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PHARMACOKINETICS OF CEFTAZIDIME IN LOGGERHEAD SEA TURTLES (*CARETTA CARETTA*) AFTER SINGLE INTRAVENOUS AND INTRAMUSCULAR INJECTIONS

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Abstract: The pharmacokinetics of ceftazidime in yearling loggerhead sea turtles (*Caretta caretta*) following single i.m and i.v. injections were studied. Eight juvenile 1.25 ± 0.18 kg turtles were divided into two groups. Four animals received 20 mg/kg of ceftazidime i.v. and four received the same dose i.m. Plasma ceftazidime concentrations were analyzed by reverse-phase high-performance liquid chromatography. The i.v. and i.m. administration half-lives were 20.59 ± 3.24 hr and 19.08 ± 0.77 hr, respectively. The volume of distribution was 0.42 ± 0.07 L/kg, and the systemic clearence was 0.217 ± 0.005 ml/min/kg. Ceftazidime was detected in all blood samples and its concentration exceeded the minimum inhibitory concentration for *Pseudomonas* for 60 hr after i.m. and i.v. injections.

Key words: Caretta caretta, ceftazidime, loggerhead sea turtle, pharmacokinetics.

INTRODUCTION

Safe and effective antibacterial dosage regimens are poorly established for many reptiles, including sea turtles. Most are empirical and are based on extrapolations from other species. Differences in antibiotic disposition between reptiles and mammals and even among different species of reptiles makes cross-species extrapolation risky.

Ceftazidime, a third-generation cephalosporin, is active against such gram-negative bacteria as *Vibrio* spp., *Pseudomonas* spp., and *Aeromonas* spp., which are often associated with morbidity and mortality of sea turtles.[7,16] We studied ceftazidime pharmacokinetics following single i.v. and i.m. injections in juvenile loggerhead sea turtles (*Caretta caretta*) to establish effective clinical dosing regimes.

MATERIALS AND METHODS

Subjects and sample collection

Eight yearling 1.25 ± 0.18 kg juvenile loggerhead sea turtles were studied. Prior to the drug trial, each animal was weighed and examined visually, and blood was collected for a complete blood count and serum chemistry analysis using an eosinophil unopette (Unopette, Becton Dickinson, Rutherford, New Jersey 07070, USA). The turtles were held individually in 190-L aquariums arranged in two groups of four, with each group connected by a recirculating system consisting of a 1/8 horsepower pump (Little Giant TE5MD-HC pump®, Aquanetics, San Diego, California 92110, USA) and a 60-W flow-through ultraviolet light sterilizer (Aquanetics). Two 300-W in-line heaters (Aquanetics) kept the water temperature at approximately 24°C.

The turtles were randomly divided into i.v. and i.m. treatment groups of equal sizes. Each animal received a single dose of 20 mg/kg of ceftazidime (Tazidime®, Eli Lilly, Indianapolis, Indiana 46284, USA) using a 25-ga needle attached to a 1-ml syringe. Intravenous doses were administered in the left cervical sinus, and i.m. doses were injected into the left deltoid muscle.

Blood collection sites alternated between right and left cervical sinuses at 0 (predose sample), 0.5, 1.5, 3, 6, 12, 24, 48, 96, and 120 hr after injection. Approximately 0.5 ml of blood was collected each time using a 1-ml tuberculin syringe with a 26-ga needle. The syringe and needle interiors were rinsed before use with 0.1 ml of 1,000 IU/ml sodium heparin solution (Elkins-Sinn, Cherry Hill, New Jersey 08003, USA) as an anticoagulant. Blood was placed into polyethylene microcentrifuge tubes (Fisher Scientific, Pittsburgh, Pennsylvania 15219, USA), which were capped and immediately submerged in ice water. The blood was then centrifuged to harvest approximately 0.3 ml of plasma, which was placed in polyethylene microcentrifuge tubes via micropipet. The tubes were capped and stored at −70°C until high-performance liquid chromatography (HPLC) analysis.

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Equipment and reagents

Ceftazidime in plasma was quantitated using HPLC. The apparatus consisted of a pump (Waters Model 600 Solvent Delivery System, Millipore Corp., Milford, Massachusetts 01757, USA), autosampler (Series 1050 Autosampler, Hewlett-Packard, Palo Alto, California 94304, USA), and a variable wavelength ultraviolet light detector (Series 1050 VWD, Hewlett-Packard) set at a wavelength of 260 nm. Data were recorded on computer software (HPLC^{2D} ChemStation, Hewlett-Packard).

HPLC conditions

Ceftazidime and the internal standard, cefuroxime sodium, were eluted on a C-18 reverse-phase column (Waters NovaPak C-18 4μ Radial Compression Cartridge Millipore Corp.) with an isocratic mobile phase consisting of 28% methanol: 72% 0.01 M acetate buffer (pH 4.2–4.5) at a flow rate of 1.5 ml/min. All solvents were filtered and degassed prior to use. The mobile phase was periodically sparged with a flow of helium (10 ml/min) during analysis. The retention times for ceftazidime and the internal standard were approximately 3.5–3.7 and 4.4–4.6 min, respectively.

Preparation of stock solutions and calibration standards

Ceftazidime was obtained as ceftazidime pentahydrate and the internal standard, cefuroxime, as cefuroxime sodium, both as USP reference standards (U.S. Pharmacopoeia, Rockville, Maryland 20852, USA). A 1 mg/ml (calculated for the base) stock solution was prepared for each compound in distilled water. The ceftazidime stock solution was further diluted with distilled water to prepare calibration standards. Stock solutions were stable in tightly capped glass vials for 48 hr in the refrigerator. Blank plasma was fortified with ceftazidime and used for quality control samples and calibration standards. Calibration standards for the calibration curve ranged from 0.5 to 100 μg/ml. Quantitation was performed by calculating a ratio of the ceftazidime peak height (mAU) to internal standard peak height (mAU) and plotting on a linear graph against the true concentration using least-squares linear regression. Fresh calibration standards were prepared for each day's analysis. The calibration curve was linear with a r^2 value of at least 0.99, and all calibration standards could be back-calculated to within 15% of the true value.

Blank samples (plasma collected prior to drug administration) from each experimental animal were analyzed to ensure that there were no interfering peaks in the chromatogram. The baseline noise from blank samples was determined for each animal and the limit of detection (LOD) and limit of quantitation (LOQ) were established as 3 times and 10 times the baseline noise, respectively.

Sample preparation

Plasma samples collected during the experiment and samples prepared for quality control were prepared for HPLC injection by pipetting 300 μl of plasma into a clean glass tube. To this tube was added 10 μl of a solution of 1.0 mg/ml cefuroxime, which served as the internal standard. To this mixture was added 300 μl of 0.8 M perchloric acid to precipitate plasma proteins. The tube was centrifuged at 2,000 rpm at 4°C for 10 min, and the clear supernatant was transferred to an autosampler injection vial. Injection volume was 50 μl.

Pharmacokinetic analysis

Noncompartmental pharmacokinetic methods were used to determine the following parameters from concentration vs. time curves for disposition of ceftazidime after i.v. and i.m. administration: first order elimination rate constant (K_{el}) using proportionately weighted least squares linear regression of the $\log_{(10)}$ plasma from y-axis intercept (C_0), terminal half-life ($t_{1/2}$), area under the curve (AUC) from time zero to infinity area under the moment curve (AUMC) from zero to infinity, and mean residence time (MRT). From the i.v. administration, the following parameters were calculated: volume of distribution area method (Vd_{area}), volume of distribution at steady-state (Vd_{ss}), and total systemic clearance (Cl_s).[8] Values for the elimination rate constant and intercept of the curves were calculated with the use of a nonlinear curve-fitting program (Fig.P Software Corp., Durham, North Carolina 27717, USA). AUC was calculated using the trapezoidal method. Percent systemic availability from the i.m. injection (%F) was calculated from the ratio of $AUC_{i.m.}/AUC_{i.v.}$. The values for the detected maximum plasma concentration (C_{MAX}) and time of maximum detected plasma concentration (T_{MAX}) were the observed values. Statistical differences between routes of administration were measured with Student's t-test for independent samples (SAS Institute, Cary, North Carolina 27513, USA). The level for significance was a P-value ≤ 0.05.

Concentrations were compaired to minimum inhibitory concentration (MIC) of a susceptible reference *Pseudomonas aeruginosa* (American Type Culture Collection [ATCC] 27853, Rockville, Maryland 20852, USA) and a *Pseudomonas* species isolated from loggerhead sea turtles.

Table 1. Mean (±SE) pharmacokinetic values for intravenous and intramuscular ceftazidime in juvenile loggerhead sea turtles (*Caretta caretta*) after a 20 mg/kg dose.

Parameter	Intravenous	Intramuscular
k_{el} (/hr)	0.03 ± 0.01	0.04 ± 0.0
C_0 (μg/ml)	48.9 ± 7.8	46.5 ± 5.2
$t_{½}$ (hr)	20.6 ± 3.2	19.1 ± 0.8
$t_{½}$ harmonic (hr)	20.2	19.1
AUC (μg·hr/ml)	1,567 ± 330	1,393 ± 66
AUMC (μg·hr²/ml)	49,860 ± 12,509	44,169 ± 2,680
Cl_s (ml/min/kg)	0.22 ± 0.05	
MRT (hr)	31.7 ± 3.4	31.7 ± 1.0
Vd_{area} (L/kg)	0.4 ± 0.03	
Vd_{ss} (L/kg)	0.42 ± 0.07	

RESULTS

Ceftazidime was detected in all samples. The LOD and LOQ were approximately 0.2 and 0.5 μg/ml, respectively. The accuracy of the method was within 85% of the true value, and the precision had a coefficient of variation of < 10%.

Pharmacokinetic values are presented in Table 1. Ceftazidime administration produced almost identical plasma concentration profiles by both routes of administration (Fig. 1). Ceftazidime from i.m. injection was absorbed rapidly and completely. The C_{MAX} was almost identical from both routes of administration and the mean %F was 89.9%. The $t_{1/2}$ was 20.2 hr and 19.05 hr after the i.v. and i.m. administration, respectively. There were no statistically significant differences between routes of administration for the parameters of k_{el}, C_0, AUC, and MRT. Plasma concentrations were maintained above the MIC of 4.0 μg/ml for the susceptible ATCC *Pseudomonas aeruginaosa* for 72 hr and above the 8 μg/ml for the sea turtle *Pseudomonas* species for 60 hr after each injection route of administration.

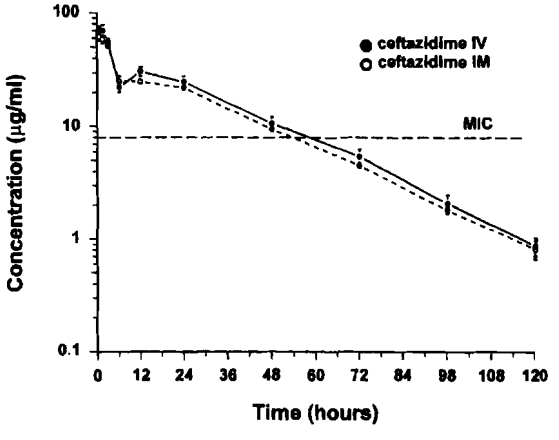

Figure 1. Mean ceftazidime plasma concentrations (± SE) after i.v. and i.m. injection of 20 mg/kg (n = 4 in each group) in juvenile loggerhead sea turtles (*Caretta caretta*). MIC = 8 μg/ml.

DISCUSSION

A rapid and accurate HPLC technique for analyzing ceftazidime concentration in 300 μl of sea turtle plasma was developed that had a LOQ below the level detected in all samples of this study.

This study indicates good absorption and distribution of ceftazidime and long half-life when given i.m. to juvenile loggerhead sea turtles. Statistically indistinct i.v. and i.m. parameters along with a systemic availability of 89.9% indicates excellent i.m. absorption.

The i.v. data deviated from the expected curve in linearity at 6 hr; this deviation was consistent for all the animals treated i.v. and to a lesser extent for all the animals treated i.m. The hemodynamics of chelonian cervical sinuses have not been well studied, but variations in plasma drug concentrations could be due to altered blood flow during diving responses.[2,5,15]

Bacteria causing infections in sea turtles include *Vibrio* spp., *Pseudomonas* spp., and *Aeromonas* spp.[1,7] According to some experts, beta-lactam antibiotics require peak concentrations approximately 4–8 times the MIC of the target bacterium and should remain above the MIC between doses for clinical cure.[11,12,14] At a 20 mg/kg dose, peak plasma levels for both groups exceeded the MIC by a factor of 17. The C_{MAX} was 69.74 ± 11.14 μg/ml for the i.v. route and 69.90 ± 8.53 μg/ml for the i.m. route. Following the dose of 20 mg/kg i.m. or i.v., plasma concentrations were maintained above the reference *P. aeruginosa* MIC (4 μg/ml) for at least 72 hr and above the sea turtle *Pseudomonas* isolate

MIC (8 μg/ml) for approximately 60 hr (Figures 1, 2).

Ceftazidime is excreted by glomerular filtration in mammals.[12] One explanation for ceftazidime's long half-life in sea turtles could be the turtle's slow renal clearance rate of approximately 0.24 ml/min/kg (green sea turtles, *Chelonia mydas*), which is a much slower than the canine renal clearance rate (3.08 ml/min/kg).[3,10,13] The green sea turtle clearance rate closely agrees with our findings of an elimination rate of 0.217 ± 0.05 ml/min/kg after i.v. ceftazidime administration, which is slower than the 3.08 ml/min/kg clearance rate of ceftazidime in dogs.[10] This finding suggests renal clearance is responsible for the elimination of ceftazidime in loggerhead sea turtles.

Antibiotic clearance in some reptiles is affected by body temperature.[4,6,9] Despite efforts to control water temperature, a 2°C variation was present between the two groups of tanks. The lack of differences in plasma concentrations between the i.m. and i.v. groups where individual animals were housed in each system suggests that a temperature difference of this magnitude did not affect ceftazidime's disposition.

A rapid and simple HPLC analysis for plasma ceftazidime in loggerhead sea turtles showed that ceftazidime plasma concentrations were above the MIC for *P. aeruginosa* (8 μg/ml) for at least 60 hr after an i.m. or i.v. dose. This finding suggests that 20 mg/kg every 72 hr should be a reasonable starting dosage for treating most *Pseudomonas* spp. but may have to be increased to 22 mg/kg. Multidose trials for ceftazidime in loggerhead sea turtles are needed to establish true clinical treatment regimes.

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LITERATURE CITED

1. Aguirre, A. A., G. H. Balazs, B. Zimmerman, and T. R. Spraker. 1994. Evaluation of Hawaiian green turtles (*Chelonia mydas*). J. Wildl. Dis. 30: 8–15.

2. Berkson, H. 1966. Physiological adjustment to prolonged diving in the Pacific green turtle (*Chelonia mydas agassizii*). Comp. Biochem. Physiol. 18: 101.

3. Bovée, K. C., and T. Joyce. 1979. Clinical evaluation of glomerular function: 24-hour creatinine clearance in dogs. J. Am. Vet. Med. Assoc. 174: 488–491.

4. Bush, M., J. M. Smeller, P. Charache, and R. Arthur. 1978. Biological half-life of gentamicin in gopher snakes. Am. J. Vet. Res. 39: 171–173.

5. Butler, P. J., W. K. Milsom, and A. J. Woakes. 1984. Respiratory cardiovascular and metabolic adjustments during steady state swimming in the green turtle, *Chelonia mydas*. J. Comp. Physiol. B. 154: 167.

6. Caligiuri, R., G. V. Kollias, E. Jacobson, B. McNab, G. H. Clark, and R. C. Wilson. 1990. The effects of ambient temperature on amikacin pharmacokinetics in gopher tortoises. J. Vet. Pharmacol. Ther. 13: 287–291.

7. Campbell, T. W. 1996. Sea turtle rehabilitation. *In:* Mader, D. R. (ed.). Reptile Medicine and Surgery. W. B. Saunders Co., Philadelphia, Pennsylvania. Pp. 427–435.

8. Gibaldi, M., and D. Perrier. 1982. Pharmacokinetics, 2nd ed. Marcel Dekker, New York, New York.

9. Mader, D., G. Conzelman, and J. D. Baggot. 1985. Effects of ambient temperature on the half-life and dosage regimen of amikacin in the gopher snake. J. Am. Vet. Med. Assoc. 187: 1134–1136.

10. Matsui, H., M. Komiya, C. Ikeda, and A. Tachibana. 1984. Comparative pharmacokinetics of YM-13115, ceftriaxone, and ceftazidime in rats, dogs, and rhesus monkeys. Antimicrob. Agents Chemother. 26: 204–207.

11. Nichols, W. W. 1988. Towards a fundamental understanding on the MIC of β-lactam antibiotics. J. Antimicrob. Chemother. 22: 275–283.

12. Richards, D. M., and R. N. Brogen. 1985. Ceftazidime: a review of its antibacterial activity, pharmacokinetic properties and therapeutic use. Drugs 29: 105–161.

13. Schmidt-Nielsen, B., and L. E. Davies. 1968. Fluid transport and tubular intercellular spaces in reptilian kidneys. Science 159: 1105–1108.

14. Soriano, F. 1992. Optimal dosage of β-lactam antibiotics: time above the MIC and inoculum effect. J. Antimicrob. Chemother. 30: 566–569.

15. West, N. H., P. J. Butler, and R. M. Bevan. 1992. Pulmonary blood flow at rest and during swimming in the green turtle, *Chelonia mydas*. Physiol. Zool. 65: 287.

16. Westly, G., and B. Pfohl (eds.). 1994. Physician's Desk Reference. Medical Economics Data Production. Montvale, New Jersey. Pp. 1011, 1571.

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