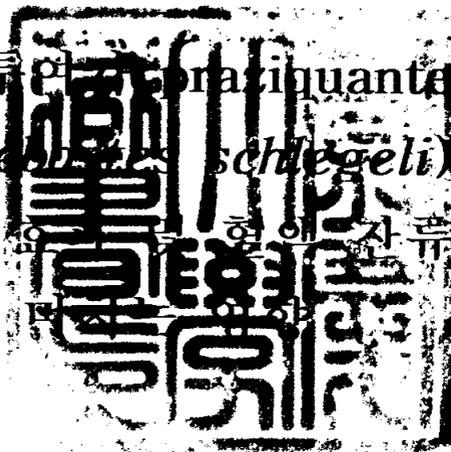


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Cimetidine enhances the plasma
praziquantel concentration and
treatment efficacy against *Microcotyle
sebastis* in cultured black rockfish,
Sebastes schlegeli

Cimetidine 투약과 praziquantel 의 양식
조피볼락 (*Sebastes schlegeli*) 아가미
흡충증 치료 실험에 관한 연구 농도에



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by

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Cimetidine enhances the plasma praziquantel concentration and treatment efficacy against *Microcotyle sebastis* in cultured black rockfish, *Sebastes schlegeli*

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ABSTRACT

The aim of the present study was to determine the depletion of praziquantel in black rockfish, *Sebastes schlegeli*, under laboratory and field conditions, and to investigate the effect of cimetidine coadministration on the praziquantel concentration in the blood and treatment efficacy against *Microcotyle sebastis*. Plasma and muscle tissue sample were analyzed for praziquantel by reversed-phase HPLC using diazepam as the internal standard. In the laboratory study, a single dose of 200 or 400 mg of praziquantel kg⁻¹ fish body weight (bw) was administered by intubation of the stomach. A bath treatment at 100 ppm of praziquantel for 4 min was carried out, also. The residue concentrations of praziquantel in plasma and muscle of black rockfish, administered orally at a dose of 200 mg of praziquantel kg⁻¹ bw, were highest 6 h after treatment and were eliminated within 120 h after treatment. Treatment with 400 mg of praziquantel kg⁻¹ bw, praziquantel was detected in plasma and muscle tissue until 96 h after treatment. In plasma the praziquantel concentration was highest at 9 h post-administration and declined sharply after 48 h. The concentration of praziquantel in the muscle tissue were lower than those in the plasma, and the highest value was found at 9 h post-treatment. Following bath treatment, praziquantel was found in plasma and muscle tissue until 72 and 24 h after treatment, respectively. In plasma, praziquantel concentration was highest

at 12 h post bath and declined sharply thereafter. The concentrations of praziquantel in the muscle tissue were significantly lower than those in the plasma, and the concentrations declined consistently in time. In the commercial net-pen farm conditions, the level of praziquantel were determined after the administration of three doses of either 200 or 400 mg of praziquantel kg^{-1} of bw given at 24 h intervals. Following treatment with 200 mg of praziquantel kg^{-1} bw, praziquantel was detected in muscle tissue until 1 day. Following treatment with 400 mg of praziquantel kg^{-1} bw, praziquantel was found in muscle tissue until 5 days post treatment.

The effect of cimetidine on the praziquantel concentration in the blood and the consequent effect on the treatment efficacy against *M. sebastis* were investigated. Fish were divided into 7 groups and orally administered praziquantel alone (200 and 100 mg of praziquantel kg^{-1} bw) or in combination with cimetidine (in dose of 200, 100 or 50 kg^{-1} of cimetidine bw with a dose of 100 mg of praziquantel kg^{-1} bw). The fish in the sixth group were coadministered 50 mg of praziquantel and 200 mg of cimetidine kg^{-1} bw. The fish in the control group were administered only saline. The plasma sample were collected at 24 h post-treatment and analyzed by HPLC using diazepam as the internal standard, and the gills were examined to confirm the effectiveness of each treatment. The praziquantel concentration in plasma of fish administered 100 mg of praziquantel + 200 mg of cimetidine kg^{-1} bw was not significantly different from that of fish treated with 200 mg of praziquantel kg^{-1} bw and was significantly higher (about 2 times) than that of fish administered 100 mg of praziquantel kg^{-1} bw. The treatment efficacies of the groups of fish coadministered 100 mg of praziquantel kg^{-1} bw and various concentrations of cimetidine (200, 100 and 50 mg kg^{-1} bw) were not significantly different from that of the group of fish administered 200 mg of praziquantel kg^{-1} bw.

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 & Melhorn, 1985; Moser et al., 1986; Buchmann, 1987; Schmahl & Taraschewski, 1987; Schmahl et al., 1989; Buchmann et al., 1990; Szekeley & Molnar, 1990; Thoney, 1990; Sanamarina et al., 1991). Recently, Kim et al. (1998) and Kim & Cho (2000) reported that oral administration of praziquantel was effective in treating *Microcotyle sebastis* infestations in cultured black rockfish, *Sebastes schlegelii*.

Because *M. sebastis* is a blood-sucking polyopisthocotylean, the parasite inevitably absorbs praziquantel in the blood of treated fish in the process of blood feeding. However, all *M. sebastis* worms on the gills of a black rockfish would not feed blood at the same time and the levels of praziquantel in the blood would decline with the lapse of time. When praziquantel was administered in a single oral dose in mammals, the drug disappeared in the plasma within 3 h (Bittencourt et al., 1990; Jung et al., 1991). Although little work has been done on the pharmacokinetics of praziquantel in fish administered orally, the highest residue level of praziquantel in the blood of rainbow trout which were given a single oral dose (10 mg kg⁻¹ body weight) of praziquantel was obtained 7 h after medication according to the preliminary study of Rogstad et al. (1987). Considering this fast drop in praziquantel levels in the blood, maintaining high levels of praziquantel in blood during the treatment period and therefore increasing the amount of time to which the parasite is exposed to parasitocidal doses of the drug is essential for increasing treatment efficacy.

Cimetidine is a histamine H₂ receptor antagonist and has been shown to differentially inhibit a variety of cytochrome P₄₅₀ isoforms (Knodell et al., 1991). Administration of cimetidine has been reported to results in clinically significant pharmacokinetic

interactions with a variety of drugs, including praziquantel. (Dachman et al., 1994; Ebeid et al., 1994; Metwally et al., 1995; Jung et al., 1997).

Although Kim et al. (2001a) have reported that coadministration of cimetidine with praziquantel led to a significantly increased treatment efficacy of the latter drug against *M. sebastis*, there are no experimental data on the pharmacokinetic interactions between praziquantel and cimetidine in fish.

The use of praziquantel to edible fish may lead to residues in fish tissues, and the public health authorities require safe drug withdrawal periods. Compared with the wealth of information available on the pharmacokinetics of praziquantel in mammals (Andrews, 1976; Leopold et al., 1978; Groll, 1984; Bittencourt et al., 1990; Jung et al., 1991; González-Esquivel et al., 1993; Morovján et al., 1998), only few studies have reported on those of praziquantel in fish.

Concentrations of praziquantel in plasma and tissues of fish were determined by HPLC by Xiao et al. (1993), Rodstad et al. (1987) and Hormázabal & Yndestad (1995). Björklund & Bylund (1987) analyzed the pharmacokinetics of praziquantel in rainbow trout, *Salmo gairdneri* by means of a bioassay method using cercariae of *Diplostomum spathaceum* as test organisms for the drug concentrations.

The aim of the present study, therefore, was to determine the depletion of praziquantel in black rockfish under laboratory and field conditions, and to investigate the effect of cimetidine coadministration on the praziquantel concentration in the blood and the consequent effect on the treatment efficacy against *M. sebastis*.

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-ph-enyl-3H-1,4 benzodiazepin-2[1H]-one), were kindly donated by Shin-poong Pharma. Co. Ltd. (Seoul, Korea). Cimetidine was purchased from Sigma. Acetonitrile and distilled water were used of chromatographic grade (E. Merck, Germany). Standard solutions of praziquantel were made by dilution of stock solution with mobile phase (10 μg of praziquantel mL^{-1} mobile phase). The internal standard solution was prepared by dissolving 10 μg of diazepam into 1 mL of mobile phase.

Experiment I

Fish

To investigate depletion of praziquantel, seventy untreated, clinically healthy black rockfish, *Sebastes schlegeli* weighing 150 to 180 g were obtained from local black rockfish farm. The fish were separated into 3 groups (oral treatment ; 200 mg of praziquantel kg^{-1} fish body weight (bw), 400 mg of praziquantel kg^{-1} bw and bath treatment ; 100 mg of praziquantel ℓ^{-1}) and were acclimatized for 7 d before experiments in semi flow-through 500 ℓ aquaria. The water temperature was 21 to 22 $^{\circ}\text{C}$ and the pH was 7.0 to 7.2. The fish were starved during both the acclimation and the experimental period to avoid differences in drug kinetics owing to maleffective in nutritional status.

Treatment regimen

Oral treatment

Just before treatment, the fish were anaesthetized with methane sulfonate salt (MS 222, 150 mg ℓ^{-1} , Sigma, St. Louis, MO, USA). A single dose of 200 or 400 mg of praziquantel kg^{-1} bw was administered orally by intubation into the stomach. Fish were observed individually for disgorgement until 10 min after drug administration. Control fish received exactly the same treatment as drug treated fish without drug application. At 3, 6, 12, 24, 48, 72, 96, 120, 144 and 168 h post-treatment, three fish were taken randomly from the each aquarium. After anaesthesia with MS222, blood was drawn from the caudal vein and a piece of muscle tissue was collected from each fish. Blood samples were centrifuged immediately to obtain plasma. The plasma and muscle tissue sample were kept frozen at $-70\text{ }^{\circ}\text{C}$ until analyzed. Just before analysis, each muscle tissue sample was defrosted and homogenized with acetonitrile.

Bath treatment

Fish were bathed in an aquarium containing a concentration of 100 mg of praziquantel ℓ^{-1} seawater for 4 min. During the treatment the flow-through system was stopped and the water aerated vigorously to maintain a high oxygen concentration and to prevent the drug from forming a sediment. At 3, 6, 12, 24, 48, 72, 96, 120, 144 and 168 h post-treatment, three fish were taken randomly from the each aquarium. Plasma and muscle sample from each fish were collected as described previously.

Experiment II

Fish

Three net-pens, each containing about 10,000 individuals of black rockfish weighing about 200 g, in a local black rockfish farm were used for the experiment.

Treatment regime

Before feeding praziquantel-supplemented diet, the consumed amount of food given before the noon was enumerated for 3 days, and the quantity of praziquantel added to the food was calculated so that the amount of praziquantel ingested daily by fish was 200 or 400 mg kg⁻¹ bw. Each group of fish in the three net-pens was designated as control (C), 200 mg of praziquantel kg⁻¹ bw (200 P), and 400 mg of praziquantel kg⁻¹ bw (400 P). The fish in group C were fed a moist-pelleted fish meal (control diet) throughout the experiment. The fish in groups 200 P and 400 P were fed diets supplemented with praziquantel at a rate of 200 or 400 mg of praziquantel kg⁻¹ bw, respectively, for three times at 24 h intervals. The medicated diet was made by adding praziquantel to the feed mix prior to pelleting. The water temperature was 19–20 °C. As 1–8 days after last treatment, ten fish were taken randomly from each net-pen daily, and were kept frozen at -70 °C until analyzed.

Experiment III

Fish

To analyze on synergic effect of praziquantel and cimetidine coadministration, netpen-reared juvenile black rockfish *S. schlegeli* (average bw 110 g) were obtained from a local black rockfish farm. The presence of *M. sebastis* on the gills was confirmed by examination of 5 fish. After a week's acclimation, thirty five fish were randomly divided into 7 groups of 5 fish in each group. The volume of each experimental aquarium was 50 l, the water temperature was 20 ± 1 °C, and the salinity was 33 ‰. Fish were not fed throughout the experiment.

Treatment regime

Fish were anaesthetized with MS222 and were intubated directly into the stomach for the administration of varying concentrations of praziquantel and cimetidine. The treatment regime was shown in Table 1. At 24 h post-treatment, all fish in each group were sampled and anaesthetized with MS222. Blood samples drawn from the caudal vein were centrifuged immediately to get plasma sample and were kept frozen at -70 °C until analyzed. The gills of fish in each group were examined to confirm the effectiveness of each treatment.

Table 1. Treatment regime of praziquantel (P) and cimetidine (C) for analyze of synergic effect.

(unit : mg kg⁻¹ bw)

Group	Treatment regime
1	200 P
2	100 P
3	100 P + 200 C
4	100 P + 100 C
5	100 P + 50 C
6	50 P + 200 C
7	0.7 % saline

Chromatographic conditions

The instruments used were a Hewlett-Packard (HP1100 Series, USA) HPLC equipped with a QUAT pump (HP1100 Series G1311A), an automatic gradient controller (HP1100 Series G1324A), an injection valve fitted with a 5 ml sampling loop, a variable-wavelength UV detector and a data module. Analysis was performed on an ODS2C18 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 to 24 °C) and the flow rate was kept constant at 1.0 ml min⁻¹. The detector wavelength was set 217 nm. Between each 200 µl injection the column was washed for 15 min in plasma or 30 min in muscle tissue with 100 % acetonitrile.

Sample preparation

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 °C, then centrifuged at 10,000 × g for 10 min at 4 °C. The collected supernatant was evaporated to dryness with a speed vacuum (Heto-Holten A/S, Copenhagen, Denmark). The mobile dry residue was dissolved in 1 ml of mobile phase, and a portion of 200 µl was injected into the HPLC.

Muscle tissue

A 2 g portion of muscle tissue was weighed into a 15 ml corning tube, and 8.6 ml of 100 % acetonitrile was added. The sample was ground using a homogenizer (ART-Moderne Labortechnik, Mulheim, Germany), and then 0.4 ml internal standard solution was added. After it was allowed to stand for 10 min at 4°C, the sample was centrifuged at $10,000 \times g$ for 10 min, and the supernatant was collected. The collected supernatant was evaporated to dryness with a speed vacuum. The dry residue was dissolved in 1 ml of mobile phase, and a portion of 200 μl was injected into the HPLC.

Calibration curves and recovery rates

Either plasma or muscle tissue homogenate was spiked with standard solutions of praziquantel and internal standard to yield concentrations of 0.25, 0.5, 1.0, 2.0, 4.0 $\mu\text{g ml}^{-1}$ praziquantel and 4.0 $\mu\text{g ml}^{-1}$ internal standard. Samples were prepared according to the above procedure, and each concentration was assayed in triplicate. The recovery rate were determined by comparing results of the HPLC analysis of the spiked plasma and muscle tissue with those of standard solutions.

Statistical analysis

The plasma and muscle praziquantel concentrations were analyzed using Student's *t*-test, and the abundances of *M. sebastis* were analyzed using the Mann-Whitney *U*-test.

RESULTS

Calibration curves and recovery rate

Chromatograms of plasma and muscle tissue homogenate are shown in Fig. 1. The retention times for praziquantel and the internal standard were 6.08 and 7.20 min for plasma, and 6.09 and 7.08 min for muscle tissue homogenate, respectively. A linear relation ($R^2 = 0.997$) was found when the ratio of the peak height of praziquantel in plasma to that of the internal standard was plotted against the concentration of praziquantel in the range 0.25 to 4.0 $\mu\text{g ml}^{-1}$. A linear relation ($R^2 = 0.990$) was obtained also for the muscle tissue homogenate. The average recoveries of praziquantel, assessed by comparison of peak height from the biological fluids with those from standard solutions were 99.2 % in plasma and 82.7 % in muscle tissue homogenate (Table 2).

Experiment I

Oral treatment

The concentrations of praziquantel found in plasma and muscle tissue after oral administration of 200 or 400 mg of praziquantel kg^{-1} fish body weight (bw) are shown in Table 3 and 4, and chromatograms are shown in Fig. 2 and 3. The residue concentrations of praziquantel in plasma and muscle of black rockfish, administered orally at a dose of 200 mg of praziquantel kg^{-1} bw, were highest 6 h after treatment and were eliminated within 120 h after administration. Treatment with 400 mg of praziquantel kg^{-1} bw, praziquantel was detected in plasma and muscle tissue until 96 h after administration. In plasma the praziquantel concentration was highest at the 9 h sampling time and had declined sharply at the 48 h sampling point. The concentrations of praziquantel in the muscle tissue were lower than those in the plasma, and the highest value was found at the 9 h sampling time.

Bath treatment

The concentrations of praziquantel found in plasma and muscle tissue after praziquantel bath treatment are shown in Table 5, and chromatograms are shown in Fig. 4. Following bath treatment, praziquantel was found in plasma and muscle tissue until 72 and 24 h after bath treatment, respectively. In plasma the praziquantel concentration was highest at the 12 h sampling time and declined sharply thereafter. The concentrations of praziquantel in the muscle tissue were significantly lower than those in the plasma, and the concentrations declined consistently with time.

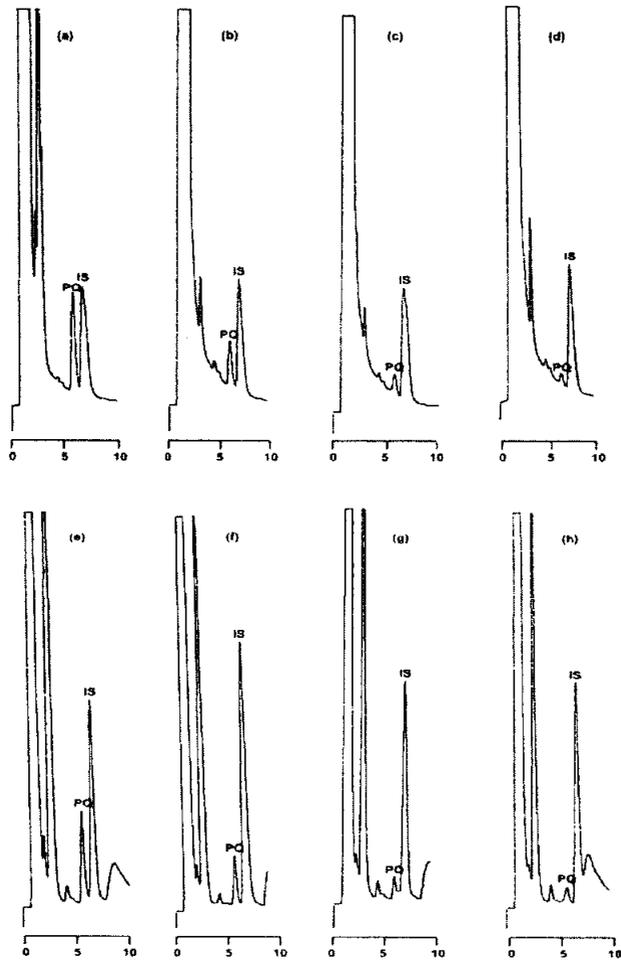


Fig. 1. Chromatograms of praziquantel (PQ) determination in black rockfish, *Sebastes schlegeli* compared with diazepam as an internal standard (IS). Plasma spiked with 4, 2, 1, 0.5 μg of PQ ml^{-1} [(a), (b), (c), (d)]. Muscle spiked with 4, 2, 1, 0.5 μg of PQ ml^{-1} [(e), (f), (g), (h)].

Table 2. Mean recovery of praziquantel in spiked sample of plasma and muscle tissue

Amount added ($\mu\text{g ml}^{-1}$)	Mean recovery (%)	
	plasma	Muscle
4.00	101.5	85
2.00	99.4	69.4
1.00	95.4	77.8
0.50	101.2	92.3
0.25	98.4	88.9
Average (%)	99.2	82.7

Table 3. Praziquantel concentration in black rockfish, *Sebastes schlegeli* plasma and muscle tissue samples after oral treatment with the drug at a dosage of 200 mg of praziquantel kg⁻¹ fish body weight (3 fish per sampling time). Values are mean \pm SD. nd: not detected

Sampling time (hours after treatment)	Plasma ($\mu\text{g ml}^{-1}$)	Muscle ($\mu\text{g g}^{-1}$)
3	7.85 \pm 3.10	2.66 \pm 1.04
6	8.22 \pm 2.95	4.30 \pm 1.41
9	6.39 \pm 1.25	4.15 \pm 1.18
12	3.83 \pm 0.58	1.91 \pm 0.50
24	2.22 \pm 0.07	1.35 \pm 0.24
48	1.54 \pm 0.06	0.50 \pm 0.16
72	1.86 \pm 0.28	0.32 \pm 0.02
96	1.04 \pm 0.51	0.16 \pm 0.02
120	nd	nd
144	nd	nd
168	nd	nd

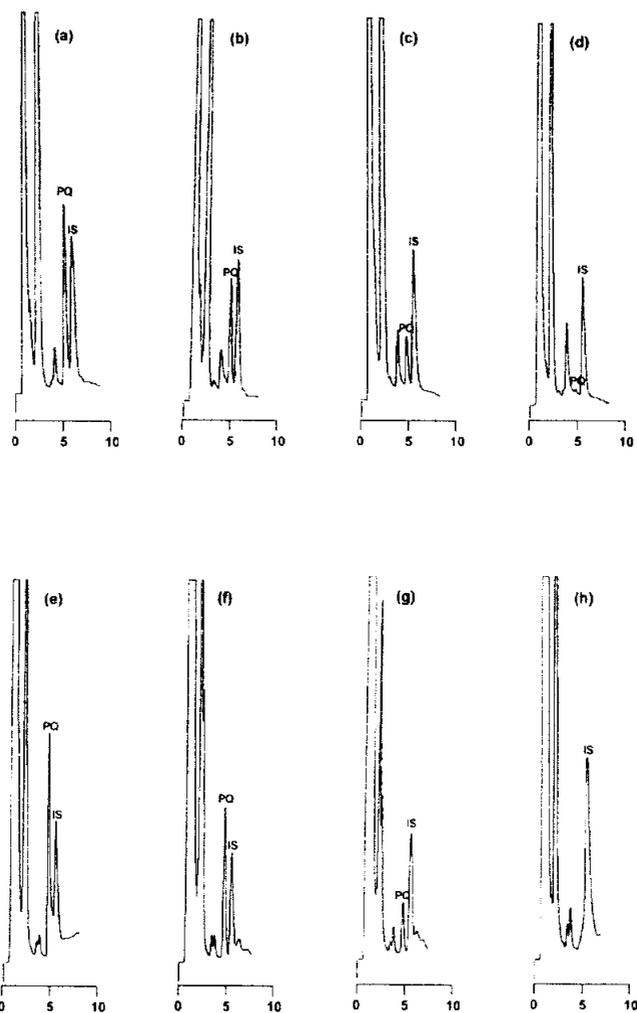


Fig. 2. Chromatograms on 200 mg of praziquantel (PQ) kg⁻¹ fish body weight oral administration in black rockfish, *Sebastes schlegeli* compared with diazepam as an internal standard (IS) (a) Plasma, 6 h after oral treatment; (b) Plasma, 9 h after oral treatment; (c) Plasma, 24 h after oral treatment; (d) Plasma, 120 h after oral treatment; (e) Muscle, 6 h after oral treatment; (f) Muscle, 9 h after oral treatment; (g) Muscle, 24 h after oral treatment; (h) Muscle, 120 h after oral treatment.

Table 4. Praziquantel concentration in black rockfish, *Sebastes schlegeli* plasma and muscle tissue samples after oral treatment with the drug at a dosage of 400 mg of praziquantel kg⁻¹ fish body weight (3 fish per sampling time). Values are mean ± SD. nd: not detected

Sampling time (hours after treatment)	Plasma ($\mu\text{g ml}^{-1}$)	Muscle ($\mu\text{g g}^{-1}$)
3	6.69 ± 1.33	2.49 ± 0.81
6	7.57 ± 3.36	3.82 ± 1.50
9	8.59 ± 1.83	4.20 ± 1.59
12	7.11 ± 0.05	2.51 ± 1.05
24	8.47 ± 1.23	3.61 ± 0.24
48	2.45 ± 0.92	0.22 ± 0.10
72	1.88 ± 0.42	0.08 ± 0.13
96	1.86 ± 0.42	0.06 ± 0.11
120	nd	nd
144	nd	nd
168	nd	nd

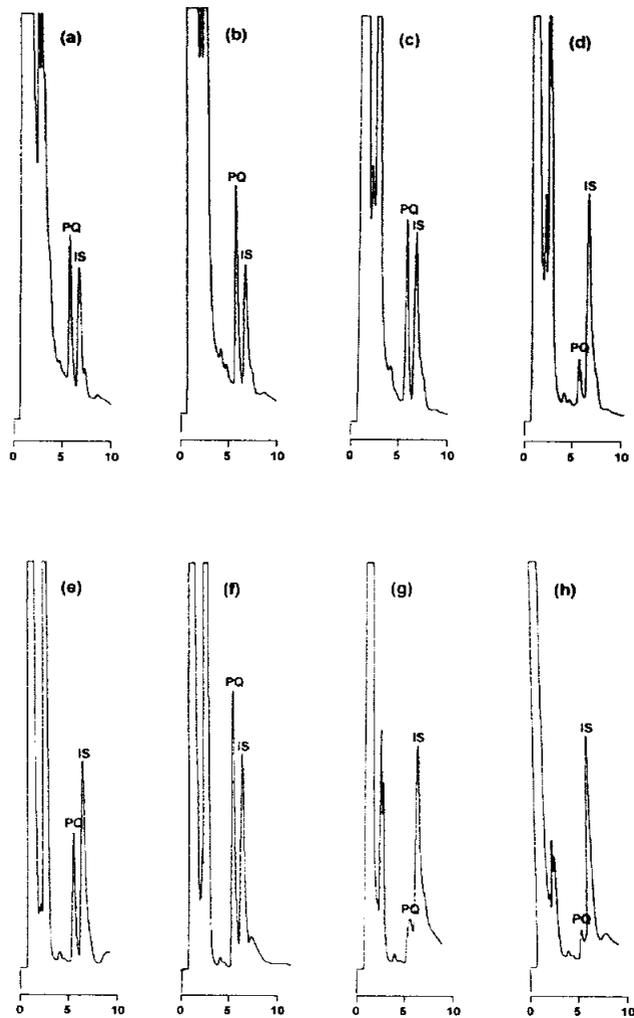


Fig. 3. Chromatograms on 400 mg of praziquantel (PQ) kg^{-1} fish body weight oral administration in black rockfish, *Sebastes schlegeli* compared with diazepam as an internal standard (IS) (a) Plasma, 3 h after oral treatment; (b) Plasma, 9 h after oral treatment; (c) Plasma, 24 h after oral treatment; (d) Plasma, 72 h after oral treatment; (e) Muscle, 3 h after oral treatment; (f) Muscle, 9 h after oral treatment; (g) Muscle, 24 h after oral treatment; (h) Muscle 72 h after oral treatment.

Table 5. Praziquantel concentration in black rockfish, *Sebastes schlegeli* plasma and muscle tissue samples after bath treatment with the drug at 100 ppm for 4 min (3 fish per sampling time). Values are mean \pm SD. nd: not detected

Sampling time (hours after treatment)	Plasma ($\mu\text{g ml}^{-1}$)	Muscle ($\mu\text{g g}^{-1}$)
3	4.10 \pm 0.83	0.49 \pm 0.05
6	4.48 \pm 0.78	0.44 \pm 0.13
9	4.06 \pm 0.45	0.30 \pm 0.17
12	5.96 \pm 3.84	0.28 \pm 0.04
24	1.85 \pm 0.11	0.04 \pm 0.07
48	1.25 \pm 0.50	nd
72	0.29 \pm 0.51	nd
96	nd	nd
120	nd	nd
144	nd	nd
168	nd	nd

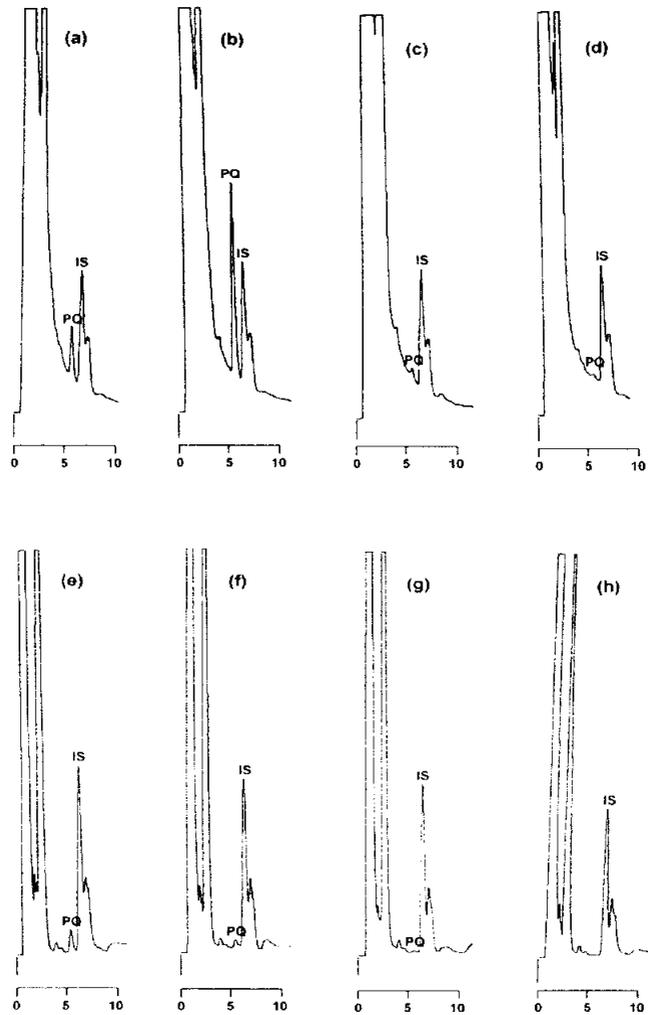


Fig. 4. Chromatograms on 100 ppm of praziquantel (PQ) bath treatment in black rockfish, *Sebastes schlegeli* compared with diazepam as an internal standard (IS) (a) Plasma, 3 h after bath treatment; (b) Plasma, 12 h after bath treatment; (c) Plasma, 24 h after bath treatment; (d) Plasma, 48 h after bath treatment; (e) Muscle, 3 h after bath treatment; (f) Muscle, 12 h after bath treatment; (g) Muscle, 24 h after bath treatment; (h) Muscle, 48 h after bath treatment.

Experiment II

Praziquantel concentration in muscle

The levels of praziquantel found in muscle tissue after oral administration of praziquantel are shown in Table 6, and its chromatogram is shown in Fig 5. In the group of fish fed 200 mg of praziquantel kg^{-1} bw, praziquantel was detected in muscle tissue until 1 day post treatment. Following treatment with 400 mg of praziquantel kg^{-1} bw, praziquantel was found in muscle tissue until 5 days post treatment. The levels of praziquantel in muscle tissue was sharply declined at 2 days post-treatment, and consistently declined with the lapse of day in both praziquantel-administered groups.

Table 6. Praziquantel concentration in black rockfish, *Sebastes schlegeli* muscle tissue sample after three oral doses 200 (200 P) or 400 mg of praziquantel kg⁻¹ bw (400 P) at 24 h intervals (10 fish per each sampling day). Values are mean \pm SD. nd: not detected

Sampling day (days after treatment)	Muscle ($\mu\text{g g}^{-1}$)	
	200 P	400 P
1	0.24 \pm 0.27	1.20 \pm 0.80
2	nd	0.68 \pm 0.39
3	nd	0.73 \pm 0.50
4	nd	0.67 \pm 0.89
5	nd	0.11 \pm 0.19
6	nd	nd
7	nd	nd
8	nd	nd

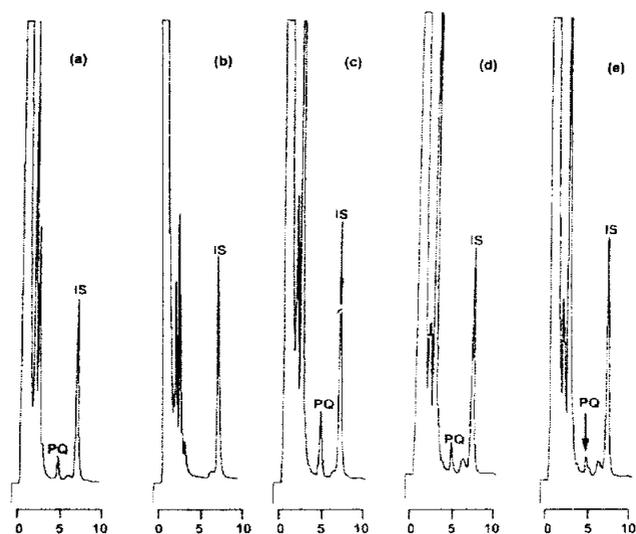


Fig. 5. Chromatograms of praziquantel (PQ) determination in black rockfish, *Sebastes schlegeli* muscle tissue after oral administration of three doses of either 200 (200 PQ group) or 400 mg of praziquantel kg^{-1} fish body weight (400 PQ group) given at 24 h intervals. After (a) 1 day of 200 PQ group; (b) 2 days of 200 PQ group; (c) 1 day of 400 PQ group; (d) 3 days of 400 PQ group; (e) 5 days of 400 PQ group. IS, internal standard.

Experiment III

Praziquantel concentrations in plasma

After 24 h each treatment, the praziquantel concentration in plasma of fish administered 100 mg of praziquantel + 200 mg of cimetidine kg^{-1} bw was not significantly different from that of fish treated with 200 mg of praziquantel kg^{-1} bw and was significantly ($p < 0.05$) higher (ca. $2\times$) than that of fish administered 100 mg of praziquantel kg^{-1} bw (Fig. 7 & Table 7). Although the fish administered 100 mg of praziquantel + 100 mg of cimetidine kg^{-1} bw or 100 mg of praziquantel + 50 mg of cimetidine kg^{-1} bw showed slightly higher plasma praziquantel concentrations than fish administered 100 mg of praziquantel kg^{-1} bw, there were no statistical significances. However, the group of fish administered 50 mg of praziquantel + 200 mg of cimetidine kg^{-1} bw showed similar plasma praziquantel concentration to the fish treated with 100 mg of praziquantel kg^{-1} bw. No praziquantel was detected in the plasma of control fish (Fig. 6).

Treatment efficacy

All the groups of fish administered praziquantel alone or coadministered praziquantel and cimetidine simultaneously showed significantly lower abundances of *M. sebastis* on the gills than those of the control fish (Fig. 8 & Table 8). The treatment efficacies of the groups of fish coadministered 100 mg of praziquantel kg^{-1} bw and various concentrations of cimetidine (200, 100 and 50 mg of cimetidine kg^{-1} bw) were not significantly different from those of the group of fish administered 200 mg of praziquantel kg^{-1} bw but were significantly higher than those of the groups of fish fed 100 mg of praziquantel kg^{-1} bw alone. Because of large variations in treatment efficacy in the fish coadministered 50 mg of praziquantel + 200 mg of cimetidine kg^{-1} bw, there were no significant differences between this group and other experimental groups except the control group.

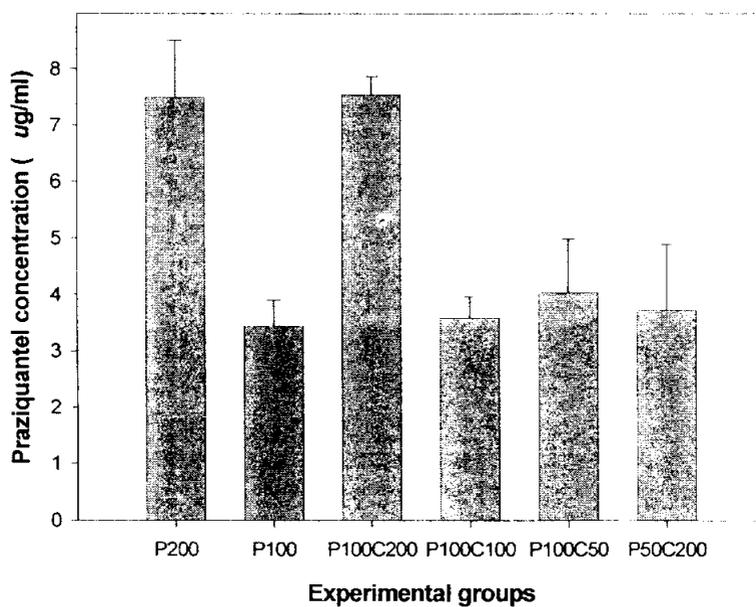


Fig. 7. Praziquantel (P) concentration in plasma (mean \pm standard error) of black rockfish, *Sebastes schlegeli* 24 h after oral administration of P (mg kg^{-1} fish body weight, bw) alone or coadministered with cimetidine (C, mg kg^{-1} bw) in various combinations.

Table 7. Significance in plasma praziquantel concentration between experimental groups calculated using Student's *t*-test 24 h after oral administrations. C, cimetidine (mg kg^{-1} bw); P, praziquantel (mg kg^{-1} bw)

Group	P200	P100	P100+C200	P100+C100	P100+C50	P50+C200
P200	-	0.022	0.969	0.062	0.067	0.071
P100		-	0.002	0.848	0.609	0.838
P100+C200			-	0.004	0.025	0.034
P100+C100				-	0.745	0.934
P100+C50					-	0.846

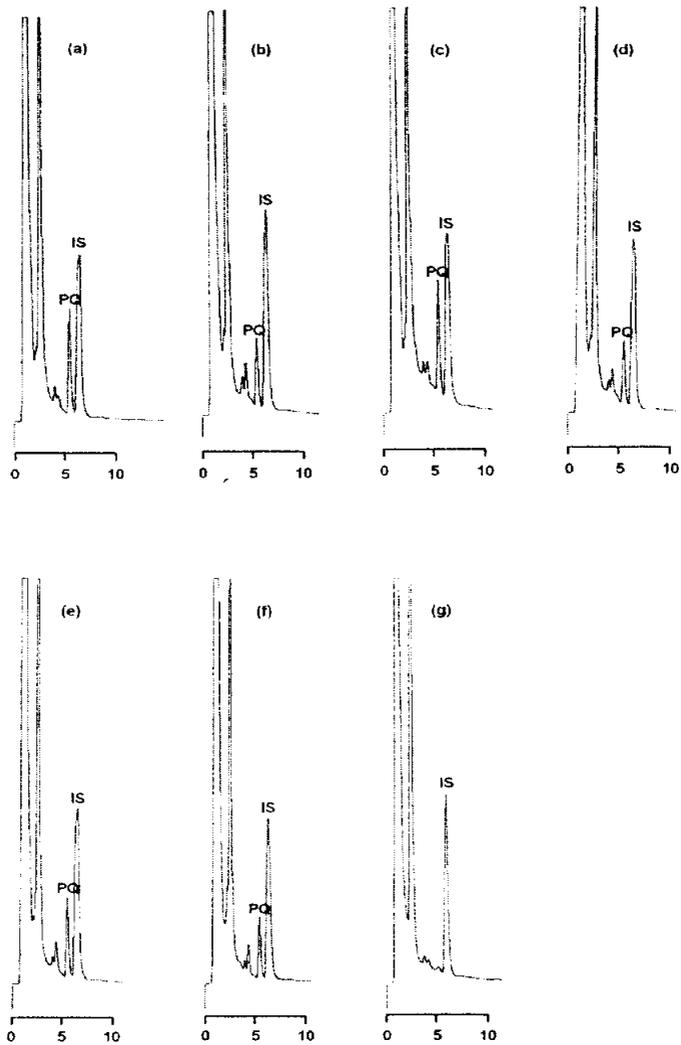


Fig. 6. Chromatograms of praziquantel (PQ) in plasma of black rockfish, *Sebastes schlegeli* compared with diazepam as an internal standard (IS) 24 h after each oral treatment with (a) 200 mg of praziquantel kg^{-1} fish body weight, bw, (b) 100 mg of praziquantel kg^{-1} bw, (c) 100 mg of praziquantel + 200 mg of cimetidine kg^{-1} bw, (d) 100 mg of praziquantel + 100 mg of cimetidine kg^{-1} bw, (e) 100 mg of praziquantel + 50 mg of cimetidine kg^{-1} bw, (f) 50 mg of praziquantel + 200 mg of cimetidine kg^{-1} bw, and (g) with control.

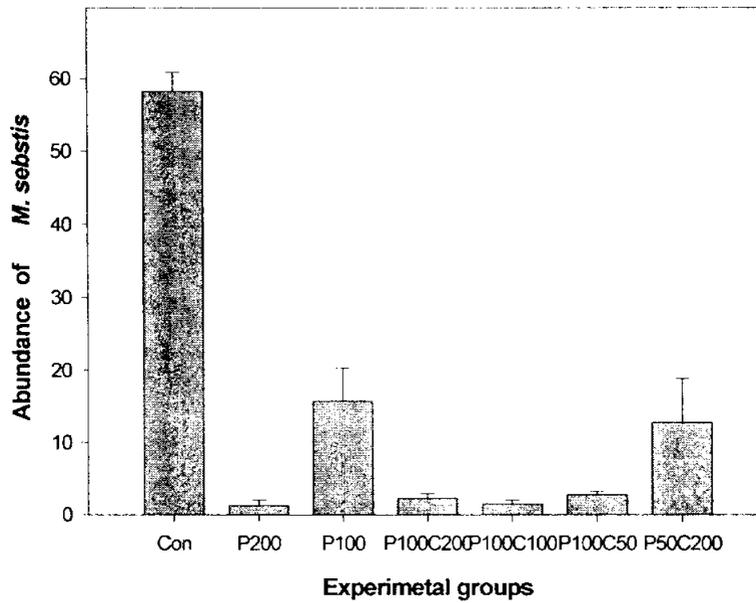


Fig. 8. Abundance (mean \pm SE) of *Microcotyle sebastis* on the gills of black rockfish, *Sebastes schlegeli* 24 h after oral administration of praziquantel (P; mg kg⁻¹ bw) alone or coadministered with cimetidine (C; mg kg⁻¹ bw) in various combinations.

Table 8. Significance in abundance of *Microcotyle sebastis* between experimental groups calculated using Mann-Whitney *U*-test 24 h after oral administrations. C, cimetidine (mg kg⁻¹ bw); P, praziquantel (mg kg⁻¹ bw)

Group	Control	P200	P100	P100+C200	P100+C100	P100+C50	P50+C200
Control	-	0.000	0.000	0.000	0.000	0.000	0.000
P200		-	0.020	0.346	0.791	0.143	0.111
P100			-	0.026	0.020	0.029	0.707
P100+C200				-	0.387	0.550	0.138
P100+C100					-	0.121	0.116
P100+C50						-	0.153

DISCUSSION

Sample preparation and HPLC methods in the present study were fast and simple for the analysis of praziquantel, and showed good recovery of the drug in black rockfish plasma and muscle tissue. The recovery of praziquantel in the study of Rogstad et al. (1987) varied from 79 to 93 % for muscle of rainbow trout. In the work of Hormazabal & Yndestad (1995), the recovery of praziquantel varied from 91 to 92 % for plasma, and from 99 to 100 % for muscle of salmon and rainbow trout.

Although the absorption and depletion of praziquantel in black rockfish in this study were slower than those in mammals, the data were similar to those of previous pharmacokinetic studies of praziquantel in rainbow trout (Bjorklund & Bylund, 1987; Rogstad et al., 1987). The residue concentrations of praziquantel in plasma and muscle of black rockfish, administered orally at a dose of 200 mg of praziquantel kg^{-1} fish body weight (bw), were highest 6 h after treatment and were eliminated within 120 h after treatment. Treatment with 400 mg of praziquantel kg^{-1} bw, praziquantel was detected in plasma and muscle tissue until 96 h after treatment.

The present results indicate that the withdrawal time of praziquantel in muscle of black rockfish when fed 200 mg of praziquantel kg^{-1} bw for three times at 24 h intervals was less than 4 days. The residue levels of praziquantel in muscle of black rockfish administered 400 mg of praziquantel kg^{-1} bw, at a day's interval for three times were eliminated within 6 days post treatment. According to the preliminary study of Rogstad et al. (1987), the highest residue concentration of praziquantel in muscle and serum of rainbow trout, which were given a single oral dose (10 mg kg^{-1} bw) of praziquantel, was obtained 7 h after treatment, and no residue were found 48 h after medication. Using a bioassay with parasitic cercariae as test organisms for determination of praziquantel concentrations, Bjorklund & Bylund (1987) reported that the peak values of praziquantel

in different tissues (serum, muscle, liver, bile fluid, kidney) of rainbow trout were reached 4 to 16 h after a single oral administration at a dose of 500 mg of praziquantel kg^{-1} bw and by 32 h after administration, 67 to 96 % of the maximum amounts had been eliminated from the tissues.

In the present study, the absorption and depletion of praziquantel in the blood of black rockfish were fast and the residual concentration of praziquantel declined below 4 $\mu\text{g ml}^{-1}$ within 24 h post treatment. The fast depletion of praziquantel in the blood of black rockfish was correlated with the survival of some *M. sebastis*. During the experimental period, the survived worms would suck the blood when the praziquantel residues were sub-parasitocidal concentrations. Considering the sharp decrease in praziquantel concentration in plasma after 24 h after oral administration in the present study, retreatment at an interval of 24 h would be effective for eradication of *M. sebastis*.

It is known that the gill is a main route of absorption for drugs administered in water (Treves-Brown, 2000). However, direct absorption via the skin cannot be excluded. Bathing black rockfish with 100 ppm of praziquantel for 4 min in the present study was the same scheme as that used in the treatment of *M. sebastis* in field conditions (Kim & Cho, 2000). In the present study, praziquantel residue concentrations in muscle of black rockfish after bath treatment were about 10 times lower than those in plasma and showed a consistently decreasing pattern with time. This results suggests that absorption of praziquantel in black rockfish following bath treatment is largely through the gills, but a small amount of the drug can be absorbed via skin.

Although the concentrations of praziquantel in plasma after bath treatment were lower than those after oral treatment in the present experimental schemes, not only direct contact of *M. sebastis* with praziquantel but also feeding by the parasites on blood containing the drug resulted in high treatment efficacy of *M. sebastis* by bath with praziquantel in the study of Kim & Cho (2000)

The results of the present study agree with a previous report which indicated that coadministration of cimetidine with praziquantel led to a significantly increased treatment efficacy of the latter drug against *M. sebastis* infestation in black rockfish (Kim et al., 2001a). Moreover, in the present study we first demonstrated in fish that these increased treatment efficacies by cimetidine supplementation were through elevation of praziquantel concentration in the blood of treated fish. Masimirembwa & Hasler (1994) reported that praziquantel was metabolized by phenobarbitone-inducible isoforms of cytochrome P₄₅₀, and cimetidine was an effective inhibitor of the metabolism of praziquantel. Diekmann et al. (1989) also reported that cimetidine was an effective inhibitor of praziquantel metabolism at a dose of 200 mg kg⁻¹ bw in rats. In the treatment of human neurocysticercosis, increase of praziquantel's bioavailability by coadministration of cimetidine and consequently increase of treatment efficacy were well demonstrated by many studies (Overbosch, 1992; Dachman et al., 1994; Jung et al., 1997; Sotelo & Jung, 1998; Al-Khodairy et al., 1999).

The present results showed that coadministration of 200 mg of cimetidine kg⁻¹ bw with 100 or 50 mg of praziquantel kg⁻¹ bw raised the plasma praziquantel level to that achieved by administration of praziquantel alone at dosages of 200 or 100 mg kg⁻¹ bw, respectively. Although addition of 100 mg of cimetidine or 50 mg kg⁻¹ bw to 100 mg of praziquantel kg⁻¹ bw did not significantly affect the plasma praziquantel levels 24 h post-administration when compared with oral administration of 100 mg of praziquantel kg⁻¹ bw alone, the treatment efficacies against *M. sebastis* were not significantly different from those of the group coadministered 100 mg of praziquantel + 200 mg of cimetidine kg⁻¹ bw. In mammals, cimetidine is rapidly absorbed following oral administration, peak plasma levels being attained after approximately 2 h when taken with food, or after 1 h when taken without food, and about two-thirds of the oral dose is excreted within 24 h (Kelly et al., 1995). According to present the pharmacokinetic study of praziquantel was

also sharply decreased in plasma of black rockfish after 24 h of oral administration. Therefore, the results of the present study suggest that oral administration of cimetidine at doses of 100 or 50 mg kg⁻¹ bw to black rockfish can inhibit praziquantel metabolism in liver and can raise plasma praziquantel levels enough to kill *M. sebastis* within 24 h.

**Cimetidine 투여가 praziquantel의 양식 조피볼락 (*Sebastes schlegeli*) 아가미 흡충증
치료 효과 및 혈액 잔류 농도에 미치는 영향**

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어병학과

요 약

양식 조피볼락에서 praziquantel의 잔류량 검사 및 cimetidine을 혼합 투여가 praziquantel 잔류 및 아가미 흡충증 치료효과에 미치는 영향에 대해서 연구하였다. Plasma와 muscle 조직은 diazepam을 internal standard로 사용한 HPLC system에 의해 분석하였다. 실험실 환경에서 praziquantel의 잔류량을 조사하기 위해서 어체중 kg당 200, 400 mg 및 100 ppm을 경구 투여 및 약육하였다. 200 mg의 praziquantel을 경구 투여한 경우 plasma와 muscle에서 6 시간째에 가장 높은 양이 검출되었고 120 시간 후에 완전히 소멸되었다. Praziquantel 400 mg을 경구 투여한 경우 plasma와 muscle에서 투약 후 96 시간까지 검출되었다. Plasma에서 praziquantel의 plasma 농도는 투약 후 9 시간째에 가장 높았고 48 시간 이후부터 두드러진 감소가 나타났다. Muscle에서 praziquantel의 농도는 plasma 보다 낮았고, muscle 내의 최고 농도는 투약 후 9 시간째에 나타났다. Praziquantel을 약육한 경우, praziquantel은 plasma와 muscle에서 각각 72 및 24 시간까지 검출되었다. Plasma에서 praziquantel의 농도는 투약 후 12 시간째에 가장 높았으며 이후 두드러진 감소가 나타났다. Muscle에서 praziquantel의 농도는 plasma 보다 낮았으며 시간에 경과함에 따라 감소하였다. 현장 환경에서는 어체중 kg당 praziquantel 200 또는 400 mg을 24 시간 간격으로 3회 경구투여하였다. 어체중 kg당 praziquantel을 200 mg을 투약한 경우 muscle에서는 투약 후 24 시간까지 검출되었고 400 mg을 투약한 경우는 muscle에서 투약 후 120 시간까지 검출되었다.

혈중 praziquantel의 농도와 아가미 흡충의 치료에서 cimetidine의 영향이 연구되었다. 경

구로 praziquantel을 단독 투약한 실험구와 cimetidine을 혼합 투여한 실험구로 나누었다. 투약 후 24 시간째에 plasma와 gill을 수집하여 분석하였다. 어체중 kg당 praziquantel 100 mg에 cimetidine 200 mg을 혼합투여한 경우 plasma에서 praziquantel의 농도는 praziquantel을 단독으로 200 mg 투약하였을 때와 유의적인 차이가 나타나지 않았고 praziquantel 100 mg을 단독으로 투약한 실험구와는 유의적인 차이가 나타났다. 아가미 흡충의 치료 효과는 어체중 kg당 praziquantel 100 mg에 각각 200, 100 및 50 mg의 cimetidine을 혼합투약한 경우 praziquantel을 단독으로 200 mg 투약하였을 때와 유의적인 차이가 나타나지 않았다. 그러므로 cimetidine은 혈중 praziquantel의 농도를 지속시켜주어 아가미 흡충의 치료에 영향을 주는 것으로 여겨진다.

감사의 글

언제나 열정을 가지고 일하시면서 부족한 저에게 학문에 대한 길을 열어주시고 깨우쳐 주신 존경하는 김기홍 선생님께 감사하다는 말 외에 다른 표현방법이 없다는 것이 정말 안타깝습니다. 큰산처럼 계시면서도 세세하게 조언을 아끼지 않으셨던 박수일 선생님, 바쁘신 와중에도 논문심사를 위해 애쓰신 정준기 교수님, 언제나 따뜻하게 대해주시고 격려해주시는 정현도, 강주찬 교수님께 진심으로 감사 드립니다.

힘든 실험실 생활이었지만 함께 동고동락하면서 같이 지낸 찬휘 재혁, 실험과 인생에 있어서 많은 조언을 해준 실험실 맏형 안경진 선배님, 수많은 부탁과 불평을 잘 이해해준 재범, 힘든 내색 한번 하지 않고 묵묵히 도와준 착한 성미, 불평불만은 많았지만 항상 끝까지 웃으면서 도와준 지영, 약한 몸으로 실험실을 위해 애쓰는 은혜, 선배들 뒤치다꺼리에 바빴던 도선, 경이, 막내에서 이제 막 자기 역할을 하는 현진, 혜영, 아직 실험실에 대해 잘 모르지만 열심히 해나가고 있는 막내들, 동기이기 때문에 같이 있는 것만으로도 즐거웠던 영재, 갑민에게 감사의 말을 전합니다.

실험에 많은 도움을 주신 한규석, 최성재, 김영수, 배찬민, 장명덕, 문재석 선배님께 감사드리며, 힘들 때마다 위로의 말과 함께 사기를 북돋아 준 강형길, 고석형, 서정수, 이근의, 우승호, 장석우 선배님께 감사 드립니다. 또 마음을 툭 터놓고 이야기 할 수 있었던 준범, 려진에게 감사의 말을 전합니다.

언제나 따뜻한 휴식처가 되어주며 물심양면으로 도와준 친구 주형, 성오, 우환, 문규, 문현, 동엽, 가장 가까운 곳에서 지켜봐주며 자기 일처럼 도와주고 곁에 있다는 것만으로도 힘이 되어준 사랑하는 소정에게 감사의 말을 전합니다.

부족한 저를 위해 항상 기도해주신 김해 어머님께 감사 드립니다. 지금의 김천수가 있기까지 누구보다도 많은 도움을 주시고 사랑과 믿음으로 지켜봐 주신 부모님과 사랑스러운 동생들께 감사 드리며 이 논문을 바칩니다.

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