 ± 7 hours. After administration of multiple doses of praziquantel, mean values of T max, AUC i, and MRT i were greater than those values observed after administration of a single dose of 25 mg/kg (Table 1); however, only MRT i was significantly greater after 3 doses of 25 mg/kg, compared with administration of a single dose of 25 mg/kg.

Discussion

In sea turtles, the recommended dose of praziquantel is reported as 10 to 20 mg/kg. Praziquantel is available in injectable and oral forms. In the only efficacy study reported for reptiles, green turtles with spontaneous spirorchid infection were treated with praziquantel PO at a dosage of 50 mg/kg, administered 3 times in 1 day (at 0, 7, and 9 hours). In that study, the authors selected the dosage on the basis of estimates of the effective dose (ED95) of praziquantel against trematodes in different species of animals. Treated turtles were subsequently euthanatized on days 3 and 7 after treatment. Cardiovascular trematodes were absent or dead in treated turtles; untreated control turtles were infected. While this regimen was effective, the dosage was subsequently considered excessive. The study reported here was designed to provide information from which to derive an appropriate oral dose of praziquantel in loggerhead sea turtles.

Our study had direct clinical relevance for wild ill loggerhead sea turtles admitted into sea turtle rehabilitation facilities in Florida, particularly in light of a recently identified epidemic of neurosprirochiadiasis. Because loggerhead sea turtles are federally protected in the United States, a pharmacokinetic study was designed instead of an efficacy study that would necessitate euthanasia of treated animals. Clinically healthy loggerhead sea turtles in a rehabilitation facility in Florida were used in our study prior to their release into the wild. The orally administered form of praziquantel was selected for comparison with results of the only efficacy study of praziquantel in sea turtles known to the authors. Additionally, the oral formulation of praziquantel was used rather than the injectable formulation because of concerns that injection of large volumes of solution could be associated with pain at the injection site and other adverse effects in the sea turtles. The injectable formulation for use in small animal practice is marketed at a concentration of 56.8 mg/mL. The volume needed to achieve doses of 25 and 50 mg/kg in large sea turtles in our study was considered excessive. For example, the largest turtles in the 25 and 50 mg/kg single-dose groups weighed 61 and 81 kg, respectively. To achieve the study doses, these turtles would require volumes of 26 and 71 mL of injectable praziquantel, respectively. In the multiple-dose group, even greater volumes of praziquantel would be required per turtle. Signs of pain and irritation have been reported in animals receiving injectable praziquantel, and a maximum injection volume of 3 mL is recommended in dogs. Lower doses could possibly be used if given by injection, but this would need to be confirmed in another study.

In the single and multiple dose groups, there was considerable variability in plasma praziquantel concentrations among turtles at each sampling time. Although increases in AUC and dose appeared to be possibly associated, AUC was not significantly different at any dose because of the variability within each group. Interindividual variability may have been influenced by factors such as body weight, gastrointestinal motility, and gastric-emptying rate.
enced by the disparity in metabolic sizes associated with the wide range in body mass of turtles in each group. Metabolic scaling of drugs has been recommended in reptiles, but problems have been identified that limit broad application of groups of drugs in different species of reptiles. The influence of body size on uptake and elimination of praziquantel was beyond the scope of our study.

One turtle in the 50-mg/kg single-dose group developed necrotizing skin lesions that, microscopically, resembled toxic epidermal necrosis. Lesions included areas of epidermal necrosis with detachment that developed within 48 hours after administration of praziquantel and resolved 11 days later. Because these lesions developed in 1 turtle in the higher single-dose group, the lower dose of praziquantel was selected for the multiple-dose study.

In our study, a lack of a clear elimination phase was problematic when attempting to estimate AUC and, by extension, MRT. Because of this, we chose to estimate AUC and MRT, as reported in other studies. Although AUC is not an accurate representation of total AUC extrapolated to infinity, this method allowed comparisons among plasma concentration data at different doses to be made. These calculations indicated that MRT and, therefore, drug exposure increased with increasing dose.

In the multiple-dose study, turtles received 25 mg/kg of praziquantel orally 3 times in 1 day at 3-hour intervals (at 0, 3, and 6 hours) on the basis of a similar dosing regimen recommended in humans with schistosomiasis. Mean plasma concentrations of praziquantel in the multiple-dose group were comparable to those in turtles that received praziquantel in a single dose of 50 mg/kg. However, no skin lesions were observed in any of the turtles that were administered praziquantel in 3 doses of 25 mg/kg. For the single-dose group, plasma praziquantel concentrations were not quantifiable > 24 hours after administration, while plasma praziquantel concentrations were still measurable 48 hours after the first dose in the multiple-dose group. In a study performed in human volunteers that were given praziquantel at 25 mg/kg every 2 hours for 3 treatments, plasma praziquantel concentrations were maintained for 12 hours. This was considered a benefit compared with findings of single-dose studies. Similarly, in loggerhead sea turtles, MRT was greater and plasma praziquantel concentrations persisted for a longer period when multiple doses of praziquantel were administered, compared with a single orally administered dose of 25 mg/kg. We expect that this will allow prolonged contact between the parasiticidal agent and the parasite and, thus, have benefits similar to those predicted for humans.

Results of our study indicated that uptake of praziquantel was slower and that the drug remained in the plasma for a longer period in loggerhead sea turtles than in humans. In fasted humans given therapeutic doses of praziquantel, Cmax was 319 ng/mL with a Tmax of 1.39 hours. Mean Cmax for turtles that received praziquantel at doses of 25 and 50 mg/kg were 109 and 342 ng/mL, respectively. Mean Tmax were 10 and 14 hours for groups receiving doses of praziquantel of 25 and 50 mg/kg, respectively. In humans, the MRT was 4.39 hours; in the turtles of our study, MRT, were 9 and 11 hours after single doses of 25 and 50 mg of praziquantel/kg, respectively. This difference can be partially explained by the lower body temperature and lower metabolic rate of reptiles, compared with mammals. Differences in kinetics of other drugs administered to reptiles, compared with similar doses administered to mammals, have been reported; plasma concentrations of those drugs were also less than those achieved in humans that received comparative doses. This may be a reflection of a slower uptake of drug from the gastrointestinal tract. Until additional studies are performed, oral administration of praziquantel at 25 mg/kg 3 times at 3-hour intervals may be an appropriate treatment regimen for spirochidiasis in loggerhead and other sea turtles. To demonstrate the efficacy of this dosing regimen, sea turtles that are treated with this dose and subsequently die would have to be submitted for postmortem evaluations to determine the presence or absence of spirochid adults and eggs in these turtles.

In humans, diet is known to influence the bioavailability of praziquantel. Humans consuming diets high in carbohydrates or fats had greater plasma praziquantel concentrations compared with those of fasted volunteers. The composition of squid is approximately 18% protein, 1.6% fat, 3.6% carbohydrate, and 75% water. Increasing the amount of fat or carbohydrate in the diet at the time of praziquantel administration may similarly improve gastrointestinal tract uptake in loggerhead sea turtles.

Cimetidine is a histamine H2 receptor antagonist and also an important inhibitor of hepatic microsomal enzymes. While it has been reported to potentiate serum concentrations of certain drugs, including praziquantel, in humans and other animals, the specific mechanism is unknown. Administration of cimetidine may be another approach to further optimize plasma praziquantel concentrations in sea turtles.

References


