Enrofloxacin serum bioactivity in bottlenose dolphins, *Tursiops truncatus*, following oral administration of 5 mg/kg in whole fish

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Eight adult bottlenose dolphins *Tursiops truncatus* (six male, two female) were employed in a single-dose study of orally administered enrofloxacin dosed at 5 mg/kg body weight. Blood samples were obtained from all animals at 0, 2, 4, 8, 12 and 24 h following administration of the dose in the animals morning ration of fish. Serum antimicrobial activity concentrations (SAAC) were determined using bioassay.

The mean elimination half-life ($t_{½}$) of enrofloxacin and its major metabolites was 6.4 ± 2.0 h with a range of 3–9.4 h. The time of maximal serum concentration (t_{max}) occurred at approximately 4 h with a range of 2–8 h following a single oral dose of 5 mg/kg. This variation in t_{max} most likely resulted from individual differences in absorption because of variations in the storage and digestion of the fish ration containing the drug dose within the compartmentalized cetacean stomach.

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INTRODUCTION

Enrofloxacin is a fluoroquinolone antimicrobial agent structurally related to nalidixic acid and norfloxacin and is licensed for veterinary use only. The fluoroquinolones have a bactericidal spectrum encompassing gram-negative, gram-positive aerobic bacteria and mycoplasmas (Reeves *et al.*, 1984; Scheer, 1987a). Enrofloxacin is an important antimicrobial agent that is proving to be very useful in treatment of infectious diseases in many domestic animal species (Scheer, 1987a; Jenkins & Friedlander, 1988; Prescott & Yielding, 1990; Van Landuyt *et al.*, 1990).

The primary mechanism of action of the fluoroquinolones appears to be interference with the bacterial enzyme DNA gyrase (Vancutsem *et al.*, 1990; Hooper & Wolfson, 1991). This unique mechanism of action differs from that of β–lactams, tetracyclines, aminoglycosides, macrolides, chloramphenicol and folic acid antagonists and could explain the lack of cross-resistance between these other antimicrobial agents and the fluoroquinolones (Scheer, 1987a).

Enrofloxacin has close to 100% oral bioavailability and attains excellent body tissue and fluid compartment concentrations in canines and felines (Scheer, 1987b). These characteristics make this antimicrobial, when given orally, particularly promising in use with marine mammals. Although there have been infrequent reports of neurological, haematological, gastrointestinal and cartilaginous toxicities associated with enrofloxacin, the drug has been very well tolerated in a variety of domestic animal species (Altreuther, 1987; Jenkins & Friedlander, 1988).

In this study we investigated the serum antimicrobial activity concentrations (SAAC) attained when enrofloxacin tablets were given to bottlenose dolphins in their morning ration of fish. This protocol would determine if this method of dosing enrofloxacin is feasible and would assist veterinarians involved in marine mammal practice to expand their antimicrobial treatment armamentarium.

MATERIALS AND METHODS

Study design

Following approval from the Animal Care Committee at our facility, eight healthy adult bottlenose dolphins, *Tursiops truncatus* (six male, two female) were selected for this study. They ranged in weight from 154 to 331 kg and were on no other medical treatments. Each animal received a single oral dose of enrofloxacin at a dosage of 5 mg/kg body weight. The dose was rounded to the nearest half tablet or 34 mg increment (68 mg tablets) and placed intact (not crushed) into a whole fish and administered with the morning fish ration. Experimental drug received from Bayer Corporation for our study consisted of the original 'purple tablet' oral preparation of enrofloxacin, which dissolves rapidly in water. The new chewable tablets now available do not disintegrate as easily as the former purple tablets and therefore may cause a greater lag between administration and absorption. This method of drug delivery with these animals is standard practice which is convenient and eliminates drug loss

to the aqueous environment (Ridgway, 1972). The animals continued to receive their normal food ration for the day but at the time of dosage had not received any food for 12 h. The tablets are commercially available under the trade name Baytril® (Bayer Corp. Agriculture Division, Animal Health, Shawnee Mission, KS).

Sampling and analyses

Blood samples were obtained immediately before and at 2, 4, 8, 12 and 24 h after administration of enrofloxacin. Blood samples were collected, allowed to clot, centrifuged and the serum was immediately separated, frozen (–7 °C) and transported to a commercial laboratory for determination of the serum antimicrobial activity concentration (SAAC) *via* bioassay (Barry, 1991; Chapin-Robertson & Edberg, 1991). The SAAC was measured using an agar plate diffusion assay, using *Bacillus subtilis* as the test organism. The calibration of the test was done with a pure standard enrofloxacin powder. The sensitivity of the assay was 0.1 mg/mL. The mean values ± SD are reported in Table 1.

Pharmacokinetic analysis

The concentration–time curve for each dolphin was analysed to estimate the time to peak concentration (t_{max}) and peak concentration (C_{max}). The terminal elimination half-life ($t_{½}$), area under concentration curve (*AUC*) from 0 to 24 h following dose administration ($AUC_{0–24}$) and mean residence time (*MRT*) were analysed and determined by using a noncompartmental pharmacokinetic model (Laub & Gallo, 1996). The mean values ± SD are reported in Table 2.

RESULTS

The mean time to peak SAAC (t_{max}) was 4 h with individual values ranging from 2 to 8 h. The mean peak SAAC (C_{max}) observed was 1.4 ± 0.3 μg/mL. The mean elimination half-life ($t_{½}$) was 6.4 ± 2.0 h with a range of 3–9.4 h. The mean SAAC at 12 and 24 h post dose was 0.6 ± 0.34 μg/mL and 0.2 ± 0.14 μg/mL, respectively. The mean *AUC* was 15.4 ± 5.5 μg·h/mL. Figure 1 is the semilogarithmic plot of plasma (SAAC) concentration–time curve of dolphin 2 after 5 mg/kg oral dose.

Table 1. Plasma concentrations (mean ± SD μg/mL) of enrofloxacin and its active metabolites (SAAC) after oral (p.o.) administration (5 mg/kg b.w.) in 8 bottlenose dolphins

Time (h)	Enrofloxacin + Metabolites (SAAC) mean ± SD (μg/mL)
2	0.83 ± 0.68
4	1.29 ± 0.34
8	0.96 ± 0.42
12	0.63 ± 0.34
24	0.20 ± 0.14

Table 2. Pharmacokinetic parameters (mean ± SD) of enrofloxacin and its active metabolites (SAAC) after oral (p.o.) administration of 5 mg/kg b.w. in 8 bottlenose dolphins

Parameter	Unit	Mean ± SD
$t_{½}$	h	6.4 ± 2.0
$AUC_{0–24}$	μg·h/mL	15.4 ± 5.5
MRT	h	11.8 ± 2.8
t_{max}	h	4
range	h	2–8
C_{max}	μg/mL	1.4 ± 0.3
range	μg/mL	0.9–1.9

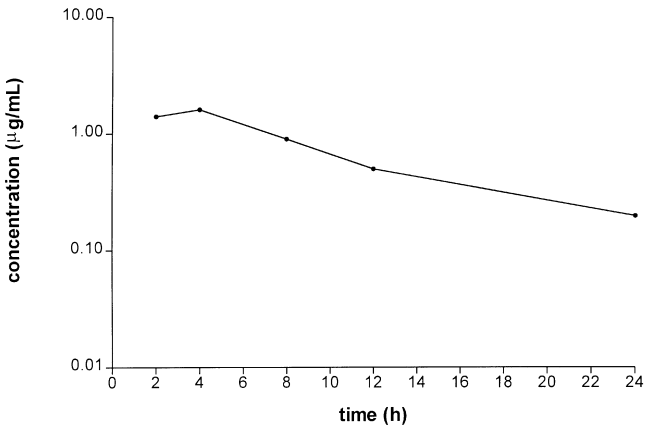

Fig. 1. Semilogarithmic plot of the plasma concentration–time curve (enrofloxacin and active metabolites mg/mL) of dolphin no. 2 after oral (p.o.) administration of enrofloxacin (5 mg/kg b.w.).

DISCUSSION

The results of this study indicate that enrofloxacin administered orally within whole fish at 5 mg/kg body weight to bottlenose dolphins will produce maximal serum antimicrobial activity concentrations (SAAC) occurring at approximately 4 h. This observation differs from similar studies performed on terrestrial mammalian species which indicate the time of maximal serum concentration to be between 1 and 2.5 h (Scheer, 1987b). This observed lag time (delay in absorption) is most likely because of differences in the storage and digestion of the fish ration containing the enrofloxacin within the forestomach compartment of the dolphin stomach. Breakdown of the fish containing the enrofloxacin tablets is required before dissolution makes the drug available for absorption in the fundic portion of the dolphin stomach (Green, 1972). The mean peak SAAC was determined to be 1.4 ± 0.3 which is above the (MIC_{90}) of many bacterial pathogens commonly dealt with in veterinary practice (Scheer, 1987a; Watts *et al.*, 1997). In addition, the following bacteria have been isolated from dolphins at our facility and reported by the lab to be sensitive to enrofloxacin (no minimum inhibitory concentration (*MIC*) studies were done): *Klebsiella* sp., *Aeromonas* sp., *Aeromonas hydrophila*, *Proteus mirabilus*, *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli* and *Vibrio alginolyticus*. It is

important to remember that much of the MIC data in the literature are based upon enrofloxacin and not its metabolites. The SAAC values produced in this study consist of enrofloxacin and its active metabolites. Ciprofloxacin is a metabolite of enrofloxacin and can contribute significantly to the SAAC (Küng *et al.*, 1993), potentially causing an overestimation of the concentration.

The mean elimination half-life ($t_{½}$) of enrofloxacin and its active metabolites was 6.4 ± 2.0 h. Intravenous (i.v.) studies were not done at the time of this study so confirmation of the elimination half-life ($t_{½}$) cannot be absolutely verified. The possibility of an absorption rate-limited elimination (Rowland & Tozer, 1995) must be considered when interpreting the delay to t_{max} reported in this study.

The pharmacodynamic properties of the fluoroquinolones indicate that these antimicrobial agents utilize concentration-dependent bactericidal activity. The peak/*MIC* and/or *AUC*/*MIC* ratios are the best predictors of efficacy in treatment (Dudley, 1991; Meinen *et al.*, 1995; Craig, 1998).

In studies where the 24-h *AUC*/*MIC* was compared to survival rates in animal models infected with various gram-positive or gram-negative bacteria, a 24-h *AUC*/*MIC* of $\geqslant 100$ was associated with greater survival (Leggett *et al.*, 1989). In another study involving humans, a 24-h *AUC*/*MIC* of $\geqslant 125$ in seriously ill patients treated with intravenous ciprofloxacin resulted in greater positive outcomes (Forrest *et al.*, 1993).

An optimal dosing strategy would provide a successful clinical outcome, minimize drug toxicity and reduce the emergence of bacterial resistance. Maximizing fluoroquinolone serum concentrations to a peak/*MIC* ratio of 8–10 reportedly minimizes development of bacterial resistance (Blaser *et al.*, 1987; Cambau & Gutmann, 1993).

This study indicates that a 5-mg/kg oral dose every 24 h in *Tursiops* would be appropriate and convenient for sensitive bacteria (*MIC* less than 0.14 μg/mL). This would yield a peak/*MIC* of 10 and a 24-h *AUC*/*MIC* of 110 both based upon the respective mean values. However, for organisms with a higher *MIC* such as *Pseudomonas* sp. using a 5 mg/kg oral dose every 24 h could result in a therapeutic failure. In this case it would be necessary to increase the dose and possibly decrease the interval to maximize the peak/*MIC* ratio and/or increase the 24-h *AUC*/*MIC* for this organism.

The method of placing enrofloxacin tablets in whole fish to provide a dose is an appropriate and convenient method that will provide therapeutic serum antimicrobial activity concentrations (SAAC). Specific dosing recommendations need to be based upon sound pharmacodynamic/pharmacokinetic principles that would maximize peak/*MIC* and/or 24-h *AUC*/*MIC* ratios. It should be noted that a delay or lag time in absorption will take place. With serious or life-threatening infections or less sensitive organisms other dosage routes such as i.v., intramuscular (i.m.) or direct oral dosing may need to be utilized, at least for the initial dose.

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