

Bioavailability and pharmacokinetics of a praziquantel bolus in kingfish *Seriola lalandi*

L. A. Tubbs^{1,3,*}, M. D. Tingle²

¹School of Biological Sciences, and ²Department of Pharmacology & Clinical Pharmacology, University of Auckland, Private Bag 92019, Auckland, New Zealand

³National Institute of Water and Atmospheric Research (NIWA), PO Box 109 695, Auckland, New Zealand

ABSTRACT: Oral praziquantel (PZQ) preparations have recently been investigated for the treatment of monogeneans that infect the skin and gills of kingfish *Seriola lalandi* cultured in sea-cages. To evaluate an oral PZQ dosing strategy, the pharmacokinetics of a dissolved and in feed oral PZQ preparation (40 mg kg⁻¹ body weight) were compared with an intravenous bolus in kingfish plasma and skin using HPLC. Compared with intravenous administration, PZQ bioavailability (area under curve, AUC_{0–24h}) was slightly improved when the drug was administered with food in both kingfish plasma (56.8% in feed vs. 50.8% in solution) and skin (55.5% in feed vs. 50.3% in solution). After oral dosing, maximum drug concentrations in skin were approximately one-third of those achieved in plasma and higher when the drug was administered in solution (5.26 µg ml⁻¹) than in feed (3.96 µg ml⁻¹); additionally, the time to achieve maximum PZQ concentration was similar in plasma and skin, although markedly reduced when the drug was administered in solution (1 h) than in feed (6 h). However, clearance of the drug was delayed in skin; administered as an oral formulation, PZQ concentrations in the systemic circulation fell below the limit of quantification after 24 h, but remained quantifiable (0.3 µg g⁻¹) in skin at this time. These initial studies indicate that a daily treatment interval will lead to the exposure of parasites to highly variable anthelmintic concentrations, which may be sub-optimal for the treatment of monogeneans in this finfish species.

KEY WORDS: Bioavailability · Pharmacokinetics · Monogenea · Anthelmintics · Praziquantel

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INTRODUCTION

Praziquantel (PZQ) is widely recognised as a useful chemotherapeutic against helminths of captive fish. With its broad spectrum activity, PZQ has proven effective against monogeneans infecting the gills, skin or branchial cavities (Schmahl & Mehlhorn 1985, Schmahl & Taraschewski 1987, Thoney 1990, Kim et al. 1998, Hirazawa et al. 2000, Chisholm & Whittington 2002, Janse & Borgsteede 2003, Stephens et al. 2003, Hirazawa et al. 2004, Sharp et al. 2004), larval and encysted digeneans infecting the eyes (Bylund & Sumari 1981) and skin (Mitchell 1995), plus intestinal cestodes (Sanmartin Duran et al. 1989) of teleosts and elasmobranchs. Typically the drug is administered as a bath treatment, at concentrations of 2.5 to 20 mg l⁻¹

PZQ for 3 to 48 h (Schmahl & Taraschewski 1987, Schmahl 1991, Sharp et al. 2004), or orally at doses of 50 to 400 mg kg⁻¹ body weight (BW) daily for up to 20 d (Hirazawa et al. 2000, Kim & Cho 2000, Tubbs 2002). Of the most problematic helminths are monogeneans, because of their direct life cycle that allows them to propagate rapidly in some culture situations.

A number of *Seriola* species (Carangidae) cultured in sea-cages internationally become infected with skin and blood feeding monogenean parasites that can compromise growth and cause morbidity and mortality (Ogawa & Yokoyama 1998, Ernst et al. 2002). Infections by *Benedenia seriolae* and *Neobenedenia girellae* damage the skin of Japanese yellowtail *Seriola quinqueradiata* and amberjack *S. dumerili* (Ogawa & Yokoyama 1998); *B. seriolae* also infect kingfish

*Email: l.tubbs@niwa.co.nz

S. lalandi in Australia and New Zealand (Ernst et al. 2002, Sharp et al. 2003). Among gill flukes, *Heteraxine heterocerca* and *Zeuxapta japonica* infect yellowtail and amberjack in Japan, whilst *Zeuxapta seriolae* infect kingfish in Australia and New Zealand plus amberjack in the Mediterranean (Grau et al. 2003, Montero et al. 2004). Management of these parasites can be expensive: up to 20% of the total production cost of Japanese *Seriola* species has been attributed to *B. seriolae* management alone (Ernst et al. 2005). Parasite management strategies are therefore an important aspect of *Seriola* species culture; ideally these strategies should incorporate effective treatments that are administered under the most efficient regimen.

Current monogenean treatments typically include bathing fish with freshwater or hydrogen peroxide (Ogawa 1996, Ernst et al. 2002), although PZQ baths have also proved effective (Sharp et al. 2004). Logistically, it is difficult to administer bath treatments in sea-cages; thus, medicated feeds, which require little additional labour to administer other than that required for regular feeding, would be beneficial in this culture environment.

In establishing an oral dosing regimen, pharmacokinetic data such as drug bioavailability and tissue drug concentration, particularly at the site of activity, can be useful for optimising treatment efficiency. Although well characterised in mammals (Andrews et al. 1983), relatively little is known about the pharmacokinetics of orally administered PZQ in fish. Following administration of a 10 mg kg⁻¹ BW oral bolus to rainbow trout, peak PZQ concentration in serum and muscle occurred after 7 h and fell below detectable limits (5 ng g⁻¹) after 48 h (Rogstad et al. 1987). Similarly, plasma and muscle PZQ concentrations peaked 9 h after a 400 mg kg⁻¹ BW bolus dose was orally administered to rockfish *Sebastes schlegeli*, although the drug was still detectable 96 h after treatment in this species (Kim et al. 2001). Currently, no published pharmacokinetic data are available for PZQ in any *Seriola* species. The present study aimed to establish the bioavailability and pharmacokinetics of PZQ administered as an oral bolus dose in this species as a first step to optimise PZQ dosing regimens for *Seriola lalandi*.

MATERIALS AND METHODS

Chemicals and reagents. Praziquantel (2-cyclohexyl-carbonyl-4-oxo-1, 2, 3, 6, 7, 11b-hexahydro-4H-pyrazino[2,1-a]isoquinoline) was purchased from Novartis. The internal standard diazepam (7-chloro-1-methyl-5-phenyl-3H-1, 4-benzo-diazepin-2[1H]-one) and 2-phenoxyethanol were from Sigma Chemical. All solvents used were of chromatographic grade (Burdick

& Jackson). Standard stocks were prepared by dissolving 1 mg PZQ or 1 mg diazepam (IS) in 1 ml ethanol diluted to a final concentration of 100 µg ml⁻¹ with mobile phase. Stock praziquantel solution (100 µg ml⁻¹) was further diluted with mobile phase for calibration curves.

Fish. All fish used in these experiments were 1 to 1½ yr old (1 to 2 kg) hatchery-reared juvenile kingfish *Seriola lalandi* supplied by the National Institute of Water and Atmospheric Research (NIWA). Fish were maintained at NIWA's Bream Bay Aquaculture facility (35° 49' S, 174° 30' E) in a standard 10 000 l flow-through filtered seawater holding tank at ambient temperature (18 to 19°C) with an exchange rate of approximately 10% h⁻¹. To avoid differences in drug kinetics being caused by different nutritional status, the fish were starved for 4 d prior to conducting experiments.

Praziquantel administration. 143 kingfish were transferred in blocks of 3 to 5 individuals to a 200 l tank containing 300 ppm 2-phenoxyethanol anaesthetic for 4 to 5 min. After reaching Stage III anaesthesia, each fish was weighed and randomly allocated to 1 of 3 treatment groups. The first 2 groups received 40 mg kg⁻¹ BW PZQ dissolved in DMSO (0.5 ml kg⁻¹ BW). One group received the dose orally by intubation directly to the stomach. The second group received PZQ intravenously via the caudal vein; a small amount of blood was aspirated into the syringe prior to injecting the PZQ solution to ensure proper location of the needle. If the oral gavage tube became dislodged during drug delivery or the vein was not cleanly located on the first attempt, the fish was removed from the experiment and replaced. The third group received PZQ (40 mg kg⁻¹ BW) in Nova diet (Skretting) blended with 5% distilled H₂O (0.5 ml kg⁻¹) by oral gavage as above. No fish disgorged the oral preparation within a 10 min observation period post treatment. After treatment the fish were placed in 4000 l tanks with seawater (as above) to recover.

Sampling regime. Fish were removed ½, 1, 1½, 2, 3, 4, 6, 12, 24, 48 or 72 h post treatment (PT) and killed by spinal severance. Blood from the pulmonary artery was collected into heparinised 5 ml vials and plasma separated by centrifugation (3000 g for 5 min). Samples of skin (approximately 5 g) were collected in duplicate from the anterior dorsal region. All samples were placed immediately on dry ice then transferred to a -80°C freezer for storage until analysed.

Chromatographic conditions. The equipment used was an Agilent HP1100 HPLC with UV detection. Separation was performed on a Zorbax Extend-C18 column (4.6 × 150 mm internal diameter, 5 µm particle size; Agilent Technologies) using an isocratic mixture of 35:65% acetonitrile: water as the mobile phase with

a constant flow rate of 1 ml min⁻¹. Eluant was monitored at 210 nm (reference 360 nm). Between injections from different sample periods and treatments the column was rinsed for 15 min with 100% acetonitrile.

Sample preparation. Plasma: Internal standard (800 ng) was added to plasma (200 µl), mixed thoroughly and left to stand for 5 min before liquid extraction with ethyl acetate (2 × 1 ml). The displaced supernatants (3000 × g for 5 min) were dried in a rotary evaporator under vacuum (SC 110A SpeedVac, Thermo Savant). The dried residue was resuspended in ethanol (200 µl) and an aliquot (50 µl) injected into the HPLC.

Sample preparation. Skin: The procedure for drug extraction from skin was modified from the method of Rogstad et al. (1987). Briefly, skin (0.5 g) was finely chopped with scissors, placed in a 10 ml glass tube with IS (800 ng) and left to stand for 5 min. Ethyl acetate (1 ml) was added, the sample homogenised (IKA T25, Wolf Laboratories) then centrifuged as above. The supernatant was separated and liquid extraction repeated with ethyl acetate (1 ml), except the sample was vortex mixed instead of homogenised; the supernatant was then evaporated as above. The dried residue was resuspended in hexane (2.5 ml), vortex mixed and loaded onto an activated silica column (Isolute 500 mg SI, IST). The column was then rinsed with 15% v/v diethyl ether-hexane (2.5 ml). PZQ was eluted from the column with 70% acetone in hexane (2.5 ml) and evaporated as above. The dried residue was resuspended in ethanol (200 µl) and an aliquot (50 µl) was injected into the HPLC.

Validation and calibration curves. To determine calibration curves, blank plasma and skin samples were spiked with standard solutions to yield final concentrations between 0 to 20 µg ml⁻¹ PZQ (plasma) and 0 to 6 µg ml⁻¹ PZQ (skin). Samples were prepared and injected into the HPLC as above. Linear relationships were established between PZQ and IS area under curve (AUC) ratios for plasma ($r^2 = 0.999$) and skin ($r^2 = 0.995$). Accuracy and precision were determined by analysis of 5 replicate samples spiked with 25, 500 and 1000 ng PZQ (plasma) or 50, 500 and 750 ng PZQ (skin), extracted as above; these fell within acceptable limits, i.e. 15% of actual values for those other than the lower limit of quantification (25 ng on column), which fell within 20% of the actual value. Quality control (QC) samples (50, 500 and 750 ng PZQ) were included at the start and finish of each HPLC run.

Data analysis. Pharmacokinetic analysis was performed using a non-compartmental model with Win-Nonlin® software (Pharsight). Bioavailability (F) was determined using:

$$F = (\text{AUC}_{\text{oral}} / \text{AUC}_{\text{intravenous}}) \times 100 \quad (1)$$

Table 1. Pharmacokinetic parameters of a praziquantel bolus (40 mg kg⁻¹ body weight) administered to kingfish intravenously (IV), orally in solution (Oral) or orally co-administered (OC) with feed. T_{max} : time after dosing to maximum plasma concentrations; C_{max} : maximum plasma concentrations; $t_{1/2}$: elimination half-life; AUC: area under curve

| | | T_{max} (h) | C_{max} (µg ml ⁻¹) | $t_{1/2}$ (h) | AUC _{0-24h} (µg ml ⁻¹) |
|--------|------|-------------------------|--|------------------|--|
| Plasma | IV | 0.5 | 20.30 | 8.57 | 177.87 |
| | Oral | 1.0 | 12.73 | 6.57 | 90.42 |
| | OC | 6 | 10.62 | 7.91 | 101.12 |
| Skin | IV | 1.5 | 8.70 | 7.23 | 74.26 |
| | Oral | 1.5 | 5.26 | 5.78 | 37.38 |
| | OC | 6 | 3.96 | 4.72 | 41.22 |

RESULTS

Praziquantel pharmacokinetics in plasma

Maximum plasma concentrations (C_{max}) achieved after oral dosing were similar when PZQ was given in solution or feed (12.7 and 10.6 µg ml⁻¹, respectively) compared with a peak plasma concentration of 20.3 µg ml⁻¹ following intravenous administration (Table 1). Interestingly, the clearance of PZQ did not follow a simple decay model, with plasma concentrations rising again at the 6 h time point after all 3 dosing regimens (Fig. 1a).

Distribution of praziquantel occurred rapidly in kingfish. In oral preparations, PZQ concentration fell below detectable limits after 24 h, while intravenously administered PZQ was not detectable after 48 h. The elimination half-life $t_{1/2}$ of the drug ranged between 6.6 and 8.6 h when administered orally in solution and intravenously respectively (Table 1).

Administering PZQ with feed led to a minor improvement in the bioavailability of the drug (56.8%) compared with administration in solution (50.8%) relative to the intravenous dose.

PZQ pharmacokinetics in skin

The pharmacokinetics of PZQ in the skin of kingfish resembled that of plasma (Fig. 1b), although C_{max} values in this tissue were approximately 40% of the plasma values (Table 1). The time taken to reach C_{max} in the skin was slightly delayed when the drug was administered in solution, but was the same as plasma (6 h) when co-administered with feed (Table 1). Again, there appeared to be an increased concentration at the 6 h time point after all 3 dosing regimens.

The relative exposure to PZQ in the skin of kingfish reflected the trend observed in plasma: the AUC_{0-24h} was 50.3% of the intravenous dose when administered in solution and 55.5% when administered in the feed preparation.

DISCUSSION

Monogeneans have the ability to multiply rapidly in intensive farming facilities because of their direct life-cycle (Ernst et al. 2002), which necessitates early diagnosis coupled with rapid and effective treatment to prevent benign infections from becoming patho-

genic. Traditionally, an empirical approach based on parasite clearance has been used to develop treatment regimens, which may lead to some regimens producing toxicity while others appear ineffective (Tojo & Santamarina 1998a,b,c). However, by examining the underlying pharmacokinetics of a drug, existing dosing regimens can be evaluated to establish inefficiencies in drug dissolution, absorption or disposition. Potential benefits of this approach include minimising waste, reducing the time and cost associated with drug administration as well as minimising the potential development of anthelmintic resistance caused by extended exposure of parasites to sub-curative doses.

The current study has demonstrated that PZQ can be readily detected in both the plasma and skin of kingfish after a dose of 40 mg kg^{-1} either intravenously or orally. Although absorption, and hence peak plasma concentrations of PZQ, were delayed when co-administered with food, importantly, the oral bioavailability of the drug (~50%) did not appear to be significantly affected by administration of the drug in solution compared with as a solid in food. The lack of difference in exposure (AUC_{0-24h}) despite delayed C_{max} may simply be a reflection of the transit time in the stomach. Alternatively, as PZQ is extremely hydrophobic it is highly likely that some of the drug precipitated out of solution post-administration as it came into contact with the aqueous environment of the gut. However, given that the drug is readily detected in tissues, with no differences in bioavailability between the 2 preparations, suggests that complete dissolution of the drug occurs in the kingfish gut: indeed absorption of the drug, as determined by plasma C_{max} , is noticeably faster than that reported for other finfish species, which ranged between 4 and 7 h for rainbow trout (Bjorklund & Bylund 1987, Rogstad et al. 1987) and 9 h for rockfish (Kim et al. 2001) at similar water temperatures.

Several interesting similarities were observed between the drug concentration profiles of PZQ in kingfish skin and plasma. The drug distributed into the skin, and although no difference was found between the AUC_{0-24h} of either oral formulation, administering the drug with food lengthened the time to reach, and slightly decreased the C_{max} in this tissue. Interestingly, the concentration profiles for both plasma and skin revealed a second peak ~6 h post-dose irrespective of formulation or route. This may be a consequence of re-absorption across the gills following excretion of the drug into water, although given the volume of the tanks and the magnitude of the effect it is far more likely to indicate active reabsorption of the drug from bile fluid via enterohepatic recirculation. This phenomenon was proposed for PZQ in rainbow trout

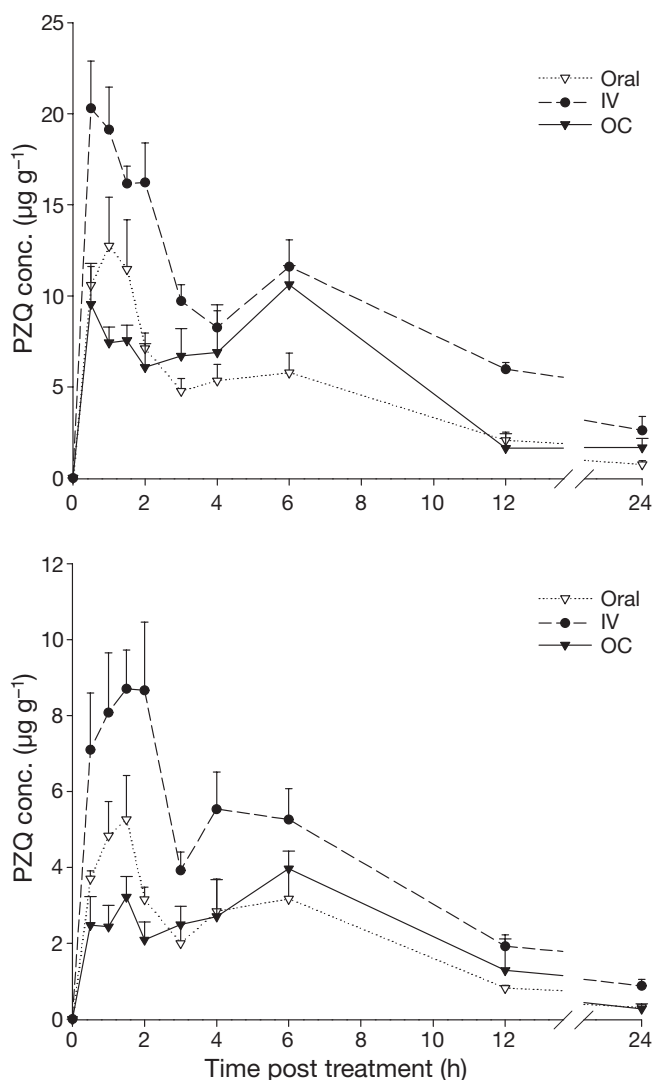


Fig. 1. *Seriola lalandi*. (A) Plasma and (B) skin concentrations of praziquantel (PZQ) following bolus administration (40 mg kg^{-1} body weight, BW), dissolved in 0.5 ml kg^{-1} BW DMSO (oral and intravenous, IV; plasma $n = 5$, skin $n = 3$) or in 0.5 ml kg^{-1} BW feed preparation (orally co-administered, OC; $n = 3$). Values are mean + SE

(Bjorklund & Bylund 1987) and was also apparent in the data presented for rockfish by Kim et al. (2001).

It was difficult to make an accurate determination of the half-life of PZQ in kingfish tissues because of the limited number of sample times available in the elimination phase. However, the range of 4.7 to 7.2 h for skin and 6.6 to 8.6 h for plasma indicates that it may be shorter than with other species, such as rockfish and rainbow trout that have been studied to date (Bjorklund & Bylund 1987, Kim et al. 2001). Further studies are required to clarify whether it is a threshold of exposure (i.e. C_{max}) or cumulative duration of exposure (i.e. AUC) that is important for achieving a therapeutic response. Additionally, the effects of temperature and the influence of repetitive oral dosing on praziquantel pharmacokinetics are worthy of investigation. Whilst it was still possible to detect PZQ in the skin of fish 24 h after dosing, these data suggest that a dosing interval shorter than 24 h may avoid the risk of exposing kingfish monogeneans to highly variable and potentially sub-curative drug concentrations.

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