PHARMACOKINETICS OF SINGLE-DOSE ORALLY ADMINISTERED CIPROFLOXACIN IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

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PHARMACOKINETICS OF SINGLE-DOSE ORALLY ADMINISTERED CIPROFLOXACIN IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

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Abstract: Ciprofloxacin is commonly selected for clinical use due to its broad-spectrum efficacy and is a frequently administered antibiotic at The Marine Mammal Center, a marine mammal rehabilitation facility. Ciprofloxacin is used for treatment of California sea lions (*Zalophus californianus*) suffering from a variety of bacterial infections at doses extrapolated from other mammalian species. However, as oral absorption is variable both within and across species, a more accurate determination of appropriate dosage is needed to ensure effective treatment and avoid emergence of drug-resistant bacterial strains. A pharmacokinetic study was performed to assess plasma concentrations of ciprofloxacin in California sea lions after a single oral dose. Twenty healthy California sea lions received a single 10-mg/kg oral dose of ciprofloxacin administered in a herring fish. Blood was then collected at two of the following times from each individual: 0.5, 0.75, 1, 2, 4, 8, 10, 12, 18, and 24 hr postingestion. Plasma ciprofloxacin concentration was assessed via high-performance liquid chromatography. A population pharmacokinetics model demonstrated that an oral ciprofloxacin dose of 10 mg/kg achieved an area under the concentration vs. time curve of 6.01 μg hr/ml. Absorption was rapid, with ciprofloxacin detectable in plasma 0.54 hr after drug administration; absorption half-life was 0.09 hr. A maximum plasma concentration of 1.21 μg/ml was observed at 1.01 hr, with an elimination half-life of 3.09 hr. Ciprofloxacin administered orally at 10 mg/kg produced therapeutic antibacterial exposure for only some of the most susceptible bacterial organisms commonly isolated from California sea lions.

Key words: Antibiotic, California sea lion, ciprofloxacin, fluoroquinolone, pharmacokinetic, marine mammal.

INTRODUCTION

Ciprofloxacin is a fluoroquinolone antibiotic commonly selected for its broad-spectrum efficacy against both gram-positive and gram-negative bacteria. A bactericidal and concentration-dependent agent, it is active in both stationary and growth phases of bacterial replication.[26] Ciprofloxacin is known for its variable oral absorption across and even within species, producing ciprofloxacin plasma concentrations that were low and inconsistent in dogs, too low for practical use in horses, and adequate only against some bacteria in cats.[1,3,37] The variability in oral absorption for ciprofloxacin in people is also well known.[25]

Although a variety of antibiotics are commonly used in pinniped species, information on pharmacokinetics of these drugs remains scarce, with only a handful of studies thus far published.[11,12,19,22] The Marine Mammal Center (TMMC) is a rehabilitation facility that cares for roughly 600 ill and injured marine mammals each year, approximately 60% of which are California sea lions (*Zalophus californianus*). Ciprofloxacin is currently one of the most frequently administered antibiotics at TMMC, and is commonly used for treatment of California sea lions affected by a variety of bacterial infections, including pneumonia, leptospirosis, and infected traumatic injuries, at a dosage of 5–10 mg/kg p.o. b.i.d. In vitro susceptibility tests have indicated that ciprofloxacin has good activity against several bacterial species commonly isolated from marine mammals, and thus has potential for therapeutic use in these animals.[6,13,14,18,31] Ciprofloxacin is an attractive antibiotic for use in pinniped species due to its infrequent (q24 hr) dosing regimen, broad antimicrobial spectrum, wide margin of safety, and ease of oral administration by hiding a tablet within a food source for voluntary consumption.[26] The purpose of this study was to examine the pharmacokinetics of ciprofloxacin in California sea lions after a single dose administered orally using a fish as the delivery vehicle. We hypothesized that a single oral dose of 10 mg/kg ciprofloxacin would achieve plasma concentrations that meet pharmacokinetic-pharmacody-

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namic targets for bacteria most commonly isolated from California sea lions.

MATERIALS AND METHODS

Sample collection

Twenty outwardly healthy California sea lions undergoing rehabilitation at TMMC were selected for the study. Animals were considered outwardly healthy if they had completed treatment for the disease process (or processes) for which they were admitted, were eating whole fish on their own, and had gained significant weight such that a release exam was warranted. At TMMC, a release exam, including a physical exam by a staff veterinarian and morphometric measurements, and routine blood chemistry and complete blood cell count, are performed on each outwardly healthy individual to confirm fitness for release back into the wild. Animals were removed from the study if found to be unhealthy or unfit for release back into the wild based on release exam or bloodwork results. Individuals included in the study had not received ciprofloxacin or any other medication for at least 5 days prior to the start of the study, but did receive a daily multivitamin supplement throughout the study period. Animals included 17 pups and three yearlings (11 males and nine females), ranging from 20.5 to 31.0 kg. Individuals were weighed within 5 days prior to the study to ensure accurate ciprofloxacin dosing. All individuals were housed in fenced pens with free access to a pool containing salt water and fed herring at 0800, 1400, and 2200 hours. This study was approved by the Institutional Animal Care and Use Committee of TMMC (proposal 2012-2), and samples were collected under Marine Mammal Protection Act permit 932-1905/MA-009526.

Ciprofloxacin (250-mg tablets, Aurobindo Pharma, North Dayton, New Jersey 08810, USA) was administered at the 0800 hour feeding following an approximately 10-hr fast. Each individual received an approximate 10-mg/kg (10.0 ± 0.83 mg/kg [mean ± SD]) oral dose of ciprofloxacin administered inside a herring fish and was visually monitored to confirm ingestion. This dose was chosen based on empirical use at TMMC and extrapolation from other mammalian species. Since it is not possible to sample these animals repeatedly without undue stress, a sparse sampling technique and population pharmacokinetic study design were used. Each individual was blood sampled at two of the following times: 0.5, 0.75, 1, 2, 4, 8, 10, 12, 18, and 24 hr post–ciprofloxacin administration, providing a minimum of three blood samples for each time point. A release exam was scheduled to coincide with the first sampling time point for each individual in the study. Sea lions were physically restrained for blood collection using towels or nets, and blood was collected from the caudal gluteal vein using a 20-ga 1- or 1.5-inch needle (Greiner Bio-One North America, Inc., Monroe, North Carolina 28110, USA) on a Vacutainer set (Becton, Dickinson and Company, Franklin Lakes, New Jersey 07417, USA), into a 5-ml sodium heparin tube (Greiner Bio-One North America, Inc.). Heparin tubes were centrifuged for 10 min, and plasma was collected and stored in a 2.0-ml cryovial (Simport Scientific, Beloeil, Quebec J3G 4S5, Canada) at –80°C. Samples were then shipped overnight on dry ice to North Carolina State University for ciprofloxacin detection via high-performance liquid chromatography (HPLC).

An additional 30 ml of plasma was collected in the manner described above from three sea lions who did not receive ciprofloxacin, and pooled for use as controls, standards, and diluent, as necessary to validate the assay.

Sample analysis

Plasma samples were analyzed via HPLC to determine the concentration of ciprofloxacin using a method adapted from a previous study on ciprofloxacin analysis in dogs.[24] The HPLC system consisted of a quaternary solvent delivery system (flow rate, 1 ml/min), an autosampler (Agilent 1200 Series solvent delivery system, Agilent Technologies, Wilmington, Delaware 19808, USA) and ultraviolet detector (Agilent 1200 Series Variable Wavelength Detector, Agilent Technologies) set at a wavelength of 279 nm. Chromatograms were integrated with a computer program (Agilent 1100 Series Chemstation 2D software, Agilent Technologies). The analytic column (Zorbax Rx C8 column, Agilent Technologies) was a reverse-phase C8 column that was maintained at a constant temperature (40°C). The mobile phase consisted of 78% distilled water and 22% acetonitrile. A 0.1% solution of trifluoroacetic acid was added to the mobile phase as a pH modifier.

The reference standard of ciprofloxacin (ciprofloxacin analytical reference standard, United States Pharmacopeial Convention [USP], Rockville, Maryland 20851, USA) was used to prepare a stock solution to fortify blank canine plasma. Stock solutions were sealed and stored in the dark in a refrigerator. The calibration curve for ciprofloxacin consisted of eight standard solutions that

ranged between 0.05 and 10.00 μg/ml and included a blank (0 μg/ml) sample. The blank sample was used to detect interfering peaks that eluted into the window of the chromatographic peak of interest and to measure background interference. The calibration curve was accepted if the linear coefficient of determination (r^2) was ≥0.99 and if the calibration curve concentrations could be back-calculated to ≤15% of the true concentration of the standard.

All plasma, calibration, quality-control, and blank-plasma samples were prepared in an identical manner. Five hundred microliters of each plasma sample were added to a conditioned solid-phase extraction cartridge (Oasis HLB, 1 ml, Waters Corporation, Milford, Massachusetts 01757, USA). The eluate from the cartridge was collected into a clean glass tube by elution with 1 ml of 100% methanol. The eluted samples were evaporated to yield a dry residue by heating the tubes at 40°C under a flow of air for 20 min. The residue of each tube was reconstituted by the addition of 200 μl of the mobile phase; solutions were vortexed briefly and transferred to an HPLC injection vial. Forty microliters of each sample was used for injection into the HPLC system.

Retention time for ciprofloxacin was 3.9 to 4.1 min. Fresh calibration and blank samples were prepared for analysis each day. The lower limit of quantification (LLOQ) for the drug in canine plasma was 0.05 μg/ml, which was determined from the lowest point on a linear calibration curve that yielded an acceptable accuracy and was within accepted guidelines for signal/noise ratio (International Conference on Harmonisation and USP guidelines).[17,32] Concentrations below the LLOQ are inherently more uncertain and thus it is the protocol in our laboratory to exclude them from pharmacokinetic analysis.

Data analysis

Plasma drug concentrations were pooled using the naïve pooled data approach because of sparse sampling and limited samples per individual. Pooling of the data was necessary due to insufficient data density for analysis. This technique is common and has been used successfully for population pharmacokinetic analysis in harbor seals for another fluoroquinolone antibiotic.[19] Analysis of curves and pharmacokinetic modeling were then performed by use of a commercial pharmacokinetic program (Phoenix software, version 6.0, Pharsight Corporation, Sunnyvale, California 94086, USA). Compartmental analysis was performed using the following formula:

$$C = \frac{k_{01} F D}{V(k_{01} - k_{10})} \left[e^{-k10} - e^{-k01t} \right],$$

where C is the plasma concentration, t is time, k_{01} is the nonintravenous absorption rate (assuming first-order absorption), k_{10} is the elimination rate constant, V is the apparent volume of distribution, F is the fraction of drug absorbed, and D is the nonintravenous dose. In this model, it is assumed that $k_{01} >>> k_{10}$, or that there is no "flip-flop" effect caused by slow absorption from the gastrointestinal tract. A lag time was included in the model, which reflects time for dissolution of the tablet and gastric emptying. Secondary parameters from the model included the peak concentration (C_{MAX}), time to peak concentration (T_{MAX}), area under the plasma-concentration vs. time profile (AUC), and the respective absorption and terminal half-lives.

RESULTS

Drug administration and sample collection

All 20 sea lions were found to be clinically healthy and suitable for release back into the wild based on release exam and bloodwork. One individual was not visually confirmed to ingest the medicated fish and was removed from the study. A second individual was observed to ingest another animal's fish containing ciprofloxacin after the first blood collection, and thus a second blood collection was not performed. A third individual was difficult to blood sample during the first time point and to avoid additional stress on the animal, a second blood collection was not performed. In total, 36 plasma samples (from 19 individuals) were collected for analysis.

There were no alterations in behavior, appetite, or clinical appearance of individuals in the study. No adverse reactions were noted.

Pharmacokinetics

Twenty-nine of 36 plasma samples provided detectable ciprofloxacin concentrations (Fig. 1). In seven samples, ciprofloxacin was not detectable at all, including two samples at 45 min, one sample at 8 hr, one sample at 18 hr, and three samples at 24 hr. Eight of the 29 samples with detectable ciprofloxacin levels had concentrations below the LLOQ of the assay (one at 30 min, one at 45 min, one at 2 hr, two at 12 hr, and three at 18 hr) and thus were not included in the pharmacokinetic analysis. Pharmacokinetic parameters for 21 plasma samples from 19 California sea lions

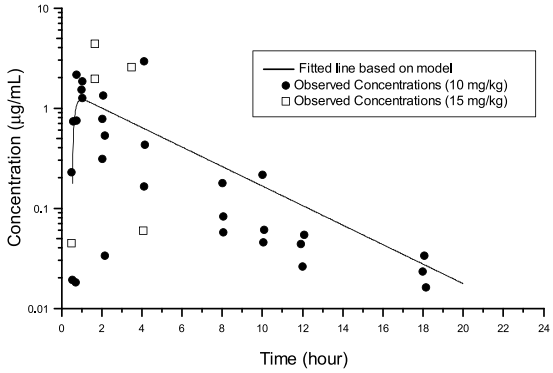

Figure 1. Plasma concentration-time profile for ciprofloxacin in 29 plasma samples from 19 California sea lions after administration of a 10-mg/kg (10.0 ± 0.83 mg/kg) single oral dose (solid circles) and in five plasma samples from five California sea lions after administration of multiple oral 15-mg/kg (14.61 ± 2.43 mg/kg) doses (hollow squares).

Table 1. Pharmacokinetic data for ciprofloxacin in 21 plasma samples from 19 California sea lions after administration of a 10-mg/kg (10.0 ± 0.83 mg/kg) single oral dose.

Parameter[a]	Units	Naïve pooled estimate
VD/F	L/kg	7.4
K_{01}	1/hr	7.80
K_{10}	1/hr	0.22
T_{LAG}	hr	0.54
AUC	hr µg/ml	6.01
Absorption half-life	hr	0.09
Elimination half-life	hr	3.09
CL/F	ml/hr/kg	1.67
T_{MAX}	hr	1.01
C_{MAX}	µg/ml	1.21

[a] VD/F indicates volume of distribution as a function of bioavailability; K_{01}, nonintravenous absorption rate; K_{10}, elimination rate constant; T_{LAG}, lag time; AUC, area under the plasma-concentration vs. time profile; CL/F, total body clearance as a function of bioavailability; T_{MAX}, time to peak concentration; C_{MAX}, peak concentration.

that received a 10-mg/kg single oral ciprofloxacin dose are summarized in Table 1. Absorption was rapid, with plasma concentrations detectable at 0.54 hr after drug administration and an absorption half-life of 0.09 hr. A C_{MAX} of 1.21 µg/ml was observed at a T_{MAX} of 1.01 hr. The elimination half-life was 3.09 hr and the AUC was 6.01 hr µg/ml.

DISCUSSION

Results of this study, involving a wild population of California sea lions undergoing rehabilitation at TMMC, demonstrated that ciprofloxacin is detectable in plasma after a single 10-mg/kg oral dose. The absorption half-life (0.09 hr) was rapid in these sea lions compared to values in cats (0.23 hr), dogs (0.31 hr), and calves (0.83 hr).[3,23,24] Maximum plasma concentrations (1.21 µg/ml) were similar to those in cats that received multiple q12 hour 10-mg/kg oral ciprofloxacin doses (1.26 µg/ml), but considerably higher than in horses that received a 20-mg/kg oral dose (0.60 µg/ml).[3,37] C_{MAX} was similar to what was observed in dogs that received a single 10-mg/kg oral dose (1.55 µg/ml), but lower than in dogs that received a single approximately 23-mg/kg oral dose (4.40 µg/ml).[1,24] T_{MAX} (1.01 hr) was earlier than in other species, including cats (1.30 hr), dogs (1.46 hr), and horses (1.46 hr).[3,24,37] The elimination half-life (3.09 hr) was shorter than what has been reported in dogs (4.91 hr), but similar to that in horses (3.60 hr).[1,37] A lag time (0.54 hr) was calculated and assumed to reflect the time for tablet dissolution and gastric emptying; however, dogs that received a 10-mg/kg oral dose of ciprofloxacin in a gel capsule on an empty stomach demonstrated a similar lag time (0.52 hr), indicating that gastric emptying time may be the main contributor to lag time in the current study.[1]

In this study, ciprofloxacin was administered inside of a herring fish, as it is the preferred method of oral administration for both wild and captive sea lions able to consume fish on their own. Whether or not concurrent feeding of herring alters the pharmacokinetics of ciprofloxacin in sea lions is undetermined. In penguins, a 15-mg/kg dosage of enrofloxacin produced a shorter T_{MAX} when given orally inside a herring fish (1.59 hr) compared to administration by pilling (4.80 hr), while C_{MAX} was not significantly affected.[35] In humans, a standard breakfast (bread, butter, jam, and tea) with a 250-mg ciprofloxacin tablet given orally produced a significantly longer T_{MAX} than when ciprofloxacin was given orally in a fasted state, while other pharmacokinetic parameters remained unaffected.[21]

Although the study's small sample size precludes statistical comparison, there was no apparent effect of gender on ciprofloxacin pharmacokinetics. Age class was restricted to pups and yearlings due to their small size, and thus ease of manual restraint, as well as to avoid potential drug interactions from sedative or anesthetic agents that larger sea lions typically

require for blood sampling. In humans, ciprofloxacin elimination occurs more rapidly in young compared to geriatric individuals, and similarly in cats, elimination rate of enrofloxacin is significantly greater in neonatal compared to adult individuals.[20,29] Although at TMMC no adverse effects have been noted in patients treated with ciprofloxacin, some fluoroquinolones, including ciprofloxacin, have been associated with a dose-dependent arthrotoxicity in young growing animals of some species.[28,30,33,34] The effect of ciprofloxacin on the articular cartilage of young sea lions is unknown. As this is the first pharmacokinetic study of an oral antibiotic in California sea lions, further research is needed to determine the potential influences of sex and age on pharmacokinetic parameters as well as potential for adverse events caused by ciprofloxacin in certain age groups.

Because of the limitations of utilizing rehabilitated sea lions for this study, and because ciprofloxacin is frequently used at TMMC, seven individuals received courses of ciprofloxacin (approximately 10 mg/kg p.o. b.i.d. for 8–15 days) prior to the start of the study, and two individuals received single doses of ciprofloxacin by eating other animals' medicated fish. All individuals were discontinued from ciprofloxacin at least 14 days prior to the start of the study except for one individual who ate another animal's medicated fish 5 days prior to the study. There was no apparent difference between animals that received or did not receive ciprofloxacin prior to the start of the study, and thus the 14-day washout period seems adequate to have precluded prior ciprofloxacin administration from affecting the study results.

Pharmacokinetic–pharmacodynamic targets for fluoroquinolones are well-established.[4,8,9] Minimum inhibitory concentration (MIC) is the lowest concentration of an antibiotic needed to inhibit bacterial growth. Using a target AUC : MIC ratio of 100, an AUC of 6.0 hr μg/ml would produce effective results for bacteria with an MIC for ciprofloxacin of <0.06 μg/ml. Common bacterial organisms isolated from pinnipeds include *Leptospira* spp., *Escherichia coli*, *Proteus* spp., *Klebsiella* spp., *Pseudomonas* spp., *Salmonella* spp., and *Aeromonas* spp., among others.[18,31] Based on MIC_{90} (the minimum inhibitory concentration to inhibit the growth of 90% of organisms) data for these bacteria, ciprofloxacin plasma concentrations produced from administration of a 10-mg/kg oral dose may be effective to treat infections caused by *Salmonella* spp., *E. coli*, and *Aeromonas* spp. Treatment of infections containing *Proteus* spp., *Klebsiella* spp., and *Leptospira* spp. would likely have intermediate susceptibility and would warrant an antibiotic susceptibility test, and infections caused by *Pseudomonas* spp. would likely be resistant to this dose.[5,10,16,27,36]

Importantly, the AUC of 6.0 hr μg/ml is derived from the naïve pooled analysis used in this study and represents the mean for our sample population. Similar to ciprofloxacin pharmacokinetics in many other species, there was substantial variation among individuals in our study (Table 1; Fig. 1). For animals with concentrations below the target AUC, a 10-mg/kg dose of ciprofloxacin may result in treatment failure and may increase emergence of resistance.[2,15] It is also important to note that the Clinical and Laboratory Standards Institute breakpoint for susceptible bacteria in human isolates is ≤ 1.0 μg/ml; thus an isolate reported by a diagnostic laboratory as "susceptible" for humans may not have an MIC <0.06 μg/ml, and may not respond to the doses of ciprofloxacin administered to sea lions in this study.[7] Therefore, clinicians should always request the specific MIC value for each bacteria isolated.

Given the considerable variability in ciprofloxacin pharmacokinetics among animals in this study, these data suggest that, if linear pharmacokinetics can be assumed (that is, increase in AUC is proportional to increase in dose), a higher dose may be necessary. Based on this supposition, clinicians at TMMC elected to increase their dose of ciprofloxacin to 15 mg/kg orally. Samples from five additional California sea lions on ciprofloxacin as a part of their treatment protocol were opportunistically collected and analyzed (Fig. 1). These individuals were on a multi-dose regimen, having received an approximately 15-mg/kg (14.61 ± 2.43 mg/kg) dose of ciprofloxacin every 12 hr for one to four doses by the time of sampling. Plasma concentrations of ciprofloxacin near T_{MAX} (1.9–4.3 μg/ml) in some of these individuals were considerably higher than values obtained given the 10-mg/kg oral dose of ciprofloxacin, suggesting that the improved plasma concentrations would likely treat infections caused by most bacteria common to California sea lions.

The data from this study of ciprofloxacin pharmacokinetics in a young healthy population of wild California sea lions indicate that oral ciprofloxacin at a dose of 10 mg/kg may be an effective treatment for infections caused by highly susceptible bacteria with MICs for ciprofloxacin <0.06 μg/ml, including some of the bacteria most

frequently isolated from California sea lions in rehabilitation settings. Oral administration of ciprofloxacin in this species was well tolerated and provided quick absorption, with plasma drug concentrations comparable to domestic cats and dogs receiving a similar dose. A 15-mg/kg oral dose may produce a greater exposure and improved clinical outcome. Larger, long-term multidose studies are needed to further elucidate ciprofloxacin pharmacokinetics, including the effects of sex, age, and fasting, and to determine a safety profile for ciprofloxacin in California sea lions. Enhanced knowledge of ciprofloxacin pharmacokinetics may lead to improved treatment of bacterial infections in California sea lions and other pinniped species at TMMC and other facilities, as well as improve the standard of care for both rehabilitating and captive marine mammals.

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