Enrofloxacin pharmacokinetics in the European cuttlefish, *Sepia officinalis*, after a single i.v. injection and bath administration

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Gore, S. R., Harms, C. A., KuKanich, B., Forsythe, J., Lewbart, G. A., Papich, M. G. Enrofloxacin pharmacokinetics in the European cuttlefish, *Sepia officinalis*, after a single i.v. injection and bath administration. *J. vet. Pharmacol. Therap.* **28**, 433–439.

Enrofloxacin pharmacokinetics were studied in European cuttlefish, *Sepia officinalis*, after a single 5 mg/kg i.v. injection or a 2.5 mg/L 5 h bath. A pilot study with two animals was also performed following a 10 mg/kg p.o. administration. The concentration of enrofloxacin in hemolymph was assayed using high-performance liquid chromatography (HPLC) and pharmacokinetic parameters were derived from compartmental methods. In the i.v. study, the terminal half-life ($t_{1/2}$), apparent volume of distribution, and systemic clearance were respectively 1.81 h, 385 mL/kg, and 4.71 mL/min/kg. Following bath administration the $t_{1/2}$, peak hemolymph concentration (C_{max}), and area under the curve to infinity ($AUC_{0-\infty}$) were 1.01 h, 0.5 ± 0.12 μg/mL, and 0.98 μg·h/mL, respectively. After oral administration, the $t_{1/2}$, C_{max}, and $AUC_{0-\infty}$ were 1.01 h, 10.95 μg/mL, 26.71 μg·h/mL, respectively. The active metabolite of enrofloxacin, ciprofloxacin, was not detected in any samples tested. The hemolymph concentration was still above minimum inhibitory concentration (*MIC*) values for shrimp and fish bacterial isolates at 6 h after i.v. administration, therefore, a dose of 5 mg/kg i.v. every 8–12 h is suggested for additional studies of efficacy. The C_{max} value for the water bath was lower than for the i.v. study, but a bath of 2.5 mg/L for 5 h once to twice daily is suggested for additional studies to test efficacy against highly susceptible organisms. Although only two animals were used for the oral study, a dose of 10 mg/kg produced hemolymph concentrations of enrofloxacin that were in a range consistent with therapeutic efficacy in other species.

(Paper received 24 November 2004; accepted for publication 15 July 2005)

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INTRODUCTION

Cephalopods represent a highly developed class of invertebrates. They have a wide range of uses that benefit people, including as laboratory animals, display animals, and food animals. They have been used as models for many years for neurophysiology and basic physiology (Oestmann *et al.*, 1997). They have also been held in captivity for public and private aquariums and are consumed across the world. Only recently have veterinarians addressed cephalopod medicine and begun identifying diseases, therapeutics, and husbandry issues (Reimschuessel & Stoskopf, 1990; Reimschuessel *et al.*, 1990; Stoskopf & Oppenheim, 1996; Sherrill *et al.*, 2000). While a cephalopod formulary has been published based on empiric experience (Forsythe *et al.*, 1990), there are currently no publications on any drug pharmacokinetics in cephalopods. In fact, there are very few pharmacokinetic papers involving any invertebrate. Some reports are available that have examined penaeid shrimp because of the importance in aquaculture but these studies have not examined enrofloxacin (Park *et al.*, 1995; Reed *et al.*, 2004; Uno, 2004). Although enrofloxacin is not approved by the US Food and Drug Administration (FDA) for use in aquaculture, it still has off-label applications for nonfood aquatic animals (Lewbart *et al.*, 1997; Stoffregen *et al.*, 1997; Linnehan *et al.*, 1999). However, extralabel use of fluoroquinolones in animals intended for food is prohibited by the FDA.

The fluoroquinolone antimicrobials have been used extensively in many animal species. They are easily administered with a good safety record in animals. They are bactericidal and are active against gram-positive and gram-negative bacteria and *Mycoplasma* spp. (Papich & Riviere, 2001).

The objectives of this study are to identify pharmacokinetic parameters of enrofloxacin in the European cuttlefish (*Sepia officinalis*) after single i.v. injection and bath administration. Cuttlefish were selected for this study because they are more easily handled for multiple hemolymph collections than octopus and squid, and have been cultured through multiple generations in laboratories. A pilot study was also performed using an oral administration in two animals. Hemolymph concentrations achieved were compared with *in vitro* minimum inhibitory concentration (*MIC*) for bacterial pathogens in order to predict effective clinical treatment protocols.

MATERIALS AND METHODS

Animals

Twenty juvenile cuttlefish, of undetermined sex, were used for these studies; eight were used for the i.v. study, eight for the bath study, two for the oral study, and two were killed to examine drug concentrations in the branchial heart to compare with concentrations in the hemolymph. Control animals were included in each group to determine if there were drug concentrations in untreated animals kept in the same environment as treated animals. The cuttlefish were randomly netted from a seventh generation, tank-raised population of 80 housed at the National Resource Center for Cephalopods at the University of Texas Medical Branch. The cuttlefish were housed individually in floating bins within an 11 370 L recirculating seawater system. Important water quality parameters were measured including temperature (25 °C), salinity (32%), pH (7.92), ammonia (0.05 p.p.m.), nitrite (0.017 p.p.m.), and nitrate (26 p.p.m.). These values were stable throughout the study. All cuttlefish were healthy and eating prior to the studies; they were fed frozen or live shrimp three times daily. Magnesium chloride ($MgCl_2{\cdot}6H_20$; EM Science, Merck KGaA, Darmstadt, Germany) was the anesthetic used for all injections and collections. A 7.5% magnesium chloride stock solution was prepared with distilled water. For each anesthetic event, 100 mL of the stock solution was mixed with 1 L of seawater for a final concentration of 6.8 g/L. The total volume of water used to anesthetize the cuttlefish ranged from 6 to 8 L. The animals were assumed anesthetized when there was a lack of response to touch or manipulation; taking approximately 6–12 min to reach that level. All injections and hemolymph collections were from the muscular portion of the cephalic vein. The animals were placed in dorsal recumbency, the funnel lifted up with forceps and then the needle was inserted midline just under the skin about 1 cm cranial to the point of insertion of the funnel. Because these were invertebrates, the study protocol was exempt from an Institutional Animal Care and Use Committee (IACUC) approval.

Experimental design

Intravenous dosing

All eight cuttlefish were weighed prior to the dosing and had a mean weight of 114.7 g (range: 87.0–138.2). Six cuttlefish were given 5 mg/kg enrofloxacin (22.7 mg/mL; Baytril®, Bayer Animal Health, Kansas City, MO, USA) i.v. in the muscular portion of the cephalic vein. Two cuttlefish were used as controls and did not receive any enrofloxacin. Three treated cuttlefish and one control cuttlefish were kept within one floating bin while the remaining cuttlefish were kept in the other floating bin, all within an 11 370 L recirculating seawater system. All animals were fed their usual dead shrimp diet three times per day. Approximately 0.3 mL of hemolymph was collected from the muscular portion of the cephalic vein at each time point using either a tuberculin or insulin syringe and needle. A naive pooling sampling protocol was employed for this study. Hemolymph was collected from three of the treated cuttlefish prior to, and at 0.5, 2, 6, 24 and 72 h after drug administration. The other three treated cuttlefish were collected prior to drug administration, and at 1, 3, 12, 48 and 72 h. This resulted in three samples collected from three separate cuttlefish at each time point and a total of six samples collected from each individual cuttlefish. This design was necessary because individual animals could not be sampled frequently enough for complete pharmacokinetic analysis, but it allowed for naive pooling of samples to determine meaningful pharmacokinetic estimates. The two controls were collected at: 0, 2, 6, 24, 48 and 72 h. The total hemolymph removed from each cuttlefish was 1.8 mL (1.6% of mean body weight).

Bath administration

All eight cuttlefish were weighed prior to dosing and had a mean weight of 98.1 g (range: 85.7–111.4). Six cuttlefish were placed in a 2.5 mg/L bath for 5 h. The sampling times started after the cuttlefish were removed from the bath. Two cuttlefish were used as controls, receiving no drug, and placed in the unmedicated water during the sampling intervals. For the bath, 8 L of seawater was transferred to a plastic bag and the calculated drug dose was added to the bag, followed by the animal, and then oxygen was added and the bag was sealed. Each animal was housed individually during the bath treatment and the bags were floated in the system to maintain the temperature during the 5 h exposure. Samples of water in which the animals were bathed were collected prior to drug administration, at 1 min after drug administration and again at 5 h. All animals were fed a live shrimp diet three times per day. Approximately 0.3 mL of hemolymph was collected from the muscular portion of the cephalic vein at each time point using either a tuberculin or insulin syringe and needle. A naive pooling sampling protocol was employed in this phase of the study with the same rationale as for the i.v. study. Hemolymph was collected from three treated cuttlefish prior to, and 0.5, 2, 6, 24 and 72 h after finishing exposure to the drug bath. Samples were collected from three other treated cuttlefish prior to, and at 1, 3, 12, 48 and 96 h after exposure to the drug bath. This resulted in three samples being taken from three separate cuttlefish at each time point and a total of six samples collected from each individual cuttlefish. Samples were collected from two controls at 0, 2, 6, 24, 48 and 72 h. The total hemolymph removed from each cuttlefish was 1.8 mL (1.8% of mean body weight). Anesthesia was used as described above.

Oral dose study

There were not enough resources to conduct a complete pharmacokinetic study using an oral dose. However, we conducted a limited pilot study in two animals. For this study, two animals were weighed prior to dosing and had weights of 95.7 and 129.7 g. Each animal was given 10 mg/kg of injectable enrofloxacin solution (22.7 mg/mL) orally. The enrofloxacin was injected into live shrimp and immediately fed to and consumed by the cuttlefish. Both animals were fed live shrimp three times per day. Approximately 0.3 mL of hemolymph was collected from the muscular portion of the cephalic vein at each time point using either a tuberculin or insulin syringe and needle. Samples were collected from both animals prior to, and at 1, 3, 6, 24 and 48 h after drug administration. The total hemolymph removed from each cuttlefish was 1.8 mL. Anesthesia was used as described above.

Killed animals

Two animals were weighed prior to dosing and had weights of 117.3 and 102.9 g. Each animal was given 5 mg/kg enrofloxacin i.v. One animal was anesthetized as described above and opened through the ventral mantle at 30 min after the injection. Approximately 0.3 mL of hemolymph was collected from the branchial heart and killing (using an overdose of $MgCl_2$) immediately followed. The other animal was anesthetized at 1 h after the injection, opened, and 0.3 mL of hemolymph was collected from the branchial heart and killing immediately followed.

Sample preparation and analysis

Hemolymph samples were centrifuged to collect supernatant. An anticoagulant was not used. The hemocyte pellet was visible only on few occasions. The samples were stored at −70 °C until analysis could be performed. Previous experience in our laboratory has shown that enrofloxacin is stable when frozen in plasma at −70 °C, but we did not measure stability in cuttlefish hemolymph under these conditions because we had little spare sample matrix. To extract the drug from the hemolymph, incurred and fortified samples were prepared using solid phase extraction cartridges (Oasis, HLB, Waters Corporation, Milford, MA, USA). The cartridges were mounted on a vacuum system and conditioned by drawing 1.0 mL of methanol, followed by 1.0 mL of distilled water through the cartridges. Two hundred microliters of sample was extracted with a vacuum pressure of −5 mmHg and washed with 95% double-distilled water/5% methanol solution. Drug was eluted with 1.0 mL of methanol into clean tubes, dried under a flow of nitrogen (20 psi) at 45 °C for 15 min, and reconstituted with 100 μL mobile phase. The mobile phase was a mixture of 15% methanol, 85% double-distilled water, and 200 μL trifluoroacetic acid (TFA) per liter of mobile phase with a 1.0 mL/min flow rate. Twenty-five microliters of each sample were injected onto the high-performance liquid chromatography (HPLC) reverse phase column (Zorbax C-8 column, Agilent Technologies, Wilmington, DE, USA) heated to 40 °C. The HPLC system consisted of an autosampler (Agilent 1000 series, Agilent Technologies, Wilmington, DE, USA), quaternary pump (Agilent 1000 series, Agilent Technologies), and UV detector (Agilent 1000 series, Agilent Technologies) set at 279 nm. Calibration curves were prepared for each day of analysis by fortifying untreated hemolymph samples with known amounts of enrofloxacin reference standard (Bayer, Bayer Animal Health, Shawnee Mission, KS, USA) over an expected range of 0.05–7.5 μg/mL and processed as described above. The retention time for enrofloxacin was 5.5 min. There were no interfering compounds identified in any of the blank samples that corresponded to the window of the enrofloxacin peak. The lower limit of detection was 0.02 μg/mL. Reference standards of ciprofloxacin [ciprofloxacin United States Pharmacopeia (USP), 12601 Twinbrook Parkway, Rockville, MD, USA] were added to standard curves to test for the presence of ciprofloxacin in the samples because ciprofloxacin is a metabolite of enrofloxacin in mammals, birds, fish, and some reptiles (Papich & Riviere, 2001). Because we had little of the blank (unfortified) hemolymph to spare from such small animals, we did not perform a full validation of this assay. We ensured that we used the same matrix as incurred samples for preparation of the calibration curve and we ensured that the curve was linear (R^2-value for calibration curve: >0.99), and that samples for the calibration curve could be back-calculated to within 15% of the true value. The limit of detection cited above was taken from analysis of the background noise of unfortified samples.

After results from naive sampling were pooled, the pharmacokinetic analysis of enrofloxacin was performed using a computer program (WinNonlin 4.01, Pharsight Corporation, Mountain View, CA, USA). Compartmental analysis parameters were calculated from equations published elsewhere (Gibaldi & Perrier, 1982). The compartmental pharmacokinetic variables calculated were: total body clearance (*Cl*), apparent volume of distribution at steady-state [$V_{d(ss)}$], area under the curve from time 0 to infinity ($AUC_{0-\infty}$), mean residence time (*MRT*), and distribution rate constants with associated half-lives. For the water bath and oral treatments, noncompartmental analyses were performed using the same computer program. Parameters calculated water bath and oral treatments included $AUC_{0-\infty}$, terminal rate constant and corresponding half-life ($t_{1/2}$), *MRT*, maximum plasma concentration (C_{max}), and time of maximum plasma concentration (T_{max}).

RESULTS

Intravenous study

All animals, including the controls, survived and the only adverse effect noted was an average weight loss of 7.8 g (range: −12.9 to 0.6), in the treated animals and the controls, within the 72 or 96 h employed in the study.

The plasma profiles for enrofloxacin administration are illustrated in Fig. 1a,b. The plasma profile for i.v. administration best fit a two-compartment model with a rapid distribution phase

followed by the terminal (elimination) phase. Pharmacokinetic results are presented in Table 1. The terminal half-life ($t_{1/2}$), volume of distribution, and clearance were respectively 1.81 h, 385 mL/kg, and 4.71 mL/min/kg. No enrofloxacin was detected in any of the controls that shared water with the treated animals.

Ciprofloxacin, an active metabolite of enrofloxacin in most vertebrates, was not detected in any samples as verified by a reference standard.

Bath administration study

No adverse effects were noted in any of the animals. All controls and treated animals, except one, gained an average weight of 5.9 g (range: −0.1 to 14.6) within the 72 or 96 h employed in the study.

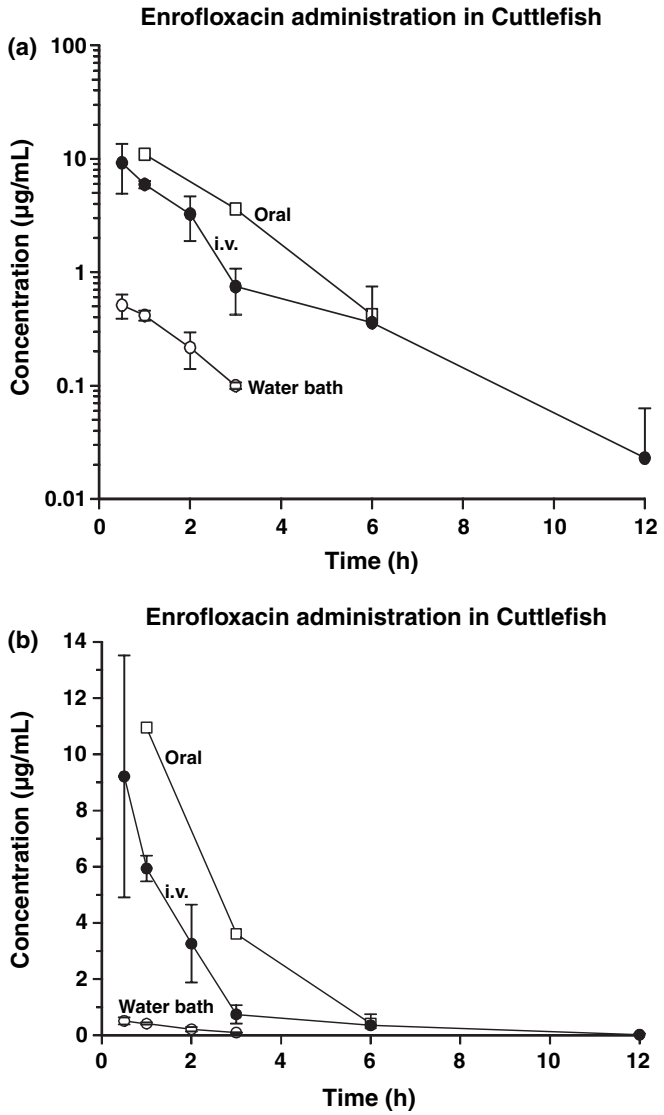

Fig. 1. (a) Semi-logarithmic axis: plasma profile of enrofloxacin administered to European cuttlefish (i.v. = 5 mg/kg; bath = 2.5 mg/L × 5 h; p.o. = 10 mg/kg). Error bars are not shown for the oral route as only two animals were in this group. (b) Linear axis: plasma profile of enrofloxacin administered to European cuttlefish (i.v. = 5 mg/kg; bath = 2.5 mg/L × 5 h; p.o. = 10 mg/kg). Error bars are not shown for the oral route as only two animals were in this group.

Table 1. Kinetic parameters from the i.v. administration of enrofloxacin (5 mg/kg)

Parameter	Value
A (µg/mL)	16.87
B (µg/mL)	2.40
α (1/hr^{-1})	1.48
β (1/hr^{-1})	0.38
$AUC_{0-\infty}$ (µg·h/mL)	17.69
$t_{1/2\alpha}$ (h)	0.47
$t_{1/2\beta}$ (h)	1.81
Cl (mL/min/kg)	4.71
MRT (h)	1.36
$V_{d(ss)}$ (mL/kg)	385.10

Pooled data from eight cuttlefish. Parameters listed are A and B, the intercepts for the distribution and terminal concentration curves, respectively; α and β represent the rate constants from the distribution and terminal concentration curves, respectively; the half-lives from these rate constants (α and β) also are included; Cl is the systemic clearance; AUC is the area under the curve from time zero to infinity; MRT is the mean residence time; $V_{d(ss)}$ is the apparent volume of distribution at steady-state.

The plasma profile from bath administration is illustrated in Fig. 1a,b. The water bath administration plasma profile was analyzed with a one- compartment model with first-order output. Following bath administration the $t_{1/2}$, C_{max}, and $AUC_{0-\infty}$ were 1.01 h, 0.5 µg/mL, and 0.98 µg·h/mL, respectively (Table 2). No detectable levels of enrofloxacin were present at 6 h postbath administration. Ciprofloxacin was not detected in any samples. The mean ± SD of water concentrations of enrofloxacin at 1 min and 5 h were 2.10 ± 0.71 and 1.76 ± 0.28 µg/mL, respectively (Table 3). This represents 84% and 70% of the total enrofloxacin concentration expected in the bath water.

Oral dose study

Adverse effects were not noted and both animals gained weight (3.4 and 2.7 g) during the 48 h employed in the study. The plasma profile from oral administration is presented in Fig. 1a,b.

Table 2. Kinetic parameters from the bath administration of enrofloxacin (2.5 mg/L × 5 h)

Parameter	Value
β (1/hr^{-1})	0.69
$t_{1/2\beta}$ (h)	1.01
T_{max} (h)	0.50
C_{max} (µg/mL)	0.51
$AUC_{0-\infty}$ (µg·h/mL)	0.98
MRT (h)	1.71

Pooled data from eight cuttlefish. β represent the rate constants for the terminal slope of the concentration curve. The half-lives from this slope is $t_{1/2}\beta$. T_{max} is the time to peak concentration; C_{max} is the peak concentration; $AUC_{0-\infty}$ is the area under the curve from time zero to infinity; MRT is the mean residence time. C_{max} SD was 0.12 µg/mL.

Table 3. Enrofloxacin concentrations (μg/mL) from the water of six cuttlefish treated by bath (the targeted initial concentration was 2.5 μg/mL)

	Animal							
Time (min)	7	8	9	10	11	12	Mean	SD
1	1.08	2.46	2.95	2.59	2.07	1.48	2.10	0.71
300	1.44	1.47	2.06	2.05	1.87	1.66	1.76	0.28

Table 4. Kinetic parameters from the oral administration of enrofloxacin (10 mg/kg p.o.)

Parameter	Value
β ($1/hr^{-1}$)	0.68
$t_{1/2\beta}$ (h)	1.01
T_{max} (h)	1.00
C_{max} (μg/mL)	10.95
$AUC_{0-\infty}$ (μg·h/mL)	26.71
MRT (h)	1.94

Data generated from pooled samples of two cuttlefish. β represent the rate constants for the terminal slope of the concentration curve. The half-lives from this slope is $t_{1/2}$ β. T_{max} is the time to peak concentration; C_{max} is the peak concentration; $AUC_{0-\infty}$ is the area under the curve from time zero to infinity; *MRT* is the mean residence time.

Following oral administration the $t_{1/2}$, C_{max}, and $AUC_{0-\infty}$ were 1.01 h, 10.95 μg/mL, 26.71 μg·h/mL (Table 4). Ciprofloxacin was not detected in any samples. No detectable levels of enrofloxacin were present at 24 h postoral administration.

Killed animals

Enrofloxacin was present in the fluid collected from the branchial heart at concentrations comparable with the concentrations in hemolymph following i.v. administration. Enrofloxacin was not detected at time 0 in either animal, and was 8.87 and 15.80 μg/mL at 0.5 and 1 h postadministration, respectively.

DISCUSSION

We anticipated that, owing to more primitive excretory systems of cuttlefish compared with vertebrate fish or mammals, enrofloxacin would be eliminated more slowly and the half-life would be longer than fish or mammals. This led to the sampling time points extending far beyond what was actually needed to measure hemolymph concentrations. To our surprise, the enrofloxacin $t_{1/2}$ after i.v. administration was considerably shorter (1.81 h), the *AUC* was smaller (17.69 μg·h/mL), the clearance was faster (4.71 mL/min/kg) and the volume of distribution [$V_{(ss)}$] smaller (0.385 L/kg) than reported for fish. Fingerling trout had a $t_{1/2}$ of 24.4 h, clearance of 1.5 mL/min/kg, and a 3.22 L/kg apparent volume of distribution following 5 mg/kg i.v. injection (Bowser *et al.*, 1992). Juvenile Atlantic salmon had a half-life of 130.6 h, clearance of 1.97 mL/min/kg, and an apparent volume of distribution of 21.53 L/kg following a 10 mg/kg i.v. injection (Stoffregen *et al.*, 1997).

One of the most surprising findings of the study was the small apparent volume of distribution in cuttlefish compared with vertebrates. This parameter is difficult to interpret, because it has no real physiologic meaning. Nevertheless, an apparent volume of distribution less than unity, compared with the much larger volume reported for vertebrates may indicate that enrofloxacin in cuttlefish has little tissue binding and possible high hemolymph protein binding. (There was not enough spare hemolymph to perform a protein-binding assay in this study.) In mammals, enrofloxacin distributes (extensively) intracellularly, which may not occur in cuttlefish. Also there may be differences in cell membrane structure or cell membrane efflux pumps between cuttlefish and vertebrates that have not yet been identified. Further studies are necessary to quantify tissue concentrations (especially skin, a frequent site of infection in captive cephalopods) compared with hemolymph concentrations to determine whether hemolymph concentrations represent tissue concentrations.

Ciprofloxacin was not detected in any samples. This is identical to findings in seabream, *Sparus aurata* L. (Della Rocca *et al.*, 2004) but opposite the findings in red pacu, *Colossoma brachypomum* (now *Piaractus brachypomus*) (Lewbart *et al.*, 1997) and most mammals. Ciprofloxacin was detected in seabass, *Dicentrarchus labrax*, liver and only occasionally in plasma (Intorre *et al.*, 2000). There is clearly species variability in relation to metabolic pathways and cuttlefish may not metabolize enrofloxacin to ciprofloxacin at concentrations detectable by our assay.

Enrofloxacin was well tolerated in all treated animals, with weight loss in the i.v. study, the only adverse effect noted. All animals survived, but long-term safety is unknown. Weight loss (mean 7.8 g) was seen with all the animals used in the i.v. study. The weight loss was initially thought to be associated with repeated handling of the animals or from the enrofloxacin itself, but the control animals, which did not receive any drug, also lost weight. It was noted that the cuttlefish stopped eating their usual dead shrimp diet during the study; therefore, the diet was changed to live shrimp. In the bath study all but one animal, controls and treated, ate regularly and gained weight. The two animals in the pilot oral study also ate live shrimp regularly and gained weight. The weight loss may be attributed more to the diet (dead shrimp), than to the repeated handling or enrofloxacin administration.

Although this study was not aimed at identifying specific bacterial pathogens associated with disease in cuttlefish, extrapolated *MIC* values from bacterial pathogens in fish and shrimp can be used to examine potential dosage regimens for enrofloxacin. There are currently no published studies identifying cuttlefish bacterial pathogens and correlating them with *in vitro* susceptibilities to enrofloxacin. Vibrios and other gram-negative organisms are common isolates from skin infections of cuttlefish and squid (Leibovitz *et al.*, 1977; Ford *et al.*, 1986; Sherrill *et al.*, 2000; Sangster & Smolowitz, 2003). *Vibrio alginolyticus*, *V. parahemolyticus*, *Vibrio* sp., *Pseudomonas putrefaciens*, and *Aeromonas hydrophila* have all been implicated as causes of myocarditis in the European cuttlefish (Reimschuessel *et al.*,

1990). *MIC* ranges for common shrimp isolates were from 0.02 to 0.5 μg/mL for the more susceptible organisms and up to 1.0 and 2.0 μg/mL for the more resistant *V. alginolyticus* and *P. aeruginosa* isolates, respectively (Mohney *et al.*, 1992). In another study of bacterial isolates from Mexican shrimp culture, 89 isolates were sensitive to enrofloxacin, three were intermediate, and none was resistant (Roque *et al.*, 2001). (Determination of resistant vs. sensitive was based on CLSI canine and feline interpretive standards.) *MIC* values for 18 bacterial fish pathogens ranged from 0.0064 to 0.16 μg/mL in one study (Bowser & House, 1990) and five fish isolates in another study ranged from 0.03 to 0.6 μg/mL (Martinsen *et al.*, 1992).

At 6 h the mean hemolymph concentration in the i.v. study was 0.36 μg/mL. This is above *in vitro MIC* values for some of the gram-negative organisms infecting shrimp and fish. Enrofloxacin also elicits a postantibiotic effect, continued bactericidal activity after the concentrations have dropped below the *MIC*. In laboratory vertebrates, a C_{max} of eight to 10 times the *MIC* or an *AUC* to *MIC* ratio of 100–125 has been shown to produce antibacterial cures. Using this guideline, the i.v. dose of 5 mg/kg produced an *AUC* that would be sufficient to treat organisms with an $MIC \leq 0.14$ μg/mL. The extrapolated peak plasma concentration (C_{max}) from the i.v. study (*y*-axis intercept of the elimination curve, B) was 2.4 μg/mL. Therefore, using a guideline of $10 \times MIC$, this implies that this dose would reach the critical C_{max}/MIC ratio for many susceptible bacteria ($MIC \leq 0.25$ μg/mL). The frequency of drug administration cannot be derived from this study, but because of the short half-life in cuttlefish compared with other animals, drug administration at least twice a day should be considered. Because i.v. administration will require anesthesia, this is probably impractical and stressful to the animals.

Bath drug administration offers another option in animals that cannot be handled for i.v. administration. Drug absorption from the medicated bath occurred across a gradient to produce the hemolymph concentrations in treated animals. However, because of our reluctance to use high concentrations in the water, the bath produced low C_{max} and *AUC* values compared with the i.v. treatment. These values would only be sufficient to treat bacteria with *MIC* values of 0.05 μg/mL (derived from C_{max}:*MIC* ratio) or <0.01 μg/mL (derived from the *AUC* ratio). For bacteria with higher *MIC* values, a larger dose (higher concentrations delivered to the water bath) would need to be investigated to determine if higher hemolymph levels are possible. Biologic filtration in the system may be compromised by this delivery method. Importantly, antibiotics administered to a water system may present an environmental health concern. Also bath treatments can be fairly expensive if a large volume of water is treated. Nevertheless, this study showed a potential for this method to treat a large population suffering from disease when drugs cannot be administered orally. Although the target bath concentration of 2.5 mg/L was not reached completely at 1 and 300 min postdrug administration, the drug still remained at 70% of the target at 5 h indicating that it is stable in seawater.

The control (untreated) animals used in both the bath and i.v. study did not have any detectable enrofloxacin in their hemolymph at any point during the study. Thus, it confirmed that enrofloxacin excreted from treated animals did not contaminate the water to such an extent that it would be absorbed by untreated animals. It is likely that the water concentration resulting from excreted drug was not high enough to be adequately absorbed by untreated animals.

The oral dosing study was a pilot study and only two animals were used. Therefore, we are hesitant to make conclusions from this portion of the study. Nevertheless, the oral dosing study revealed promising results because the C_{max} value of 10.96 μg/mL is high enough to be consistent with successful therapy in vertebrate animals. This method of delivery may be ideal if an animal is eating and the medication can be placed in the food. Future studies with more animals and time points within a shorter range of time would be beneficial for identifying an oral dosage regimen.

Two animals were killed because it was uncertain whether the hemolymph collected with our technique would represent the true value in circulating fluid. Therefore, samples were taken from the branchial heart of killed animals. Also this was important because injections and hemolymph samples were taken from the same location. Enrofloxacin was measured in the branchial heart fluid that mimicked concentrations in the hemolymph taken at similar time points of the i.v. study. Therefore, we concluded that our technique of sampling was valid.

CONCLUSIONS

This study explored methods of enrofloxacin administration to cuttlefish, and important differences in drug distribution and elimination compared with vertebrate animals that deserves further investigation. Enrofloxacin pharmacokinetics in cuttlefish differs considerably from results reported for fish, birds, reptiles, and mammals. The most striking differences were the rapid elimination rates, and the low apparent volume of distribution. Enrofloxacin was well tolerated in all treated animals. Using indices that have been derived from laboratory studies in vertebrate species, it is possible that i.v. dose of 5 mg/kg would be effective in treating some susceptible organisms, although frequency of administration may have to be greater than for vertebrate species. A bath consisting of 2.5 mg/L for 5 h once to twice daily, would be sufficient for treating highly susceptible organisms, however may not be effective for more resistant organisms. Oral administration of enrofloxacin, 10 mg/kg p.o., in live shrimp, produced high hemolymph concentrations and may be an effective method of administration, but was evaluated in only two animals. Frequently repeated i.v. injections are not practical means of drug delivery for cuttlefish, but baths and oral dosing would be more feasible.

ACKNOWLEDGMENTS

The authors are grateful to Jonathan Minton and Leigh Walsh for their assistance with the animals. The authors would also like

to thank the Merck-Merial Summer Research Internship Program, the Student Aquatic Animal Research Fund, and the Analytical Laboratory at North Carolina State University, College of Veterinary Medicine for financial support. The authors thank Ms Delta Dise of the Analytical Laboratory at North Carolina State University, College of Veterinary Medicine for assistance with the HPLC assays. Also thank Bayer Animal Health for donating the enrofloxacin reference standard.

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