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POPULATION PHARMACOKINETICS OF CEFPODOXIME IN BOTTLENOSE DOLPHINS (*TURSIOPS TRUNCATUS*)

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Abstract: Cefpodoxime proxetil is commonly used to treat cetacean patients with suspected or confirmed bacterial infections; however, pharmacokinetic data are needed to guide proper dosing in these species. Cefpodoxime proxetil is a time-dependent, semisynthetic, third-generation cephalosporin, appropriate for once-daily dosing and U.S. Food and Drug Administration–approved for use in dogs with a broad spectrum of activity including gram-positive and gram-negative species. The objective of this study was to evaluate the population pharmacokinetics of cefpodoxime in bottlenose dolphins (*Tursiops truncatus*). A sparse-sampling design was used, with serum from dolphins receiving cefpodoxime proxetil at 10 mg/kg orally every 24 h to treat suspected or confirmed bacterial infections. Serum samples ($n = 57$) from 24 dolphins were analyzed at 12 time points from 0 to 96 h postdose. Serum samples were analyzed using liquid chromatography–mass spectrometry. Population pharmacokinetic analysis was performed using nonlinear mixed-effects modeling. One- and two-compartment linear models with first order absorption were tested. Covariates including weight, age, and sex were considered for inclusion in the model, and between-subject variability was incorporated. A two-compartment model performed best, where following an oral dose of 10 mg/kg, serum concentration reached a mean maximum concentration of 23.0 μg/ml, mean time to maximum concentration of 5.0 h, and mean half-life of 11.4 h. With daily dosing, accumulation was approximately 18% and steady state was reached by the second dose. Serum protein binding was 82.8% as determined by equilibrium dialysis, similar to plasma protein binding reported in dogs. Based on the population pharmacokinetic model, once-daily oral dosing was systemically absorbed and quickly reached maximum concentrations. The half-life in dolphins appears to be longer than other species studied to date. Given the paucity of antimicrobial pharmacokinetic studies in dolphins, and limited once-daily oral antibiotic options for this species, these data are helpful for clinicians to make informed antimicrobial choices.

INTRODUCTION

There are very few pharmacokinetic (PK) studies of antimicrobials in bottlenose dolphins (*Tursiops truncatus*) and other cetaceans in the literature, with small samples sizes in the available studies.[4,7,8,10,11] PK of intramuscular cefovecin in one adult and five neonate bottlenose dolphins[7,8] and intramuscular ceftazidime in two healthy dolphins (*Tursiops aduncus*)[4] have been described in conference proceedings. There is a single peer-reviewed study describing PK of oral enrofloxacin once daily in eight bottlenose dolphins;[11] however, in recent years, there have been anecdotal reports of neurologic effects and photosensitization in cetaceans after oral enrofloxacin administration, making this option less favorable for some clinicians.[9] Given the paucity of PK data, dolphin dosing is most often extrapolated from domestic species or humans. For many drugs, this dosing extrapolation appears to be adequate, based on clinical resolution or lack of adverse effects; however, it has been shown that dolphins metabolize some drugs remarkably different from domestic species and obtaining drug levels to guide therapy may be important for safe use and monitoring in this species. Cefpodoxime proxetil is commonly used to treat cetacean patients with suspected or confirmed bacterial infections, but PK data are needed to guide proper dosing.

Cefpodoxime proxetil is an orally administered, extended spectrum, semisynthetic, time-dependent, bactericidal, third-generation cephalosporin. Simplicef® (cefpodoxime proxteil) is U.S. Food and Drug Administration–approved for treatment of skin infections (wounds and abscesses) in dogs, and it has a broad spectrum of clinically useful antibacterial activity that includes *Staphylococcus pseudintermedius, Staphylococcus aureus*, *Streptococcus canis*,

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Note: This article contains supplemental material found in the online version only.

Table 1. Demographic characteristics of dolphins included in the population pharmacokinetic analysis.

		Body weight (kg)			Age (yr)		
Sex	*n*	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Female	14	171	109	191	29	2.4	46
Male	18	190	152	254	24	5.0	43
Combined	32[a]	182	109	254	26	2.4	46

[a] Five animals represented multiple times due to different courses of medication. In total, 24 (10 female, 14 male) unique animals are represented.

and gram-negative species *Pasteurella multocida*, *Escherichia coli*, and *Proteus mirabilis*.[20] In human and domestic animal studies, cefpodoxime penetrates most tissues well;[3,13,16] in piglets, it has been documented to penetrate CSF, but not in equine foals.[1,3] Cefpodoxime proxetil is stable in the presence of many common β-lactamase enzymes;[14] therefore, many organisms resistant to other β-lactam antibiotics (penicillins and some cephalosporins) due to the production of β-lactamases may be susceptible to cefpodoxime. In dogs, cefpodoxime is eliminated from the body primarily in the urine, with an apparent elimination half-life of approximately 5–6 h after oral administration.[2,13] Delivery of cefpodoxime in its prodrug form, cefpodoxime proxetil, improves acid stability and lipophilicity.[10] Previous studies have identified some of the most common and highest risk primary bacterial isolates in managed dolphins to be staphylococci and streptococci species, making cefpodoxime's spectrum of activity ideally suited for treatment of such infections.[15,17,18]

The goal of this study was to use a sparse-sampling design to perform population PK of oral cefpodoxime in bottlenose dolphins. Based on previous clinical success with this antibiotic, the authors hypothesized that once-daily oral dosing of cefpodoxime proxetil at 10 mg/kg would be adequate to reach the minimum inhibitory concentration (MIC) for common pathogens of interest in managed dolphins.

MATERIALS AND METHODS

Animals

Twenty-four bottlenose dolphins at the U.S. Navy Marine Mammal Program were included in this study (Table 1). Participants were selected for the study if they were prescribed cefpodoxime proxetil (Simplicef®, 200-mg tablets, Zoetis Inc., Kalamazoo, MI 49007, USA) at 10 mg/kg orally every 24 h by the attending veterinarian to treat a suspected or confirmed bacterial infection. Reasons for treatment included inflammatory hemograms ($n = 8$ dolphins), skin lesions with inflammatory hemograms ($n = 8$ dolphins), pneumonia ($n = 7$ dolphins), leukopenia ($n = 4$ dolphins), and otitis media or otitis interna with an inflammatory hemogram ($n = 1$ dolphins). Antibiotics were delivered orally in a fish and given with a fish meal (≥1 lb [0.45 kg] of fish). Blood samples for use in this study included archived frozen serum samples and opportunistically collected samples as part of routine medical care, to meet three or more samples at each of the following time points: 0, 1, 2, 4, 6, 8, 12, 16, 20, 24, 48, and 96 h postdose. This sparse-sampling design was used to decrease blood sampling in individual dolphins and use previously archived samples when possible. The dataset included 57 serum samples collected across a range of 1–30 daily doses.

Navy dolphins are trained for husbandry behaviors using by operant conditioning; the majority of blood samples were collected via voluntary participation ($n = 41$), but several were collected during out-of-water procedures ($n = 16$). Navy dolphins are housed in open-water, netted enclosures in San Diego Bay, CA, and Kings Bay, GA. The Navy Marine Mammal Program is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and adheres to the national standards of the U.S. Public Health Service Policy on the Humane Care and Use of Laboratory Animals and the Animal Welfare Act. As required by the U.S. Department of Defense, the Navy's animal care and use program is routinely reviewed by an Institutional Animal Care and Use Committee and the Navy Bureau of Medicine and Surgery. The dolphins are fed mixtures of quality-controlled, frozen-thawed fish and additional vitamin supplements (Vita-Zu® Mammal Tablet #5M26, Mazuri, St. Louis, MO 63166, USA).

Blood collection

Blood samples were obtained under voluntary behavioral control (fluke present) from the ventral fluke periarterial vascular rete in routine

manner by using a 19- to 23-ga, 3/4-in. butterfly needle and collected into serum-separator tubes (BD Vacutainer®, Becton Dickinson, Franklin Lakes, NJ 07417, USA) for most samples ($n = 41$); some samples ($n = 16$) were collected during out-of-water procedures from either the fluke or ventral peduncle periarterial vascular rete. Samples were collected postdose from individual dolphins at times ranging from 15 min to 169.5 h. Blood tubes were centrifuged at 3,000 rpm for 10 min. Serum from the centrifuged tubes was aliquoted into cryovials and stored at −80°C. Samples were stored up to 1,104 d before shipment. The stability of cefpodoxime in dog plasma has been demonstrated up to 101 d (Zoetis Inc., internal data); however, lack of stability data in dolphin serum is a limitation of the current study and could be a potential source of interindividual variability. Banked, frozen serum from dolphins not receiving any medications were used as negative controls (15 serum samples from two dolphins). Batched serum samples were shipped on dry ice to Zoetis Inc. for determination of cefpodoxime concentration and PK analysis. For the protein binding study, 30 ml of banked, frozen serum was also provided from male and female dolphins who were considered healthy and not on any medications.

Analytical method

Serum samples were analyzed for the cefpodoxime concentration using a liquid chromatography–tandem mass spectrometry (LC-MS/MS) method developed for dog plasma (Zoetis Inc., internal documentation). The mass spectrometer (Sciex API4000, AB Sciex LLC, Framingham, MA 01701, USA) was set to operate in positive electrospray ionization mode for the transitions of 428 → 241 for the target analyte cefpodoxime and 454 → 241 for the internal standard (IS; cefovecin). Chromatographic separation was achieved using an Acquity UPLC system (Waters) equipped with a BEH C18, 1.7 μm, 2.1 × 50-mm column following gradient LC conditions from 5 to 45% acetonitrile (organic mobile phase) from 0 to 1.5 min. The organic phase was increased to 95% for 0.5 min and returned to starting conditions to equilibrate for 0.5 min before the next injection. The analytical run time including equilibration was 2.5 min. The aqueous mobile phase consisted of 5 mM ammonium formate with 0.3% formic acid. Peak integration was performed using Sciex Analyst® v1.7.1 software, and peak area ratios of analyte/IS were used for regression (quadratic, 1/x^2 weighting). The method was considered fit-for-purpose for the analysis of dolphin serum samples. Standards were prepared and analyzed in the 0.0100–10.0-μg/ml range with the addition of three quality control (QC) levels of 0.0300, 0.300, and 8.00 μg/ml. Serum samples above the upper limit of quantification (10.0 μg/ml) were reassayed following a fivefold dilution in control serum. All calibration standards and QC samples, including dilution QCs, were within ±15% of nominal (theoretical) concentrations.

Model development

The data, including concentration observations, dose amounts, times of dosing and blood sampling, body weight, age, and sex were assembled and formatted for analysis. Treatment of the infections almost always involved multiple doses; some animals had multiple infections separated by long periods. For animals that had multiple infections, each course was treated as an independent event. Data were analyzed using nonlinear mixed-effects modeling with the nlmixr package of the R 4.2.1. The estimation of model parameters used the First Order Conditional Estimation with Interaction method implemented in nlmixr. One- and two-compartment linear models were tested with first order absorption. Between-subject variability was also tested for inclusion on certain model parameters in the following form (for CL):

$$\log(\mathrm{CL}) = \log(\mathrm{CL}_{\mathrm{pop}}) + \eta_{\mathrm{CL}}$$

where CL is systemic clearance, $\mathrm{CL}_{\mathrm{pop}}$ is the population clearance, and η_{CL} is the deviation from the population clearance to account for individual subject variability. η_{CL} was assumed to be distributed as a normal random variable with mean 0 and variance ω_{CL}^2. Various covariates were considered for inclusion in the model. Body weight, age, and sex were all considered for inclusion relative to certain parameters. Covariates were included in the following form for continuous covariates (weight, age), example for CL with body weight covariate:

$$\log(\mathrm{CL}_{\mathrm{pop}}) = \log(\theta_{\mathrm{CL}}) + \beta_{\mathrm{BW}} \cdot [\log(\mathrm{BW}) - \log(\mathrm{sBW})]$$

where β_{BW} is coefficient relative to body weight and sBW is a standardized body weight (such as sample mean or median) and θ_{CL} is the estimated CL at the standardized covariate values. Categorical covariates for sex were renamed and categorized as male (0 = female, 1 = male). This categorical

covariate then entered the model similar to above without standardization, for example,

$$\log(CL_{pop}) = \log(\theta_{CL}) + \beta_{sex} \cdot \text{male}$$

where male is either 0 or 1 as indicated and β_{sex} represents the difference in θ_{CL} attributed to sex. Model selection was guided by various goodness-of-fit criteria, including diagnostic scatter plots, plausibility of parameter estimates, shrinkage associated with the between subject variability, and change in −2 times log likelihood (−2LL). A likelihood ratio test decided significant improvement in the fit of the model if the current model had a −2LL that was at least 6.63 less than the previous model. This amount of change in the −2LL is consistent with significance level of 0.01 assuming a chi-square distribution with 1 df.

Once the base model (one or two compartment) was decided, covariates were added in a stepwise manner. All covariates were tested for inclusion separately; the one resulting in the largest drop in the −2LL was included in the model. With this covariate included, the remaining covariates were tested again for inclusion, again including the covariate resulting in the largest significant drop in the −2LL. The stepwise process continued until no further significant improvement was made. The final model was referred to as the PopPK model.

Protein binding study

Serum protein binding was determined by equilibrium dialysis by using an HTD 96B 96-well micro-equilibrium dialysis device (HT Dialysis LLC, Gales Ferry, CT 06335, USA) with a 12–14-kDa MWCO regenerated cellulose dialysis membrane strip. Stock solutions of cefpodoxime were prepared by dissolving the drug into dimethyl sulfoxide; dilution into ultrapure water; and subsequent serial dilution to achieve concentrations of 1,500, 150, 15.0, and 1.50 μg/ml. Aliquots (1 ml) of pooled dolphin serum were spiked with the stock solutions to generate cefpodoxime concentration of 30.0, 3.00, 0.300, and 0.0300 μg/ml. Aliquots were prepared in both pooled male and female dolphin serum. The samples were divided into the equilibrium dialysis apparatus with three replicates. 150 μl of buffer (phosphate-buffered saline [PBS], pH 7.4) was added to one side of the membrane followed by 150 μl of serum to the opposite side of the membrane. The apparatus was sealed and incubated at 37°C for 4 h.

After the incubation period, 50-μl samples of serum and buffer were extracted in 96-well polypropylene plates for LC-MS/MS following the analytical test method described above. The lower limit of quantification was reduced to 0.003 μg/ml to cover the anticipated range of concentrations in PBS. Samples above the upper limit of quantification were diluted into the calibration range.

Protein binding was determined by use of the following equation:

$$\text{Protein binding}(\%) = \frac{(\text{total concentration} - \text{free concentration})}{(\text{total concentration})} \times 100$$

where total concentration is the sum of the protein-bound and protein-unbound drug concentration measured in serum and free concentration is the protein-unbound drug concentration measured in buffer.

Navy dolphin bacterial isolate MIC

The U.S. Navy Marine Mammal Program Medical Database was queried for antibiotic culture and susceptibility panels (performed at reference laboratories) containing cefpodoxime, between January 2007 and March 2023, and included culture results from all sources available in the database. Isolate data was analyzed for MIC range, MIC median, and most common bacterial isolates reported. The reference laboratories used for culture and susceptibility follow the Clinical and Laboratory Standards Institute breakpoints for MIC determinations, including guidelines for extrapolation to exotic species.[5,6]

RESULTS

No adverse effects of cefpodoxime proxetil were observed in any dolphins in the study. In total, 24 dolphins were sampled (Table 1). Five dolphins had multiple courses of therapy; one had four courses and one had three courses. Dolphins ranged in body weight from 182 to 254 kg and in age from 2.4 to 46 yr.

The two-compartment model provided significant improvement over a one-compartment model; random effect terms to account for between-subject variability were added to the model. The random effect associated with CL provided a substantial reduction to the −2LL and was therefore included in the model. All other random effect terms either had convergence problems or a high degree of

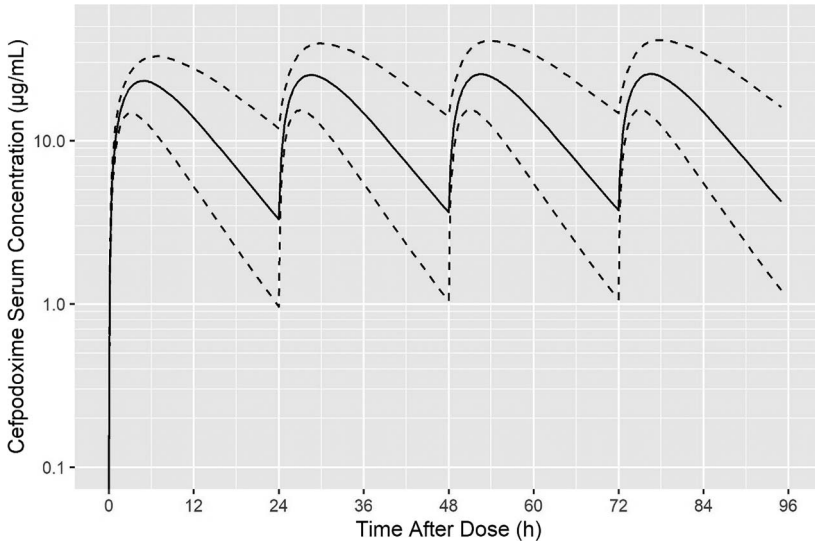

Figure 1. Simulated cefpodoxime serum profile of the first four 10-mg/kg doses. Solid line is the median, and dashed lines are the 5th and 95th percentiles.

shrinkage and were not included in the model. The fixed effect model parameters were then tested for association with covariates. There was very little data to inform the absorption rate constant, and covariates were not tested for association with this parameter. Each covariate was added, in turn, to the base model; the body weight effect associated with the apparent volume of distribution of the central compartment (Vc) provided the greatest reduction in the objective function value, this became the new base model. Covariates were again added, in turn, to the new base model. No additional covariates provided a significant reduction in the −2LL. The summary of the modeling process, goodness-of-fit plots, visual predictive check, and model fits are provided, along with the final PopPK model parameter estimates in Supplemental Tables 1 and 2 and Supplemental Figures 1–3.

To determine when steady state is reached and to estimate the PK parameters, the PopPK model was simulated at a 10-mg/kg dose at body weights that covered the observed data (Fig. 1). Table 2 provides the summary of the simulated PK parameter estimates after the first dose and after a steady-state dose. After an oral dose of 10 mg/kg, cefpodoxime serum concentration reached a mean maximum concentration (C_{max}) of 23.0 µg/ml, mean time to maximum concentration (T_{max}) at 5.0 h, and mean half-life of 11.4 h. With daily dosing, accumulation was approximately 18% and steady state was reached by the second dose (Fig. 2).

Protein binding study

Protein-bound and protein-unbound fractions of cefpodoxime in blank dolphin serum samples spiked with known concentrations of cefpodoxime are provided in Table 3. Values are reported as the mean ± SD of replicates ($n = 3$ per sex per concentration). Across all replicates ($n = 24$), serum protein binding of cefpodoxime ranged from 81.5 to 85.1%. This represents an unbound fraction of cefpodoxime ranging from 14.9 to 18.5%. A statistical comparison in Prism v8.1.1 (GraphPad Software, San Diego, CA 92108, USA), two-way ANOVA, showed there was not a statistically significant interaction between sex and concentration or significant main effects of sex or concentration, indicating protein binding was not concentration dependent. Overall mean ± SD protein binding was 82.8 ± 1.14%, with a 95% CI of 82.3–83.2% ($n = 24$).

Navy dolphin bacterial isolate MIC

The U.S. Navy Marine Mammal Program Medical Database query (January 2007–March 2023) revealed 560 bacterial isolates cultured with cefpodoxime included on the susceptibility panel (sampled from various body sites). Of these isolates, 299 isolates had MIC values reported for cefpodoxime; the cefpodoxime MIC range was 0.12–32 µg/ml and median MIC was 2.0 µg/ml. The median MICs for gram-negative and gram-positive species were the same, 2.0 µg/ml (gram-positive:

Table 2. Summary of pharmacokinetic parameters after the first dose and a steady-state dose at 10 mg/kg estimated from data simulated from the PopPK model and covering the weight and age range in the observed data.

	First dose			Steady state		
Parameter	Mean[a]	% CV	90% PI	Mean[a]	% CV	90% PI
C_{max} (μg/ml)	23.0	25.7	14.9–33.3	25.9	32.1	15.7–42.4
T_{max} (h)	5.0	26.5	2.9–7.2	4.61	22.1	2.9–6.2
AUC_{0-24h} (μg·h/ml)	305	41.5	150–553	349	49.5	158–743
$AUC_{0-\infty}$ (μg·h/ml)	341	49.1	157–715	406	58.5	169–1,006
$T_{1/2}$ (h)	NA	NA	NA	11.4	12.5	9.56–13.8

[a] Mean is the geometric mean for C_{max}, area under the curve $(AUC)_{0-24h}$, and $AUC_{0-\infty}$ and arithmetic mean for maximum concentration (T_{max}) and mean half-life ($T_{1/2}$). First dose $T_{1/2}$ not applicable because the terminal elimination phase is not reached until after 24 h. CV, coefficient of variation; PI, prediction interval (5th and 95th percentiles of simulated dataset).

median MIC, 2.00 μg/ml [range, 0.12–16.00]; gram-negative: median MIC, 2.00 μg/ml [range, 0.25–32.00]). The 10 most common isolates were *Escherichia coli* ($n = 110$), *Staphylococcus aureus* ($n = 33$), *Shewanalla putrefaciens* ($n = 24$), *Pseudomonas aeruginosa* ($n = 23$), *Staphylococcus delphini* ($n = 20$), *Vibrio* spp. ($n = 18$), *Salmonella* spp. ($n = 15$), *Enterococcus faecalis* ($n = 15$), *Vibrio alginolyticus* ($n = 15$), and *Proteus mirabilis* ($n = 12$). Of these isolates, cefpodoxime's spectrum of activity is indicated for all except *Enterococcus faecalis* and *Pseudomonas aeruginos*a, 261 of 299 isolates (87%).[20] These results represent a variety of cultures from various body sites and are not necessarily representative of primary pathogens correlated to a specific disease state.

DISCUSSION

To our knowledge, this study is the first to examine the PK of an oral cephalosporin in a cetacean species. According to the population PK model, following a single oral dose of cefpodoxime proxetil at 10 mg/kg, the drug was systemically absorbed and quickly reached maximum concentration in the dolphin (T_{max}, 5.0 h and C_{max}, 23.0 μg/ml). The half-life in dolphins (11.4 h) appears to be longer than that in other species studied to date, including dogs (approx 6 h),[2,13] equine adults and foals (approx 7 h),[3] and humans (approx 2 h).[14] As we hypothesized, once-daily dosing appears adequate based on these data for susceptible bacterial isolates. No adverse effects were seen in dolphins in the current study.

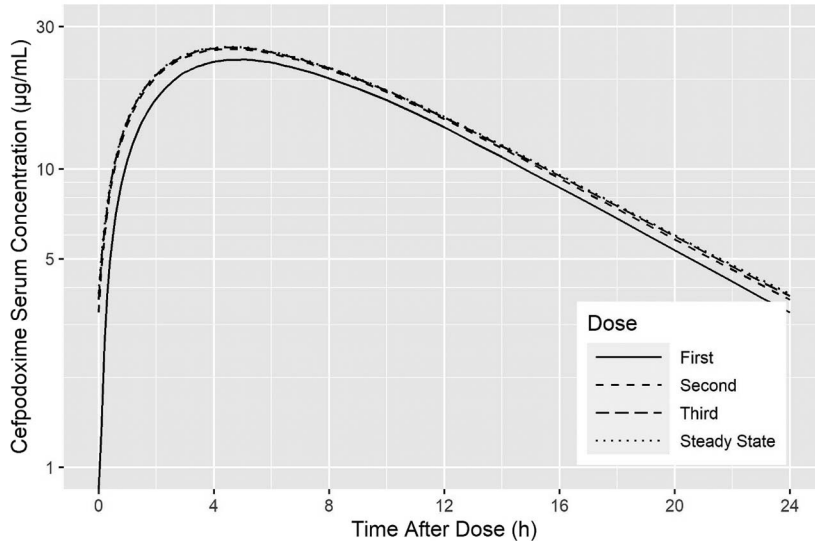

Figure 2. Median of simulated cefpodoxime serum profiles for the first three 10-mg/kg doses plus a steady-state dose.

Table 3. Summary of cefpodoxime protein binding in blank serum from healthy male and female dolphins, spiked with known concentrations of cefpodoxime.[a]

Cefpodoxime concentration (μg/ml)	Male			Female			Overall		
	Protein bound (%)		Protein unbound (%)	Protein bound (%)		Protein unbound (%)	Protein bound (%)		Protein unbound (%)
	Mean	SD		Mean	SD		Mean	SD	
0.0300	82.96	1.39	17.04	82.98	0.71	17.02	82.97	0.99	17.03
0.300	83.47	0.71	16.53	82.89	0.66	17.11	83.18	0.74	16.82
3.00	83.37	0.60	16.63	82.68	2.08	17.32	83.03	1.42	16.97
30.0	82.35	1.26	17.65	81.36	0.57	18.64	81.86	1.03	18.14
Overall	83.04	1.02	16.96	82.48	1.22	17.52	82.76	1.14	17.24

[a] $n = 3$ per concentration per sex.

The observed differences in cefpodoxime metabolism across species are unlikely to be due to a change in a metabolic elimination pathway, because human metabolism shows it to be only a minor elimination mechanism for cefpodoxime. Species differences (human, monkey, dog, rat), however, are evident for cefpodoxime proxetil in *in vitro* data that show differences in the rate and extent of conversion of the prodrug to cefpodoxime in various tissue matrices (plasma, liver, small intestine; Zoetis Inc., unpubl. data). These differences in the conversion may contribute to the *in vivo* PK differences seen across species. It has been theorized in the past that dolphins do not possess the esterase enzymes necessary for such drug conversions, but the data presented herein suggest that dolphins are able to convert the ester prodrug (cefpodoxime proxetil) to its active form (cefpodoxime).

The sparse-sampling strategy was used in this study to use a combination of archived serum and opportunistic blood sampling and reduce the number of blood collections for individual dolphins. The sample size ($n = 24$ dolphins) was relatively large in comparison with the previously available dolphin PK studies of up to eight dolphins.[4,7,8,11] The nonlinear mixed effects modeling used for PK analysis in this population allowed for estimation of the full concentration time profile and the associated variability.

Based on the retrospective bacterial culture and susceptibility data from Navy dolphins over the past 16 yr, the PK profile of cefpodoxime is very promising to target the majority of the pathogens of interest. Of the bacterial isolates with MIC data ($n = 299$), the median MIC was 2.0 μg/ml. Based on the Clinical and Laboratory Standards Institute breakpoint for susceptibility testing in dogs (broth microdilution method), MIC < 2.0 μg/ml is considered susceptible in dogs.[5] The PK/pharmacodynamics index for cephalosporins is the time above the MIC with recommendation of exceeding the MIC for 50–100% of the dosing interval.[12] In this study, the total (bound plus unbound) serum concentration remained above 2.0 μg/ml for 100% of the dosing interval (Fig. 1). The cefpodoxime protein binding was determined in dolphin serum and indicates cefpodoxime binds at a fairly high rate (82.8%) to serum proteins. This is similar to the average plasma protein binding reported in dogs (82.6%).[12] Based on the simulated model, the median unbound serum concentration is expected to exceed a MIC of 2 μg/ml for 12.5 h (52% of the dosing interval) after the first dose and 13.8 h (58%) at steady state (Fig. 3). Furthermore, gram-positive bacterial species, including *Staphylococcus* spp. and *Streptococcus* spp., have been identified as some of the most common and highest risk primary bacterial pathogens in this dolphin population,[15,17,18] which makes cefpodoxime an ideally suited antimicrobial based on its spectrum of activity. As a third-generation cephalosporin, cefpodoxime is also active against gram-negative bacteria including *Escherichia coli and Proteus* spp., which are also some of the most frequently cultured bacteria in this species, consistent with the Navy dolphin isolates presented herein, and can be primary pathogens.[17,18] With the plasma profile demonstrated herein, daily dosing of cefpodoxime proxetil should reach adequate concentrations to exceed the MIC for >50% of the dosing interval for the majority of susceptible bacterial infections in dolphins. Given that third-generation cephalosporins are considered medically important antimicrobial,[19] the authors emphasize that cefpodoxime should be used judiciously to treat susceptible infections.

Lack of cefpodoxime stability data in dolphin serum is a limitation of the current study. This could be a potential source of the interindividual variability seen, because samples were stored frozen from anywhere between 2 wk and 3 yr before analysis, which extends beyond the tested time frame in

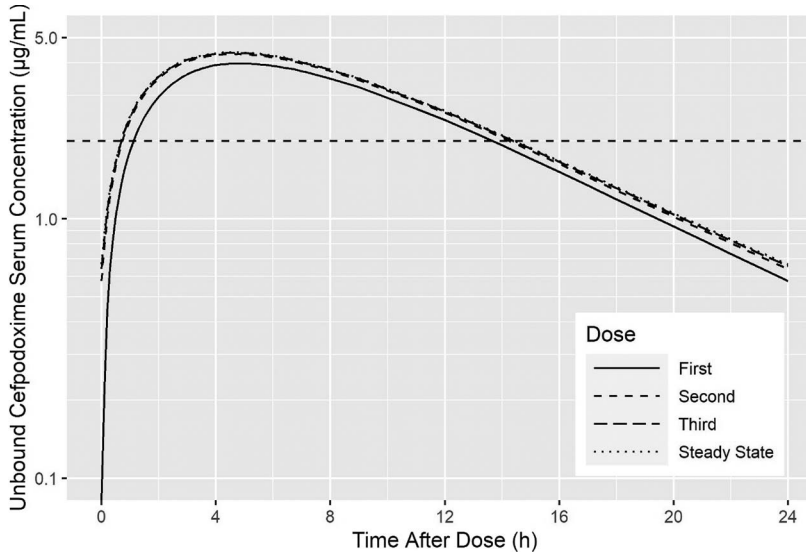

Figure 3. Median of simulated unbound cefpodoxime serum profiles for the first three 10-mg/kg doses plus a steady-state dose. The horizontal line represents an MIC of 2 μg/kg.

dogs for stability of cefpodoxime in frozen serum (up to 101 d; Zoetis, internal documentation).

In conclusion, this study demonstrated that once-daily oral cefpodoxime proxetil at 10 mg/kg in bottlenose dolphins was systemically absorbed, quickly reached maximum concentrations, and had no observable adverse effects. The half-life in dolphins appears to be longer than that of other species studied to date, and the degree of serum protein binding is similar to that reported in dogs. A sparse-sampling experimental design and nonlinear mixed effects modeling allowed for population PK analysis while reducing blood sampling frequency in individual dolphins.

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Supplemental Figure 1. Goodness-of-fit plots associated with the PopPK model.

Supplemental Figure 2. Visual predictive check, median (solid line), and 5th and 95th percentiles (dashed lines) from simulation from the final model. Approximately 90% of the observed data (black dots) are expected to fall within the 5th and 95th percentiles.

Supplemental Figure 3. Final model fit to the first two individuals; observed data represented by black dots.