Pharmacokinetics of long-acting cefovecin in copper rockfish (*Sebastes caurinus*)

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OBJECTIVE
To assess the pharmacokinetic properties of cefovecin in a cold-water teleost species.

ANIMALS
10 healthy adult copper rockfish (*Sebastes caurinus*), sex unknown.

PROCEDURES
Cefovecin (16 mg/kg) was administered SC to the rockfish. Blood samples were collected at predetermined points for measurement of plasma cefovecin concentrations (3 samples/fish). Plasma cefovecin concentrations were measured via liquid chromatography with mass spectrometry. Pharmacokinetic analysis was performed by means of naïve pooled analysis and compartmental modeling. Plasma protein binding of cefovecin was determined by ultrafiltration.

RESULTS
Cefovecin administration appeared to be well tolerated by the rockfish. Pharmacokinetic analysis resulted in a maximum plasma concentration of 104.8 μg/mL at 2.07 hours after administration. Plasma terminal half-life was 32.5 hours, and area under the curve was 5,132 h•μg/mL. Plasma protein binding was low (< 10%) for plasma concentrations of 10 and 100 μg of cefovecin/mL when assessed at 7.8° and 20°C. Plasma concentrations > 1 μg/mL persisted for the full 7-day follow-up period.

CONCLUSIONS AND CLINICAL RELEVANCE
SC administration of cefovecin to copper rockfish at a dose of 16 mg/kg yielded plasma concentrations > 1 μg/mL that persisted to 7 days, but some interindividual variability was observed. The low degree of plasma protein binding but high circulating concentration of free drug may allow an extended administration interval in rockfish. Studies are needed to assess the efficacy and safety of this dose in rockfish. (*Am J Vet Res* 2016;77:260–264)

Fish are susceptible to various bacterial diseases, and judicious use of antimicrobials is often warranted.[1] When selecting an antimicrobial, many factors need to be considered, including its spectrum of activity, frequency of administration, cost, potential for selection of bacterial resistance, legality for use in food animals, and possibility of adverse effects. In aquatic patients, additional considerations exist regarding the method of administration. Drugs are often designed to be delivered orally; via injection into the subcutaneous space, muscle, coelomic cavity, or circulatory system; or topically in an immersive bath. Fish, particularly those that are compromised, may not ingest antimicrobials reliably, so oral administration is not an ideal route. Immersion treatments can be useful, but few drugs can be administered in bath form because of poor water solubility, and there is concern about potential environmental impact, particularly in flow-through systems. As a result, fish medications may be injected, particularly in aquarium settings.

An undesirable aspect of medication injection is that handling fish can induce several stress responses, including an increase in epinephrine and cortisol production.[2] Even fairly brief stressor exposure can have long-lasting effects.[2] Furthermore, capture and handling of fish can cause trauma to the protective mucous layer and scales. To minimize distress and potential trauma when administering antimicrobials to fish, the goal is to use antimicrobials that can be administered as infrequently and uniformly as possible while maintaining therapeutic effectiveness.

Several long-acting antimicrobials are available for veterinary patients, but their effectiveness and bioavailability vary across species.[3] The third-generation cephalosporin cefovecin has a long elimination half-life in cats and dogs. As a result, drug concentrations remain at therapeutic values in those species for 14 days.[4–7] Cefovecin has antimicrobial activity against *Staphylococcus pseudintermedius* as well as gram-negative organisms such as *Escherichia coli*, *Klebsiella* spp, and *Proteus* spp.[8] This drug is currently labeled

ABBREVIATIONS

Cmax	Maximum plasma concentration
Tmax	Time to maximum plasma concentration
$T_{1/2}$	Plasma terminal half-life

in the United States for the treatment of skin infections in dogs and cats and in the United Kingdom for the management of urinary tract infections in cats.[4] Given the long half-life of the drug, cefovecin may also be indicated for extralabel use in treating various other bacterial infections.[4] Although limitations exist regarding extralabel administration of cephalosporins to food animals, fish are considered a minor species and are exempt.[9] However, extralabel use of medicated feed in aquaculture is prohibited but enforcement discretion is possible if the medicated feed is used in aquatic species that are not intended for human consumption such as fish in aquarium settings.

Pharmacokinetic studies[3–7] have shown that cefovecin remains at therapeutic concentrations in dogs and cats for 14 days, but pharmacokinetic values vary widely when the drug is used in other species. For example, the Cmax, Tmax, and $T_{1/2}$ of cefovecin in nonhuman primates, chickens, tortoises, and iguanas differ markedly from values in cats and dogs, and the rapid elimination of the drug in those species is not expected to allow extended administration intervals.[10–14] Preliminary studies[15] involving horseshoe crabs and bamboo sharks have revealed that cefovecin persists in the bloodstream for extended periods, but those studies did not identify whether the dosages used were clinically effective. To the authors' knowledge, no formal studies have been conducted to evaluate cefovecin in cold-water teleost species, although anecdotal reports[a] of its use exist. The purpose of the study reported here was to evaluate the pharmacokinetics of 1 dose of cefovecin administered SC to copper rockfish (*Sebastes caurinus*).

Materials and Methods

Animals

Ten clinically normal copper rockfish of unknown sex, weighing between 880 and 2,200 g, were included in the study. All fish were presumed to be adults on the basis of their size. Eight of the 10 fish had been used in a preliminary investigation to determine the ideal cefovecin dose for administration and points for blood sample collection, and a 21-day washout period was provided before the pharmacokinetic study began. For the purpose of the present study, fish were removed from their exhibit enclosure and housed in a 3,028-L holding tank with the same water-quality characteristics and source as in their enclosure. Fish were allowed to acclimate to the holding tank environment for 48 hours, which was a period intentionally kept to a minimum to reduce the time they were unavailable for exhibit.

To allow identification of individuals, fish were sedated by immersion in water containing 100 to 120 mg/L of tricaine methanesulfonate (MS222)[b] buffered with sodium bicarbonate[c] at a 2-to-1 ratio by weight. A small incision was made in each sedated fish between the 2 caudal rays at the base of the caudal dorsal fin. A colored cable tie was subsequently looped through the incision and secured. Microchips[d] were also inserted into the muscle on the right side to allow for permanent identification in the event that the colored tags became detached. All fish were monitored by husbandry staff for general health on a daily basis. Fish were offered food (herring, squid, clam, silversides, and mackerel) 6 d/wk. The study protocol was reviewed and approved by the Animal Care Committee at the Point Defiance Zoo & Aquarium.

Study protocol

Drug administration and blood sample collection were performed while fish were anesthetized with MS222 to reduce distress caused by handling. Ten days prior to cefovecin administration, 1 blood sample (2 to 3 mL) was collected from each of the 10 copper rockfish via the ventral tail vein by use of 22-gauge, 1.5-inch needles and a ventral or lateral approach.

On the day of administration, cefovecin[e] was reconstituted with sterile water in accordance with the manufacturer's instructions and administered SC to each fish at a dose of 16 mg/kg within 1 hour after reconstitution. Volume of injection ranged from 0.17 to 0.44 mL. The 16 mg/kg dose was chosen because, although the recommended dose of cefovecin for dogs and cats is 8 mg/kg,[4] initial investigation revealed that the 8 mg/kg dose would result in lower than expected plasma concentrations in the fish used in the present study.

Blood samples (0.4 to 1 mL each) were again collected 0.25, 0.5, 1, 4, 8, 24, 48, 72, 120, and 168 hours after drug administration, such that 3 fish provided samples at each collection point (ie, no fish contributed more than 3 blood samples [total volume, < 4 mL] during the postadministration period). Collected blood samples were transferred into lithium heparin tubes, and contents were mixed by inversion. Plasma was separated by centrifugation, frozen in polypropylene tubes within 1 hour after blood sample collection, and stored upright at −20°C until used to establish standard curves and the extent of plasma protein binding of cefovecin.

Cefovecin analysis

Plasma cefovecin concentrations were measured by means of liquid chromatography[f] with triple quadrupole mass spectrometry.[g] To determine plasma concentrations in assay standards and obtained samples, a protein precipitation method was used in which 50 µL of plasma was added to microcentrifuge tubes containing 200 µL of cephalexin (0.5 µg/mL) in methanol as an internal standard. Contents of the microcentrifuge tubes were vortex-mixed for 5 seconds and centrifuged for 5 minutes at 15,000 × *g*. Supernatant from each tube was transferred to an injection vial, and 10 µL was injected into the assay system.

The mobile phase of the assay consisted of acetonitrile and 0.1% formic acid in water with a flow rate of 0.4 mL/min. A mobile phase gradient started at 95% of 0.1% formic acid from 0 to 1 minute, with a lin-

ear gradient to 80% of 0.1% formic acid at 3 minutes, which was held until 3.5 minutes, followed by a linear gradient to 95% of 0.1% formic acid at 4 minutes. Total run time was 5.5 minutes. A C18 column[h] was used to achieve separation at 40°C.

The qualifying and quantifying ions for cefovecin were at an m/z of 454.08 and 241.00, respectively, and for the internal standard cephalexin were at an m/z of 348.09 and 158.00, respectively. The lower limit of quantification of the assay was 0.25 µg/mL, which was defined as the lowest concentration on the linear standard curve with measured concentrations within 15% of the actual concentration. The standard curve for rockfish plasma was linear from 0.25 to 100 µg/mL. Plasma samples containing concentrations > 100 µg/mL were diluted 1:1 with untreated plasma and reanalyzed. Standard curves were accepted when the measured concentration was within 15% of the actual concentration and the correlation coefficient was ≥ 0.99. Quality control samples at 0.25, 5, 50, and 100 µg/mL were within 15% of the actual concentrations (n = 1 per concentration; actual range, 90% to 115% of actual concentration).

Pharmacokinetic analysis

Pharmacokinetic analysis was conducted with computer software.[i] A 1-compartment model was generated by use of actual plasma concentrations of cefovecin following a naïve pooled method.[16] Naïve pooled analysis fits a model to all of the plasma concentration measurement points for all fish, resulting in a best fit model for the population. Absorption rate (K_{01}), elimination rate (K_{10}), absorption half-life, elimination half-life, volume of distribution per fraction of the dose absorbed, clearance per fraction of the dose absorbed, Cmax, Tmax, and area under the curve were estimated.

Protein binding

The extent of protein binding of cefovecin was estimated by use of ultrafiltration with a 10,000-Da membrane.[j] Pooled rockfish plasma was fortified with cefovecin at 10 and 100 µg/mL in replicates of 3 for each concentration and incubation temperature. The fortified pooled plasma was then incubated for 30 minutes at 7.8° and 20°C and placed in the ultrafiltration device for centrifugation at 3,000 × g for 30 minutes in a temperature-controlled centrifuge at 7.8° and 20°C. Plasma ultrafiltrate concentrations were determined by the assay method described for plasma cefovecin analysis, except that the standard curve was made for fortified plasma ultrafiltrate. To provide a control analysis, canine plasma fortified with cefovecin was processed in a similar manner at 7.8°C.

Results

No adverse effects were identified in any of the 10 copper rockfish throughout the study period. Pharmacokinetic analysis of cefovecin administered SC once to the fish at a dose of 16 mg/kg revealed a Cmax of 104.8 µg/mL and Tmax of 2.07 hours. The $T_{1/2}$ of cefovecin was 32.5 hours. Estimated volume of distribution per fraction of the dose absorbed was 146 mL/kg, and clearance per fraction of the dose absorbed was 3.12 mL/h/kg. Absorption and elimination rates were 2.28/h and 0.0213/h, respectively. Absorption and elimination half-lives were 0.304 hours and 32.5 hours, respectively. Area under the curve was 5,132 h•µg/mL

The extent of protein binding of cefovecin in fortified pooled rockfish plasma was < 10% when evaluated at 7.8° and 20°C at concentrations of 10 and 100 µg/mL. Specific results were as follows: 10 µg/mL at 7.8°C, < 5% (n = 1); 100 µg/mL at 7.8°C, 4% to 7% (3); 10 µg/mL at 20°C, < 5% (3); and 100 µg/mL at 20°C, 6% to 9% (3). Although 3 replicates were performed for each temperature and concentration, 2 samples representing 10 µg/mL at 7.8°C were lost during analysis, and not enough plasma was available to retest, so that particular condition involved only 1 sample. For

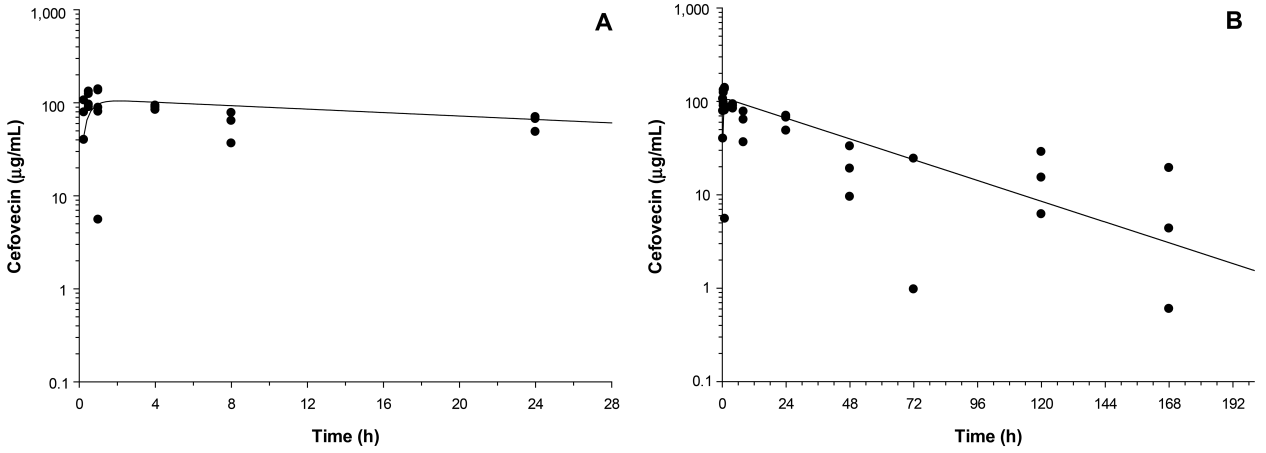

Figure 1—Measured (dots) and predicted (line) plasma cefovecin concentrations during a 24-hour (A) and 168-hour (B) period in copper rockfish (*Sebastes caurinus*) to which 1 dose of cefovecin (16 mg/kg) was administered SC (0 hours). Predicted values were calculated on the basis of naïve pharmacological analysis. Each measurement point represents data for 3 fish; 10 fish were used in total.

the canine plasma control analysis, extent of protein binding of cefovecin when evaluated at 10 µg/mL and 7.8°C ranged from 98.3% to 98.4% (n = 2), and that of cefovecin evaluated at 100 µg/mL at the same temperature ranged from 88.3% to 88.8% (2).

Pharmacokinetic predicted plasma concentrations of cefovecin exceeded 1 µg/mL throughout the 7-day sample collection period **(Figure 1)**. However, individual concentrations in 2 fish were < 1 µg/mL by day 3 or 7, suggesting interindividual variability in the persistence of cefovecin in the bloodstream.

Discussion

A long-acting antimicrobial would be useful for treatment of aquarium fish species because it would allow for infrequent handling and single-dose administration. In the study reported here, peak plasma cefovecin concentration was reached 2.07 hours after SC administration of one 16 mg/kg dose to copper rockfish. The $T_{1/2}$ was 32.5 hours, compared with a $T_{1/2}$ for dogs and cats of 133 and 166 hours, respectively.[5,6]

One reason that cefovecin persists in the bloodstream for such a prolonged period and requires such infrequent administration in cats and dogs is that it is highly protein bound.[5,6] However, the extent of protein binding in plasma samples from copper rockfish was < 10% at their natural environmental temperature of 7.8°C as well as at 20°C, which would help to explain the shorter half-life in this species. To determine whether temperature alone was responsible for differences in plasma binding, canine plasma samples fortified with cefovecin were also assessed at 7.8°C, and cefovecin in those samples remained highly bound, indicating that the difference in plasma protein binding was likely attributable to species differences in plasma rather than to temperature. These findings suggested that caution should be exercised when determining whether cefovecin is appropriate to use in species for which no label claim is available and that dosages for fish should not simply be extrapolated from dogs and cats.

A pharmacokinetic study[15] involving horseshoe crabs and bamboo sharks provided some initial information on the disposition of cefovecin (8 mg/kg, SC) in aquatic species. Mean ± SD half-lives were 37.7 ± 9.04 hours and 2.02 ± 4.62 hours in the crabs and sharks, respectively, and mean Cmax values were 26.01 ± 3.84 µg/mL and 52.08 ± 16.03 µg/mL, respectively, after 8 mg/kg SC. The extent of protein binding was also low (< 10%) in those species. Cefovecin in the copper rockfish of the present study had a half-life similar to that of the horseshoe crabs, even though this species is physiologically more similar to bamboo sharks than to crabs. On the other hand, the Cmax for copper rockfish was higher than that of the other 2 species, but so was the dose. One potential limitation of the present study was that the same fish were used for control sample collection and pharmacokinetic analysis, providing the potential for fluid shifts to have occurred that could have affected the volume of distribution of cefovecin. Pharmacokinetic differences among these 3 aquatic species with regard to cefovecin further illustrate the limitations of interspecies extrapolation.

Although the low degree of plasma protein binding in the copper rockfish of the present study may have contributed to the shorter half-life of cefovecin in this species, it suggested that more unbound drug would be available to elicit an antimicrobial effect than if greater protein binding had occurred. Surrogate markers in pharmacokinetic or pharmacodynamic modeling suggest that the amount of unbound drug better predicts effects than does total drug concentration.[17] Given that more unbound cefovecin would be available in rockfish than in dogs and cats, the antimicrobial effect in the fish could be expected to persist at lower total plasma concentrations. However, additional studies are needed to evaluate the efficacy of cefovecin in rockfish against pathogenic bacteria so that optimal dosages can be identified.

Data regarding the antimicrobial activity of cefovecin against bacterial pathogens of fish are unavailable. The minimum inhibitory concentration that inhibits 90% of *E coli* isolated from dogs is reportedly 1 µg/mL.[18] Whether minimum inhibitory concentrations against rockfish pathogens are similar is unknown, but with no data available, a target of 1 µg/mL provides a starting point for estimating potential dose administration protocols for efficacy studies. Although large numbers of fish were not used in the present study, the pharmacokinetic data obtained allowed for an estimation of the mean plasma profile of cefovecin in rockfish. Given the results of pharmacokinetic predictions, mean plasma concentrations exceeding 1 µg/mL were maintained for the 7-day follow-up period. These data suggested that a single dose may, on average, provide activity against pathogens for which the minimum inhibitory concentration of cefovecin is ≤ 1 µg/mL for at least 7 days. However, plasma concentrations in 2 fish were ≤ 1 µg/mL at days 3 and 7 after cefovecin administration, suggesting individual variation in drug exposure, which could be attributable to differences in elimination. Another possible explanation of these differences is that drug leakage occurred after injection in these 2 fish or that their volume of distribution was larger than in the other fish. It is important to note that the present study was conducted to assess feasibility and not efficacy. The findings reported here can therefore be used to assist in the design of future efficacy studies.

Results of the present study suggested that cefovecin may have some applications for treatment of cold-water fish. However, because the half-life and plasma binding profile were different in fish than in dogs and cats, it should not be presumed that the drug will persist in the bloodstream of rockfish for 7 to 14 days as it does in those other species. Further investigation regarding the efficacy and safety of cefovecin in copper rockfish is needed to establish the ideal dosage for that species.

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Footnotes

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f. Shimadzu Prominence, Shimadzu Scientific Instruments, Columbia, Md.
g. API 2000, Applied Biosystems, Foster City, Calif.
h. Supelco Discovery (50 X 2.1 mm; 5 µM), Sigma Chemical Co, St Louis, Mo.
i. WinNonlin, version 5.0, Pharsight Corp, Cary, NC.
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