Pharmacokinetics of Ticarcillin in the Loggerhead Sea Turtle,

*Caretta caretta*, after Single Intravenous and Intramuscular Injections

Charles. A. Manire, D.V.M., Mote Marine Laboratory and Aquarium, 1600 Ken Thompson Parkway, Sarasota, Florida 34236, USA, telephone 941-388-4441, fax 941-388-4317, email cmanire@mote.org

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Abstract: Pharmacokinetic studies of drugs used in endangered and threatened sea turtles are extremely limited. This study was undertaken to provide a drug dosage for sea turtles for ticarcillin, a semi-synthetic anti-pseudomonal beta-lactam antibiotic. Three captive loggerhead sea turtles, *Caretta caretta*, were used in four trials, one intravenous and three intramuscular, to determine the pharmacokinetic properties of single dose ticarcillin in the species. For the intravenous study, each turtle received 50 mg/kg of drug and blood samples were analyzed at pre-dose, 0.5, 1, 2, 4, 6, 8, and 12 hr and at 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 14 days after the injection. For the intramuscular study, each turtle received one of three dosages (25, 50, or 100 mg/kg) and blood samples were analyzed at the same time intervals. Each trial was separated by a minimum of 28 days to allow for complete drug clearance. Drug concentration in plasma was analyzed via LC/MS. For the intravenous study, the mean drug elimination half-life ($t_{1/2}$) was 5.0 hr ($\pm$ 1.57 SD), indicating a fairly slow drug distribution. For the intramuscular study, time to maximum plasma concentrations ($t_{\text{max}}$) ranged from 1.7 ($\pm$0.58) hr with the 50 mg/kg group to 3.7 ($\pm$2.5) hr with the 100 mg/kg group. Systemic availability (F) ranged from 45% ($\pm$15%) with the 50 mg/kg group to 58% ($\pm$12%) with the 100 mg/kg group and the mean residence time (MRT) ranged from 7.5 hr ($\pm$2.6) with the 25 mg/kg group to 16 hr ($\pm$6.8) with the 100 mg/kg group. One turtle showed vomiting after the intravenous injection and one of the intramuscular injections. Two turtles had slight ALT elevations at two different dosages, but otherwise, blood chemistries were unaffected. From these results, an appropriate intramuscular dosage for loggerhead sea turtles should be 50 mg/kg q24 hr or 100 mg/kg q48 hr. It is suggested that liver enzymes be monitored during treatment.
INTRODUCTION

Ticarcillin is a semi-synthetic beta-lactam developed in the early 1970's, primarily as an anti-pseudomonal antibiotic (Wise and Reeves, 1974). In humans, the drug is excreted through the urine, primarily unchanged (Brogden et al., 1980). Although it has been used for a wide variety of infections in humans (Brogden et al., 1980), its veterinary use has been limited primarily to dogs (Johnson et al., 1978; Tilmant et al., 1985; Garg et al., 1987; Nuttall, 1998) and horses (Sweeney et al., 1984; Spensley et al., 1986; Sweeney et al., 1988a, 1988b; Van Camp et al., 2000), but its use has been investigated in dairy cows (Nouws et al., 1984) and sheep (Errecalde et al., 1991), as well.

The efficacy of ticarcillin was found to be very similar to carbenicillin (Neu and Garvey, 1975), except that it is more active against *Pseudomonas aeruginosa* and Gram-negative bacilli and less active against Gram-positive cocci (Brogden et al., 1980). It also has efficacy against anaerobic bacteria, but it is inactivated by beta-lactamase producing microorganisms (Brogden et al., 1980). In veterinary medicine, its use is generally reserved for resistant *P. aeruginosa* infections where it is often used in combination with the beta-lactamase inhibitor clavulanic acid (Prescott, 2000).

In reptiles, there has been very little mention of ticarcillin in the literature. Frye (1991, 1994) suggested a dosage of 50-100 mg/kg IM q24 hr for reptiles, but cited no pharmacokinetic studies upon which to base this. The following study was undertaken to determine an appropriate dosage for use in loggerhead sea turtles, as *P. aeruginosa* infections are fairly common for sea turtles undergoing rehabilitation. With the danger of this organism developing resistance to the commonly used antibiotics, it was hoped that this drug would be usable either as
a backup when the more commonly used antibiotics failed to eliminate such an infection or in combination with them to prevent the development of resistance.

MATERIALS AND METHODS

Animals

The three clinically healthy turtles utilized in this study were housed on exhibit at Mote Marine Aquarium in Sarasota, Florida. Two were adult female loggerhead sea turtles, Caretta caretta, which had been collected as hatchlings in 1977 in North Carolina and were brought to Mote in 1998. The third, an amelanistic subadult female loggerhead, was brought to Mote as a hatchling in 1992. The two adults, weights 96.9 and 101.8 kg at the start of this study, were housed in a 87,000 l display tank and the subadult (44.5 kg) was housed in a 20,000 l display tank. Both systems were recirculating, filtered seawater that was maintained at 25-27°C. Physical examinations, hematology, and blood chemistries were all within normal limits prior to the start of the trial. The turtles did not receive any other medication during the trial.

Intravenous study

Each turtle was weighed, had a pre-dose blood sample collected, and then was given a single intravenous injection of ticarcillin disodium (Ticar, SmithKline Beecham Pharmaceuticals, Philadelphia, PA 19101, USA) into the external jugular vein at a dosage of 50 mg/kg. Blood samples were then collected at 0.5, 1, 2, 4, 6, 8, 12 hr and at 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 14 days after the injection. Blood was collected into anticoagulant blood tubes (Lithium Heparin Vacutainer, Becton-Dickinson, Franklin Lakes, NJ 07417, USA) and immediately centrifuged at 1500 G to separate out the plasma, which was then placed into
cryovials and stored at -80°C until analyzed. At occasional sampling times, additional blood was collected for health assessment via complete blood counts and chemistry profiles.

**Intramuscular study**

Following the intravenous study, each turtle was randomly assigned one of three different dosage regimens (25, 50, or 100 mg/kg IM) for three consecutive trials with each turtle finally receiving each of the different dosage regimens. For each trial, each turtle was weighed and given the appropriate dose by IM injection deep into the musculature of the shoulder. For each trial, blood samples were collected at pre-dose, 0.5, 1, 2, 4, 6, 8, 12 hr and at 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, and 14 days after the injection and processed as in the intravenous study. At occasional sampling times, additional blood was collected for health assessment via complete blood counts and chemistry profiles. The interval between trials was a minimum of 28 days to be certain all drug had cleared from the turtles’ bodies.

**Plasma analysis**

Ticarcillin concentration was determined in sea turtle samples by use of a liquid chromatographic-mass spectrometer assay. A Luna C18(2) (150 × 2.0 mm, 5 µm) column from Phenomenex (Torrance, CA) was used to separate ticarcillin and the internal standard (ceftiofur). All chemicals used were high-performance liquid chromatography grade. The mobile phase consisted of a gradient using A) 30:70 and B) 50:50 ACN / 10 mM ammonium acetate and at 0 min was 100% A, at 0.3 min 100% B, then held for 5.7 min and then 1 min to return to 100% A, the flow rate was 0.2 ml/min. The mass spectrometer used an electrospray ionization source in the positive ionization mode. To screen for ticarcillin, a mass to charge ratio (m/z) of 401.8 was
selected, then mass spectrometry with a normalized collision energy of 20% was performed and the daughter ions of 384.7 and 159.9 \( m/z \) were monitored and used for quantitation. Mass spectrometer settings were as follows: Spray/Source voltage, 4.5 kV; Sheath gas flow rate, 55 arbitrary units; Auxiliary gas flow rate, 10 arbitrary units; Capillary voltage, 17 V; Capillary temperature, 130 °C; Tube Lens Offset, 55.0 V; Intermultipole lens voltage, -18 V; Multipole 1 offset, -3.5 V; Multipole 2 offset, -5.5 V.

Quality control (QC) samples were made in bulk at three different concentrations within the range of the standard curve, then stored at -20°C until extracted with the standard curve and samples. Standard samples were prepared on the days samples were assayed and had a range from 0.1 to 75 μg/ml. Samples, standards, and quality control samples were extracted by modifying a previously reported ticarcillin extraction method (Kwan et al.). Briefly, to a 500 μl aliquot of plasma, 50 μl of internal standard (10 μg/ml ceftiofur) was added, 500 μl of 1 M formic acid, and 3 ml of ethyl acetate. Vortexed for 1 min, centrifuged for 10 min at 1000 × g, transferred the supernatant to a clean tube and dried under N₂ in a 30 °C water bath, reconstituted in 200 μl of mobile phase (B) and injected on to the liquid chromatography system. Plasma concentrations were quantitated by use of peak area ratios and slope-intercept from linear standard curves. Intra-day variation (precision) was 5.0%, 6.3%, and 5.9% and intra-day accuracy was 8.5%, 9.2%, and 9.0% for the 0.5, 5, and 50 μg/ml QC samples, respectively. Inter-day variation (precision) was: 3.7%, 6.0% and 2.0% and inter-day accuracy was 7.8%, 7.9%, and 9.1% for the 0.5, 5, and 50 μg/ml samples, respectively. Recoveries were 5.7%, 11.3%, and 10.0% for the 0.5, 5, and 50 μg/ml samples, respectively. Samples in which concentrations of ticarcillin appeared greater than the highest standard curve concentration were reassayed by diluting an aliquot of the original sample with control plasma.
Pharmacokinetic calculations

The values of the pharmacokinetic parameters were determined for each individual animal using non-compartmental analysis (Gibaldi & Perrier, 1982; Riviere 1999). Values calculated following the i.v. dose were: area under the plasma concentrations vs. time curve (AUC), area under the first moment curve (AUMC), mean residence time (MRT = AUMC/AUC), apparent volume of distribution steady-state (Vd = (Dose × AUMC)/AUC²), plasma clearance (Cl_p = dose/AUC), elimination rate constant (λ) calculated as the slope of the terminal phase of the plasma concentration curve that included a minimum of 3 time points, and terminal half-life (t_1/2 = 0.693/λ). Following i.m. administration, the following parameters were determined as above: AUC, AUMC, MRT, and bioavailability F = (AUC_{po} × D_{iv}) / (AUC_{iv} × D_{po}) × 100. The AUC and AUMC were calculated using trapezoidal rule with extrapolation to ∞.

RESULTS

Intravenous study

The mean ticarcillin concentrations vs. time was calculated for the three turtles combined (Fig. 1) and the pharmacokinetic variables were calculated (Table 1). The mean drug elimination half-life (t_1/2) was 5.0 hr (± 1.57 SD), indicating a fairly slow drug distribution. One of the turtles vomited about 12 hr following the injection but did not hesitate to eat the following morning. No other adverse reactions were noted following the intravenous administration. Blood chemistries did not change appreciably following this portion of the study.
Intramuscular study

For each dosage group, the mean ticarcillin concentration vs. time was calculated (Fig. 1) and the pharmacokinetic variables were calculated (Table 2). Among the three dosage groups, time to maximum plasma concentrations ($t_{max}$) ranged from 1.7 (±0.58) hr with the 50 mg/kg IM group to 3.7 (±2.5) hr with the 100 mg/kg group. Systemic availability (F) ranged from 45% (±15%) with the 50 mg/kg group to 58% (±12%) with the 100 mg/kg group.

The same turtle that vomited following the intravenous injection also vomited 12-24 hr after receiving the 50 mg/kg intramuscular dosage, but no vomiting was noted at the other dosages for the same turtle or at any dosage for the other two turtles. Two of the turtles showed slight increases in alanine transaminase (ALT) concentrations, one on the 100 mg/kg dosage and the other on the 50 mg/kg dosage. The increase was transient with no accompanying symptoms and no other blood chemistry elevations were evident for any of the turtles at any of the dosages.

DISCUSSION

Ticarcillin is a semi-synthetic penicillin classified as a ureidopenicillin, with activity primarily against *Pseudomonas aeruginosa*, *E. coli*, and *Proteus* spp., but is generally reserved for use against *P. aeruginosa* in animals (Prescott, 2000). The elimination half-life ($t_{1/2}$) of ticarcillin is relatively short, being 1.2 hr in humans (Dalhoff and Hoffler, 1977), 0.8-1.2 hr in dogs (Garg et al., 1987; Tilmant et al., 1985), 0.83-1.8 in adult horses (Spensley et al., 1989; Sweeney et al., 1988b), and 0.83-2.9 in foals, depending on route of administration (Sweeney et al., 1988a), 1.1 in dairy cows (Nouws et al., 1984), and 0.90-0.96 in sheep (Errecalde et al., 1991). The longer $t_{1/2}$ for the sea turtles in the current study is likely an indication of the slower
metabolism of reptiles and is a consistent finding with other drugs in sea turtles (Mallo et al., 2002; Manire et al., 2003) as well as other reptiles.

The bioavailability (F) of ticarcillin is quite variable in mammals, with studies showing a range of 54.6% to 91.37% from the foal and dog, respectively (Sweeney et al., 1988a; Garg et al., 1987). The bioavailability found in the current study (45-58%) falls at the lower end of the range in mammals. Stamper et al. found that in loggerhead turtles florfenicol had a fairly low bioavailability (62.4%) but ceftazidime had a fairly high bioavailability (89.9%) (Stamper et al., 2003, 1999).

Because ticarcillin is commonly used to treat infections caused by *P. aeruginosa*, an organism that readily develops resistance to antibiotics, it is often used with other antibiotics or with beta-lactamase inhibitors. It is commercially available in combination with clavulanic acid (Timentin, GlaxoSmithKline, Research Triangle Park, NC 27709) and there appear to be no interactions between the ticarcillin and clavulanic acid (Tasker et al., 1986). Concurrent use of ticarcillin was found to inactivate gentamicin in humans, especially with renal failure (Brogden et al., 1980).

Side effects commonly noted with ticarcillin include pain at injection site for intramuscular injections (Garg et al., 1987; Sweeney et al., 1988a, 1988b), platelet dysfunction (Johnson et al., 1978; Brogden et al., 1980), and a few other minor symptoms. Clinically, elevated transaminases have been observed in humans, but it is not known if the elevations were related to the ticarcillin or the infection (Brogden et al., 1980; Tasker et al., 1986). It is not known if the ALT elevations that were observed in two of the turtles were caused by the ticarcillin, but it warrants close observation of blood chemistries when using ticarcillin in sea turtles. The vomiting that was observed with one turtle was likely caused by the ticarcillin, as
both instances followed the injection by 12-24 hr, but it did not appear to affect the turtles appetite and may not be a serious problem with treatment. There was no evidence in these three turtles of pain following intramuscular injection. Likewise, there was no evidence of clotting disorder, but this would not be expected with a single dose of the drug. When ticarcillin is used in a clinical situation, the patients should be observed for the development of such a disorder.

It should be noted that a number of studies have shown that the pharmacokinetic values of drugs are affected by environmental (water) temperature in reptiles (Mader et al., 1985; Caligiuri et al., 1990). For this study, the turtles were maintained at 25-27°C, and it is likely that the pharmacokinetics would be different for turtles maintained at higher or lower temperatures. Therefore, drug dosage may have to be adjusted accordingly for other ambient temperatures.

CONCLUSIONS

The results of this study suggest that an intramuscular dosage of 5 mg/kg q24 hr or 10 mg/kg q48 hr would be appropriate for treating Pseudomonas aeruginosa infections in the loggerhead sea turtle maintained at 25-27°C. However, due to the high risk of development of resistance with any treatment for P. aeruginosa, it should always be used in combination with a broad-spectrum aminoglycoside or with beta-lactamase inhibitors. Also, care should be taken in monitoring liver enzyme concentrations in case of the development of hepatic toxicity.

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**LITERATURE CITED**


Table 1. Pharmacokinetic variables for ticarcillin after intravenous administration in three loggerhead sea turtles.

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<th>Mean</th>
<th>SD</th>
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<tr>
<td>AUC₀-Tₘₚₙ (h×μg/mL)</td>
<td>1414</td>
<td>941</td>
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<tr>
<td>AUMC₀-Tₘₚₙ (h²×μg/mL)</td>
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<td>17825</td>
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<td>AUC₀-∞ (h×μg/mL)</td>
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</tr>
<tr>
<td>AUMC₀-∞ (h²×μg/mL)</td>
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<td>MRT (h)</td>
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<td>V₀(area) (L/kg)</td>
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<tr>
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<td>0.0256</td>
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<tr>
<td>Clₚ (L/h/kg)</td>
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<td>0.0105</td>
</tr>
<tr>
<td>λ (h⁻¹)</td>
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<tr>
<td>t₁/₂ (h)</td>
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Table 2. Pharmacokinetic variables for ticarcillin after intramuscular administration of three different dosages in three loggerhead sea turtles.

<table>
<thead>
<tr>
<th></th>
<th>IM - 25 mg/kg</th>
<th>IM - 50 mg/kg</th>
<th>IM - 100 mg/kg</th>
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<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>AUC₀-ₜₐₐₙ (h×µg/mL)</td>
<td>572</td>
<td>182.1</td>
<td>1148</td>
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<tr>
<td>AUMC₀-ₜₐₐₙ (h²×µg/mL)</td>
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<td>2716</td>
<td>10562</td>
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<td>AUC₀→∞ (h×µg/mL)</td>
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<td>189.7</td>
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<tr>
<td>AUMC₀→∞ (h²×µg/mL)</td>
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<td>11180</td>
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<td>MRT (h)</td>
<td>7.5</td>
<td>2.6</td>
<td>9.5</td>
</tr>
<tr>
<td>MAT (h)</td>
<td>-1.9</td>
<td>5.3</td>
<td>0.14</td>
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<tr>
<td>Cₘₐₓ (µg/mL)</td>
<td>76</td>
<td>15</td>
<td>111</td>
</tr>
<tr>
<td>Tₘₐₓ (h)</td>
<td>2.7</td>
<td>2.9</td>
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<tr>
<td>λ (h⁻¹)</td>
<td>0.127</td>
<td>0.0652</td>
<td>0.105</td>
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<tr>
<td>F (%)</td>
<td>51%</td>
<td>33%</td>
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<tr>
<td>AUC₀→∞/D</td>
<td>24</td>
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<td>Cₘₐₓ/D</td>
<td>3.1</td>
<td>0.58</td>
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Figure 1. Mean plasma ticarcillin concentrations vs. time after administration to three loggerhead sea turtles for both an intravenous dosage and three different intramuscular dosages.