

## Single intramuscular administration of long-acting oxytetracycline in grouper (*Epinephelus marginatus*)

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**Abstract:** The plasma concentration of long-acting oxytetracycline (OTC-LA) was measured following a single intramuscular (IM) injection (50 mg/kg) in grouper (*Epinephelus marginatus*), a common inhabitant of marine aquaria and a potential candidate for fish farming. The experiment was carried out at 20 °C and sampling points were selected at 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h post-administration. OTC concentration in grouper circulation was maintained at high levels for the whole experiment (8.35-39.67 µg/mL) with maximum plasma concentration measured at 1 h post-injection. The area under the curve values was also promising since AUC<sub>0-24</sub> and AUC<sub>0-48</sub> were calculated to be high (363.45 and 668.73 µg h/mL, respectively). Due to the high OTC levels accomplished for prolonged period of time, a single administration of OTC-LA could be an ideal route in cases when multiple handling is unwanted and big animals such as grouper are to be treated.

**Key words:** Oxytetracycline, long acting, grouper, *Epinephelus marginatus*, injection, pharmacokinetics

### Introduction

Antibacterial agents are necessary in aquatic medicine to combat emerging bacterial diseases in captive fish. There are various routes of drug administration in aquaculture, each limited by its own practical constrains. Oral administration is the most common route in aquatic farming because it is associated with low cost, is highly convenient, and does not provoke stress to the fish but is rendered inefficient by reduced drug levels in fish circulation and financial loss when medicated feed is being

rejected by the fish either due to palatability problems or due to reduced appetite (1). Bath treatment with antibacterials including OTC is also easy to apply in land-based facilities, but results in extremely low levels in fish tissues mainly due to the fact that tetracyclines make complexes with cations in seawater and are therefore less useful for superficial infections (2). On the other hand, individual injection, either intraperitoneal (IP) or intramuscular (IM), of fish is labour intensive but is more beneficial in terms of achieving high tissue drug levels (3) and can be used to treat valuable individuals, such as broodstock or

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ornamental fish. This administrative route is usually the desirable therapeutic measure to treat infected animals kept in aquarium stations populated by other healthy species. In addition, it is cheaper and environmentally friendly because the quantity of the total administered drug is dramatically lower compared to the other routes and is not released (at least directly) into the environment.

Oxytetracycline, a member of tetracyclines, a group of broad-spectrum bacteriostatic antibacterials produced by *Streptomyces* spp. fungi, possesses antimicrobial activity by binding to the 30S ribosomal subunit of susceptible organisms. Upon binding, the oxytetracycline interferes with transfer RNA's ability to bind with messenger RNA, thereby preventing bacterial protein synthesis (4). Tetracyclines are characterized by time-dependent killing pattern with prolonged post-antibacterial effect. Oxytetracycline is widely used in aquatic medicine to combat mainly gram-negative bacteria (5). Long-acting formulations of OTC (OTC-LA) are widely used in veterinary medicine to provide prolonged broad spectrum antibacterial activity at high therapeutic blood concentrations with minimal tissue reactions in infected animals (6). This advantage is desirable in clinical situations where repeated handling of infected individuals for drug administration is unwanted.

Grouper (*Epinephelus marginatus*) is a species commonly housed in aquaria and recently introduced in the farming industry where bacterial infections in various growing stages including broodstock can be detrimental. Vibriosis is the dominant disease affecting this species (7) with several members being identified as pathogenic including *Vibrio parahaemolyticus* (8), *V. harveyi* (9), *V. alginolyticus* (10), and *V. carchariae* (junior synonym of *V. harveyi*) (7). The purpose of this study was to investigate the circulatory levels of a commercially available long acting OTC formulation following an IM injection in grouper.

## Materials and methods

### Chemicals

Oxytetracycline hydrochloride of high purity (>99%) and tetracycline were obtained from Sigma (USA). High-performance liquid chromatography

(HPLC) grade solvents were obtained from Labscan (Ireland). The columns Isolute C18 used for solid phase extraction (SPE) were bought from International Technology (Sorben, UK). The administered drug was Terramycin 200 long active injectable solution containing 217.4 mg OTC dihydrate/mL (Pfizer Inc, NY, USA).

### Fish and experimental set up

For practical purposes the experiment was not conducted on large individuals usually accommodated in aquarium or brood stock facilities but on smaller fish weighing  $285 \pm 111$  g. Seventy groupers were maintained in 10 fibre glass tanks (500 L) receiving sea water (38‰ salinity) at a water temperature of 20 °C prior to and during the experiment. Each tank contained 7 fish (marked according to their weight) and was allocated for the 10 sampling points of the experiment.

### Intramuscular administration and blood sampling

Groupers were administered 50 mg OTC/kg fish by IM injection adjusted to the weight of each fish separately. When leakage was observed those fish were excluded. Approximately 1 mL of blood was drawn from the caudal artery of randomly selected fish (7 individuals), at each of the following time points post-dosing: 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h. After collecting the heparin-treated blood samples, plasma was prepared by centrifugation at  $7000 \times g$  for 10 min and stored at  $-20$  °C until analysis.

### Drug analysis

The analysis of OTC was performed according to the procedure of Rigos et al. (11). Tetracycline was used as an internal standard. Briefly, plasma samples (200  $\mu$ L) were homogenized with 2 mL of McIlvaine buffer (citric acid 0.06,  $K_2HPO_4 \cdot 2H_2O$  0.02 M) (68:32 v/v) and centrifuged for 5 min at  $6000 \times g$ . The samples were subjected to a SPE procedure using Isolute C18 disposable columns (100 mg of 1 mL capacity). Columns were pre-conditioned with 2 mL of methanol and 2 mL of McIlvaine buffer. Samples were eluted with 2 mL of methanol/acetonitrile (50:50 v/v). Columns were then cleaned using 5 mL of high performance liquid chromatography (HPLC)-grade

water and dried under vacuum for 15 min. The eluate was evaporated to dryness under nitrogen at 50 °C, resuspended in 1 mL of HPLC mobile phase solvent (oxalic acid 0.01 M: acetonitrile) (85:15 v/v), vortexed, and filtered (0.45 µm). Exactly 100 µL of sample were injected into a Waters Alliance 2690MX HPLC system fitted with a Water 510 pump and a Waters 484 UV detector. Analytes were separated using an H&P deactivated Hypersil ODS C18 (100 x 4 mm i.d.; pore size 5 µm). The detection wavelength was 360 nm and the flow rate was 0.5 mL/min. Under these conditions, the retention time of OTC was 6 min and the detection limit of OTC was 20 ng/mL (linear range to 1000 ng/mL;  $P < 0.002$ ). OTC concentrations were extrapolated from calibration curves of different concentrations of authentic high-purity OTC standard. The percentage of recoveries of OTC from all samples was calculated by comparing OTC concentrations in spiked drug-free plasma to a standard solution. The recovery of OTC through the method was found to be 71%.

### Pharmacokinetics

The maximum plasma concentration ( $C_{max}$ ), and the time to reach maximum plasma concentration ( $T_{max}$ ) were selected from the observed data. The area under the concentration–time curves (AUC) were calculated using the trapezoidal rule (12).

### Results

The plasma OTC levels measured in grouper after the IM injection are presented in the Figure. Mean peak plasma concentration of OTC was reached rapidly (1 h post-injection). A rapid and continuous drop from 2 to 8 h followed by a second peak at 12 h was observed. High drug levels (8.2–39.67 µg/mL) were maintained for the whole experiment.  $AUC_{0-24}$  and  $AUC_{0-48}$  were calculated to be 363.45 and 668.73 µg h/mL, respectively (Table 1).

### Discussion

This is the first investigation on the kinetic profile of OTC-LA after an IM injection in warm water farmed species. Surprisingly, OTC clearance from grouper plasma displayed an unexpected non-gradual pattern. Following a peak concentration that was reached very rapidly, a sudden drop was observed, followed by a second peak at the last time points. Possibly reabsorption from intestine after biliary excretion can be an explanation for this discrepancy.

High OTC levels were maintained through the whole experiment possibly as a result of the long-acting properties of the drug formulation used. Mean peak drug concentration found in grouper is within the range (2.5–65 µg/mL) of studies involving other aquatic farmed species following either IP or IM

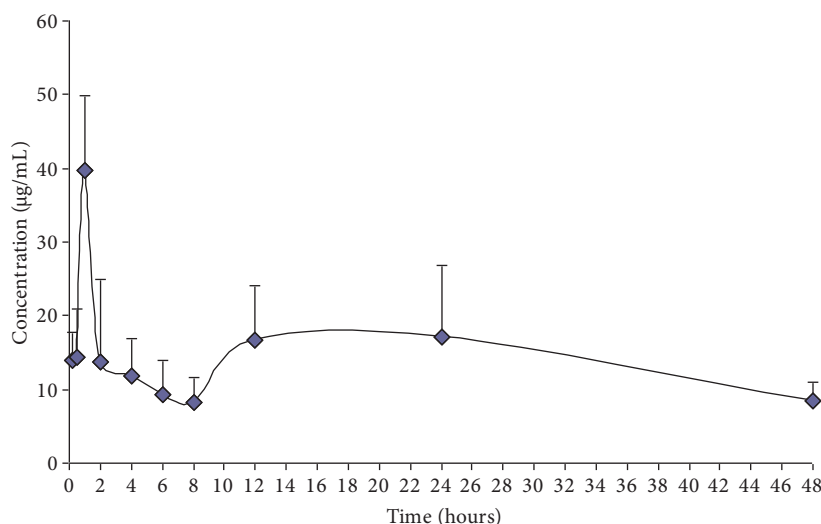


Figure. Plasma OTC levels (mean  $\pm$  st. error) against time in grouper (285  $\pm$  111 g) after an IM injection of 50 mg/kg fish at 20 °C.

Table 1. Pharmacokinetic values of OTC in grouper (285 ± 111 g after) after an IM injection of 50 mg/kg at 20 °C.

Pharmacokinetic parameters	Values
AUC <sub>0-24</sub> (µg h/mL)	363.5
AUC <sub>0-48</sub> (µg h/mL)	668.7
C <sub>max</sub> (µg/mL)	39.7 ± 10.1
T <sub>max</sub> (h)	1

injection of OTC with dosages ranging from 5 to 60 mg/kg (Table 2).

Evaluations of treatment schedules in modern veterinary medicine are commonly based on clinical breakpoints integrating both pharmacokinetic and pharmacodynamic data (MIC: minimum inhibitory concentration) (13). Early proposals have set theoretical breakpoints of drug’s antibacterial efficacy based on the requirement that C<sub>max</sub> exceeds MIC (14,15). Later approaches suggested as more appropriate for tetracyclines the inter-dosing interval during which the blood concentration exceeds MIC (t > MIC) (12). More recent theories proposed other parameters for this group of drugs such as the AUC<sub>0-24h</sub> (area under the curve from 0 to 24 h post-dosing) (16) or in more general approaches the AUC<sub>partial</sub> (AUC of particular interval) instead of the commonly calculated AUC and AUC<sub>0->∞</sub> (17).

Groupers are commonly maintained in public aquaria where bacterial outbreaks are common. Their farming, which recently entered the Mediterranean area at a pilot scale, has experienced a rapid expansion

in Asian aquaculture industry, but faces serious threats owing to a variety of pathological constrains. Among the main pathogenic bacterial agents, *V. parahaemolyticus* (8), *V. harveyi* (9), *V. alginolyticus* (10), and *V. carchariae* (7) have been identified. The pharmacodynamics of OTC against these pathogens expressed as MIC has not been determined yet. There is however related information on these pathogens from the crustacean farming industry. In particular, the MIC or MIC<sub>90</sub> of OTC to combat in vitro bacteria such as *V. harveyi*, *V. parahaemolyticus*, and *V. alginolyticus* have been found to be in the range of 0.125 to 50 µg/mL (18-20).

An integration of the lowest OTC concentration found in grouper plasma with the aforementioned MIC values resulted in ratios ranging from 0.17 to 66.8 for at least 48 h depending on isolate. Using the peak measured concentration, the ratios are much higher (0.79-316). These values are above the proposed breakpoints (C<sub>max</sub>/MIC ≥ 8) given by Blaser et al. (14) for most bacteria. Similarly, when the obtained AUC<sub>0-24</sub> or AUC<sub>0-48</sub> values of OTC in grouper are implemented with MIC data under the guideline of Turnidge and Paterson (13), ratios of 7.2-2904 and 13.4-5349 can be estimated, respectively.

Overall, IM administration of OTC-LA in grouper seemed to be promising. It is essentially important, however, that the MIC of bacterial isolates specific to groupers to be determined possibly coupled with some challenge trials prior to drawing final conclusions with respect to the evaluation of the treatment schedule proposed by the present study.

Table 2. Peak OTC levels in circulation of various species following IP or IM injection.

Species	Drug dose (mg/kg)	Route	Peak plasma or serum level (µg/mL)	References
Rainbow trout	60	IM	35	21
	50	IM	41.5	22
Atlantic salmon	20	IP	2.5	23
Yellow perch	50	IP	32	24
	50	IM	29	
Common carp	60	IP	65	21
African catfish	60	IM	43.4	25
Pacu	5	IM		26
Grouper	50	IM	39.7	Present study

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