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Correlation between Pharmacokinetics of Praziquantel and Extermination of *Microcotyle sebastis* (Monogenea) in Cultured Rockfish *Sebastes schlegeli*

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To investigate the re-treatment time of *Microcotyle sebastis* by oral administration of praziquantel, the residue levels of praziquantel in plasma of rockfish *Sebastes schlegeli* administered orally at a dose of 200 mg/kg B.W. were analyzed by reversed-phase HPLC, and the concentrations of praziquantel in the plasma were correlated with the extermination of *M. sebastis*. The absorption and depletion of praziquantel in the blood of rockfish were fast and the residual concentrations of praziquantel declined below 4 μg/mL within 24 hr post treatment. Most of worms were exterminated within 3 hr after oral administration of praziquantel, however, a small number of *M. sebastis* were not killed by the treatment until end of the experiment. Considering fast drop of praziquantel in blood and extermination pattern of *M. sebastis* in the present results, retreatment at an interval of 9~12 hr would be effective for eradication of *M. sebastis*.

Key words: Pharmacokinetics of praziquantel, *Microcotyle sebastis*, Rockfish

Introduction

Praziquantel chemotherapy has been employed to control various internal helminth infections in mammals, and has recently been used to control monogenean diseases in fish by bath treatment (Schmahl and Melhorn, 1985; Szekely and Molnar, 1990; Buchmann, 1987; Buchmann et al., 1990, Thoney, 1990). Recently, Kim et al. (1998, 2001b) and Kim and Cho (2000) reported that oral administration of praziquantel was effective in treating *Microcotyle sebastis* infestations in cultured rockfish, *Sebastes schlegeli*. Because *M. sebastis* is a blood-sucking polyopisthocotylean, the parasite inevitibly absorbs praziquantel in the blood of treated fish in the process of blood feeding. However, all *M. sebastis* worms on the gills of a rockfish would not feed blood at the same time and the levels of praziquantel in blood would be declined with the lapse of time. According to the pharmacokinetic study of Kim et al. (2001a), the residue levels of praziquantel in plasma and muscle of rockfish, administered orally at a dose of 400 mg/kg B.W., were highest at the 9 hr post treatment, then, declined sharply, and eliminated within 120 hr post treatment. The fast depletion of praziquantel in the blood of the treated fish and the differences in feeding time or interval among *M. sebastis* individuals in fish might allowed to some parasites an opportunity of avoiding parasiticidal concentrations of praziquantel in blood. Buchmann et al. (1992) reported that mebendazole-resistance in gill monogeneans *Pseudodactylogyrus* spp. from the European eels could be selected by subtherapeutic treatments. Therefore, to prevent a selection of anthelmintic resistance following use of drugs, determination of the dosages and the interval between treatment times, which can exterminate the parasites completely, is essential.

The aim of the present study was to investigate the re-treatment time of *M. sebastis* by oral admi-

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nistration of praziquantel. The residue levels of praziquantel in plasma of rockfish administered orally at a dose of 200 mg/kg B.W., which was a recommended dosage to kill *M. sebastis* parasitising on the gills of rockfish (Kim et al., 1998; Kim et al., 2001b), were analyzed by reversed-phase HPLC, and the relationship between concentrations of praziquantel in the plasma and extermination of *M. sebastis* was investigated.

Materials and Methods

Chemicals and reagents

Praziquantel (2-cyclohexylcarbonyl-4-oxo-1,2,3,6,7, 11b-hexahydro-4H-pyrazino[2,1-a]isoquinoline) and the internal standard, diazepam (7-chloro-1-methyl-5-phenyl-3H-1,4-benzodiazepin-2[1H]-one) were kindly donated by Shinpoong Pharma. Co. Ltd. (Seoul, Korea). Acetonitrile for the mobile phase and distilled water were of chromatographic grade (E. Merck, Germany).

Fish

Thirty-three untreated, clinically healthy rockfish (*Sebastes schlegeli*) weighing 110~150 g, were obtained from a local rockfish farm. The fish were acclimatized for 7 days before experiments in flow-through 500 L aquaria. The water temperature was 20~21℃ and the salinity was 33‰. The fish were starved both during the acclimation and the experimental period to avoid differences in drug kinetics owing to differences in nutritional status.

Oral administration of praziquantel

Just before treatment, the fish were anaesthetised with methane sulfonate salt (MS222, 150 mg/L, Sigma, MO, USA). A single dose of 200 mg of praziquantel per kg of body weight was administered orally by intubation of the stomach. Fish were observed individually for disgorgement until 10 min after drug administration. Control fish got exactly the same treatment as treated fish with the exception of drug application. At 3, 6, 12, 24, 48, 72, 96, 120 and 144 hr post-treatment, three fish were taken randomly from the aquarium. After anaesthesia with MS222, blood was drawn from the caudal vein and the gills were excised from each fish. Blood samples were centrifuged immediately to get plasma and were kept frozen at 70℃ until analyzed. The excised gills were examined for analysis of infection intensity of *M. sebastis*.

Chromatographic conditions

The chromagraphic analysis was performed according to the method established previously in this laboratory (Kim et al., 2001a). The instruments used were a Hewlett-Packard (HP1100 Series, DE, USA) high-performance liquid chromatograph equipped with QUAT pump (HP1100 Series G1311A), an automatic gradient controller (HP1100 Series G1324 A), an injection valve fitted with 5 mL sampling loop, a variable-wavelength UV detector and a data module. Analysis was performed on a ODS2 C18 column (125×4 mm, Hewlett Packard) with acetonitrile-water (45:55, v/v) as the mobile phase. The column was kept at room temperature (20~24℃) and the flow rate was kept constant at 1.0 mL/min. The detector wavelength was set 217 nm. Between each 200 μL injection the column was washed for 15 min with 100% acetonitrile.

Preparation of plasma

To a 1.0 mL volume of plasma, 1.0 mL of 100% acetonitrile and 0.4 mL of the internal standard solution were added. The sample was allowed to stand for 10 min at 4℃, then, centrifuged at 10,000 ×g for 10 min. The collected supernatant was evaporated to dryness with speed vacuum (Heto-Holten A/S, Copenhagen, Denmark). The dry residue was dissolved in 1 mL of mobile phase, and a portion of 200 μL was injected into the HPLC.

Statistical analysis

Significance of *M. sebastis* abundance compared to control group was analyzed using Mann-Whitney's U-test.

Results

Plasma praziquantel concentrations

The levels of praziquantel found in plasma after oral administration of praziquantel are shown in Fig. 1. Following oral treatment, praziquantel was detected in plasma until 120 hr post treatment. The praziquantel concentration was highest at the 6 hr sampling time, then, declined sharply.

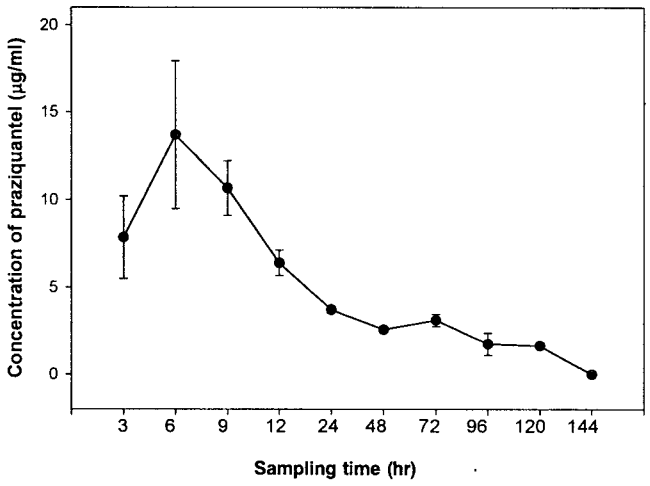

Fig. 1. Plasma concentrations (mean ± standard error) of praziquantel in rockfish *Sebastes schlegeli* after oral administration with 200 mg/kg B.W. praziquantel.

Abundance of *Microcotyle sebastis*

The fish sampled at each hour after administration of praziquantel showed significantly lower abundance of *M. sebastis* than the fish not fed praziquantel (Fig. 2). However, the treatment efficacy was not 100%, and a few worms were found on the gills of praziquantel-treated fish throughout the experiment. Most worms found from the fish sampled at 12 hr and 24 hr after treatment were exhausted and scarcely responded to stimuli.

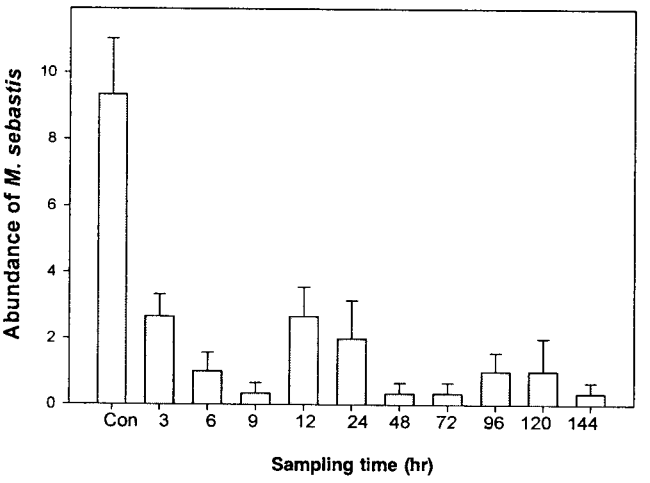

Fig. 2. Abundance (mean ± standard error) of *Microcotyle sebastis* on the gills of rockfish *Sebastes schlegeli* after oral administration with 200 mg/kg B.W. praziquantel.

Discussion

In the present study, the absorption and depletion of praziquantel in the blood of rockfish were fast and the residual concentrations of praziquantel declined below 4 μg/mL within 24 hr post treatment. Previous pharmacokinetic studies of praziquantel in fish, also, demonstrated the fast depletion of praziquantel in blood and muscle tissue (Bjrklund and Bylund, 1987; Rogstad et al., 1987; Kim et al., 2001a).

The fast depletion of praziquantel in the blood of rockfish was correlated with the survival of some *M. sebastis*. The present results showed that most of *M. sebastis* were exterminated within 3 hr after oral administration of praziquantel (200 mg/Kg B.W.). However, a small number of *M. sebastis* were not killed by the treatment until end of the experiment. The survived worms should sucked the blood when the praziquantel residues were sub-parasiticidal concentrations. Recently, Kim et al. (2001b) reported that coadministration of cimetidine with praziquantel led to a significantly increased treatment efficacy of the latter drug probably through increasing elimination half-life of praziquantel. Therfore, maintaining parasiticidal levels of praziquantel enough to surmount parasite's feeding intervals must be the key point for complete extermination of *M. sebastis*. Considering fast drop of praziquantel in blood and extermination pattern of *M. sebastis* in the present results, retreatment at an interval of 9~12 hr would be effective for eradication of *M. sebastis*.

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