



Pharmacokinetics of Oxytetracycline in the White Shrimp, *Penaeus setiferus*, Following Intravascular and Oral Administration

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INTRODUCTION

• The reason for studying the disposition of OTC in the penaeid shrimp is to benefit the aquaculture industry. Both the human population and the per capita fish consumption have nearly doubled in the past 50 years. All major fisheries are currently fished at maximum sustainable yield. So without aquaculture, the world consumption of seafood will be decreased.

• The US trade imbalance in fisheries is 3.5 billion dollars a year with shrimp constituting 40% of all edible seafood imported. Imports comprise 80% of our total shrimp supply. These imports are primarily from Asian and Latin American aquaculture. Domestic production of shrimp, although only 20% of production, is primarily wild caught.

• A major obstacle to the growth of shrimp aquaculture in this country is disease. Losses to SC farmers from 1995-1997 due to disease approached 95% mortality. In contrast, the success of terrestrial farming in the US has been due largely to the judicious use of therapeutic and prophylactic drugs. Still, there are no antibiotics approved by the FDA for shrimp. One reason is that there is little information on the disposition of pharmaceuticals in shrimp.

• Oxytetracycline (OTC) is a broad spectrum antibiotic with potential for use in farm-raised shrimp for the treatment of vibriosis and necrotizing hepatopancreatitis infections. In the entire industry it is one of only two antibiotics approved, and OTC was the first to received FDA approval for use in finfish aquaculture. OTC has shown success with non-shrimp crustaceans.

• Drug disposition data in shrimp is necessary to determine optimal dosing regimens, to achieve and maintain therapeutic drug levels, and to predict depuration time prior to human consumption of treated shrimp. Only limited pharmacokinetic data is available for drugs in crustaceans, and the pharmacokinetic data available for OTC in mammals may not apply since shrimp are poikilothermic and aquatic. The purpose of this study was to evaluate pharmacokinetics of OTC in the white shrimp, *Penaeus setiferus*, after intravascular and oral administration.

EXPERIMENTAL CONDITIONS

Pharmacokinetics were investigated in penaeid shrimp (~20g b.w.) at a water temperature of 18-20°C and a salinity of 20 parts per thousand after intravascular and oral dosing. Oral dosing was performed by delivering the OTC in the feed as the dihydrate (OXTC) or quaternary salt (TMF) form. Hemolymph and tissue levels of OTC at predetermined time intervals after dosing were measured by HPLC. The hemolymph and tissue OTC levels were fitted to appropriate pharmacokinetic models to determine the parameters.

SPECIFIC AIMS

1. Study the pharmacokinetics of OTC delivered parenterally to the penaeid shrimp, *Penaeus setiferus*. Investigate protein binding to predict the amount of free drug available for efficacy.
2. Determine tissue levels of OTC following intravascular administration to penaeid shrimp, and predict antibiotic residues and depuration times.
3. Evaluate the physicochemical properties of two forms of OTC, the dihydrate and quaternary ammonium salt, as related to biopharmaceutics and environmental impact.
4. Investigate and compare the oral bioavailability of two forms of OTC in penaeid shrimp which will allow comparison of the rate and extent of oral absorption for the two different salt forms of OTC and correlation with their loss to the environment based on results of Specific Aim 3.
5. Predict depuration time following a multiple oral dosing regimen, based on pharmacokinetic parameters generated in Specific Aims 1 and 4, in an aquaculture simulated environment. Compare observed depuration times with predicted, and thus evaluate the validity of the model.

RESULTS AND DISCUSSION

- Intravascular data represents individual shrimp being tested over a 72 hour period after receiving a single bolus IV dose. Behavior is biexponential two-compartment with a fast distribution phase followed by a slow elimination.
- Pharmacokinetic parameters determined from the mean intravascular data include a large volume of distribution, suggesting little protein binding; a 2 hour distribution half-life followed by a long >20 hour elimination half life.
- Semi-log plots of hemolymph-time data for high and low doses show linear elimination half-lives.
- Fit of oral data is triexponential for both forms of OTC. C_{max} and t_{max} were not statistically different; F and AUC were statistically different at the $p = 0.05$ level using Student's t-test.

CONCLUSIONS

- This is the first report of OTC disposition, after intravascular and oral dosing, successfully determined in shrimp without terminal sampling.
- OTC has a long elimination half-life and a peripheral compartment for drug distribution.
- OTC is well absorbed from both forms of the medicated feed (less well from TMF); bioavailability is better than reported in the literature for finfish.
- The delayed peak concentrations suggest a unique mechanism for absorption in shrimp.
- Current proposed dosing regimen is sufficient to exceed MIC. Accumulation in hemolymph does occur with this repetitive dosing scheme; less frequent administration may be better. The TMF form is more stable; therefore will remain in the environment longer.
- Overall, OTC has good potential as a pharmaceutical in shrimp to combat infections and facilitate aquaculturing of shrimp. Shrimp should be safe for the consumer by 14 days post-dosing.

Figure 1. Ventral view of penaeid shrimp showing the site for intravascular injection.

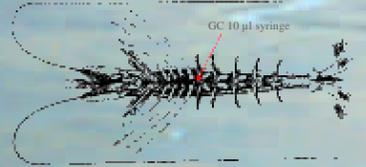


Figure 2. Photograph showing hemolymph sampling with a 250 µl Hamilton syringe at a sample size of 50 µl.



Figure 5. Schematic figure describing the pharmacokinetic model for disposition of OTC in shrimp following intravascular administration.

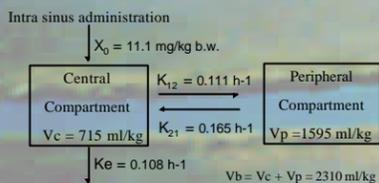


Figure 3. Typical chromatograms of extracts of blank shrimp hemolymph and hemolymph spiked with 1 µg/ml OTC. Chromatographic conditions: mobile phase, 0.01 M oxalic acid-ACN-MeOH (60 + 30 + 10, v/v/v); flow rate, 1.5 ml/min; LC column, 250 x 4.6 mm id, C_{18} , 5 µm; UV detector, 350 nm wavelength, 0.005 AUFS.

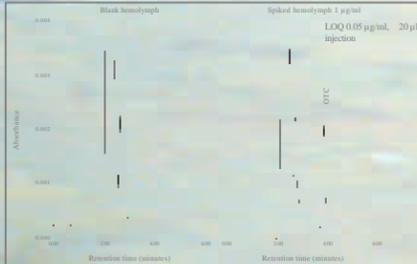
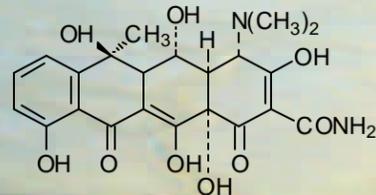


Figure 4. Structure of Oxytetracycline, 4-(Dimethylamino)-1,4a,4a,5a,6,11,12a-octahydro-3,5,6,10,12,12a-hexahydroxy-6-methyl-1,11-dioxo-2-naphthacene-carboxamide. $C_{22}H_{36}N_2O_9$ MW 460.44



EXPERIMENTAL DESIGN

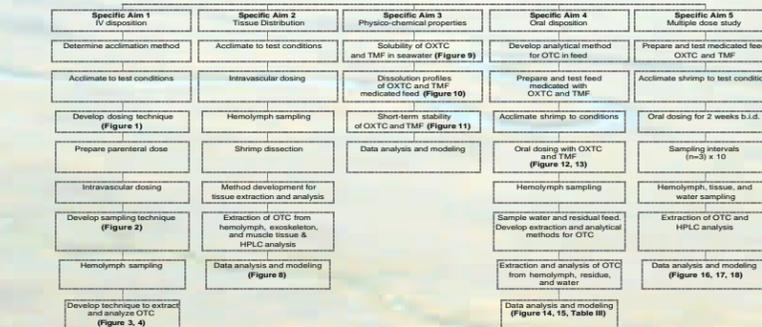


Figure 8. Tissue study: Comparison of hemolymph and muscle tissue levels of OTC plotted versus time obtained after sacrifice of an individual shrimp at predetermined time intervals after intravascular dosing. Thus, each point represents one shrimp, and the curve is the best fit to average data (n=3).

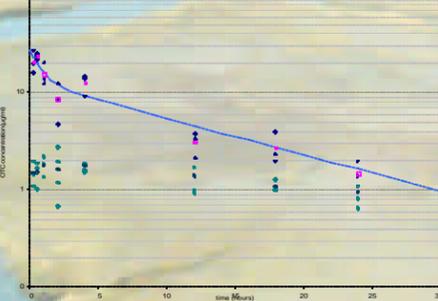


Figure 9. Comparison of solubility of OTC from OXTC and TMF premixes (n = 3) in natural seawater, 20°C, and ambient temperature. Solubility is significantly different at the p = 0.05 level by Student's t-test.

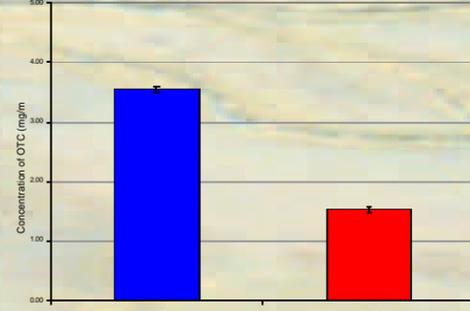


Figure 6. Effect of dose on drug disposition as studied by comparing hemolymph-time profiles. (Mean data points).

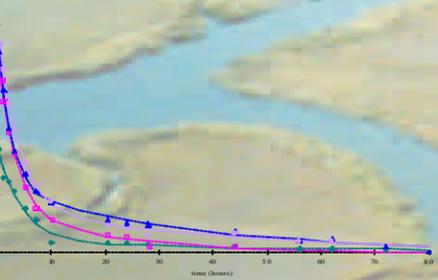


Table I. Comparison of pharmacokinetic parameters of OTC after intravascular administration of a single bolus dose of either 11.1 or 59.3 mg/kg body weight.

	high dose	low dose
X_0 = dose based on body weight	59.3	11.1 mg/kg
CL = total body clearance	31.93	77.03 ml/h/kg
VB = volume of distribution of body	991.90	2310.8 ml/kg
$t_{1/2}$ = elimination half-life	21.5	22.3 h
$t_{1/2}$ = distribution half-life	2.3	2.1 h
m = mass of shrimp	16.885	18.097 g

Table II. Protein binding of OTC in hemolymph at 20 µg/ml as a function of molecular weight cut off.

cut-off	% free OTC	% bound OTC	%recovery
3,000 MW	78.65 ± 1.01	20.96 ± 0.91	99.61 ± 1.26
10,000 MW	81.80 ± 0.68	14.05 ± 1.11	95.85 ± 0.43

Figure 7. Hemolymph-time profile of OTC after intravascular administration of a single bolus dose of 11.1 mg/kg body weight. Each curve represents fitted line for the observed data for an individual shrimp. The fitted curves were best described by biexponential equation: $C = Ae^{-\lambda t} + B e^{-\lambda' t}$, (n = 4). $R^2 > 0.99$.

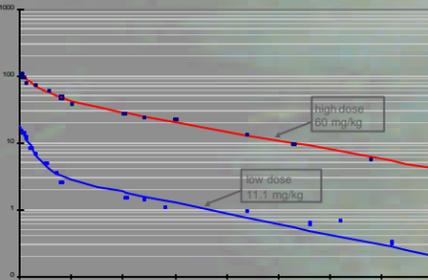


Figure 10. Average release profiles (n = 3) of OTC from OXTC and TMF medicated feeds into natural seawater using the USP dissolution apparatus II at 50 RPM and ambient temperature. The release profiles were best described by the Higuchi's square root of time kinetics and there was no significant difference (p > 0.05) in release rates from the two medicated feeds.

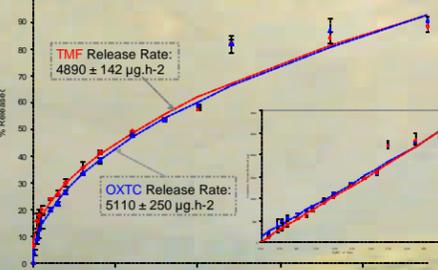


Figure 11. Degradation profile plotted on log-scale of OTC in seawater when OXTC and TMF premixes are dissolved in seawater.

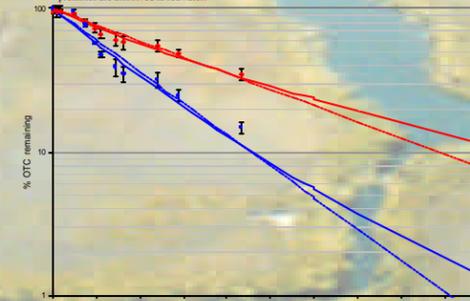


Figure 12. Mean feeding rates for shrimp using the percent body mass method

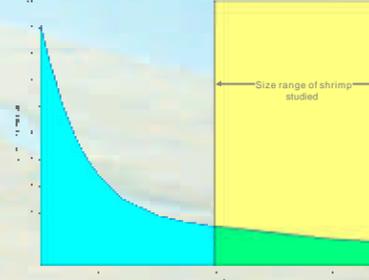


Figure 13. Photograph of shrimp grabbing feed pellets during the study.



Figure 15. Average hemolymph concentration of OTC for IV (11.1 mg/kg b.w.) and oral dosing (100 mg/kg b.w.) with OXTC or TMF form of premix.

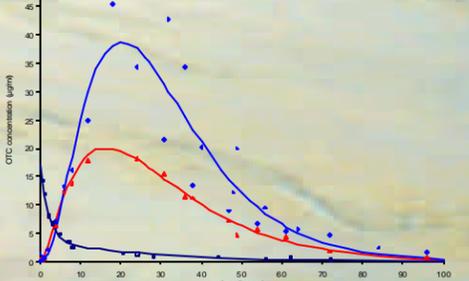


Table III. Disposition of OTC after dosing with OXTC or TMF medicated feed at a dose of 100 mg/kg b.w. (n = 5).

	OXTC	TMF
C_{max} (observed)	35.36 ± 14.66 µg/ml	19.24 ± 7.48 µg/ml
t_{max} (observed)	25.40 ± 3.13 h	19.20 ± 6.57 h
*AUC	1409.8 ± 545.9 µg·h/ml	738.72 ± 299.1 µg·h/ml
*F (apparent)	92.24 ± 32.70 %	49.50 ± 19.65 %

*Significantly different at p = 0.05 by Student's two sample t-test.

Figure 14. Comparison of oral hemolymph time profiles after dosing with either OXTC or TMF forms of OTC as medicated feed at a dose of 100 mg/kg body weight. Observed data for each shrimp fitted to curves described by first order absorption with two-compartment disposition.

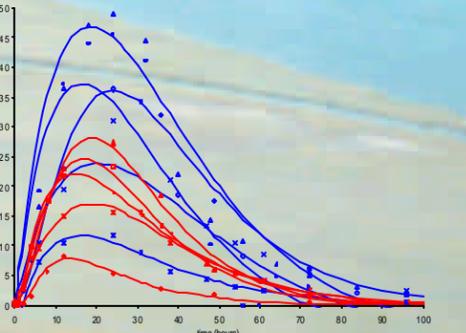


Figure 16. Predicted hemolymph-time profiles following multiple dosing with OXTC or TMF using pharmacokinetic parameters obtained from the earlier study as described in Figure 19 (n = 5).

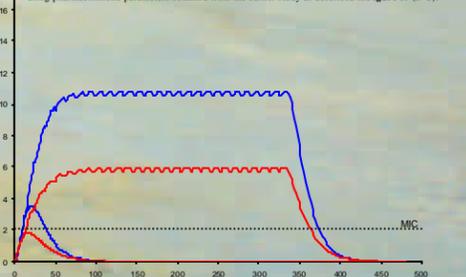


Figure 17. Observed data (n = 24) compared to predicted hemolymph-time profiles following multiple dosing with OXTC or TMF using pharmacokinetic parameters obtained from the earlier study as described in Figure 19. Tail muscle tissue OTC concentration w. time obtained after sacrifice of an individual shrimp at predetermined time intervals after multiple dosing in the depuration study with OXTC or TMF medicated feed. Each point represents one shrimp (n=24).

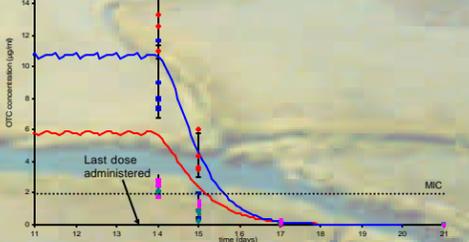


Figure 18. Photograph of raceways used for the multiple dosing with OXTC and TMF depuration study.



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