Efficacy of Oral Administration of Praziquantel and Mebendazole against *Microcotyle sebastis* (Monogenea) Infestation of Cultured Rockfish (*Sebastes schlegeli*)

Ki Hong Kim^{*1,3}, Soo-Il Park^{*1} and Bo-Young Jee^{*2}

^{*1}Department of Aquatic Life Medicine, Pukyong National University, 599-1 Dae Yeon Dong, Nam Ku, Pusan 608-737

^{*2}Pathology Division, National Fisheries Research and Development Institute, Pusan 619-900, Republic of Korea

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The efficacy of praziquantel and mebendazole against *Microcotyle sebastis* infestation in juvenile rockfish by oral administration was evaluated at low ($10 \pm 1^\circ\text{C}$) and high ($20 \pm 1^\circ\text{C}$) water temperatures. Oral administration of praziquantel or mebendazole resulted in significant reduction or complete extermination of *M. sebastis* infestation in juvenile rockfish. The treatment efficacy of praziquantel 200 mg/kg B. W. at both temperatures (10°C and 20°C) and mebendazole 200 mg/kg B. W. at 20°C was 100%. Praziquantel was more effective against *M. sebastis* than mebendazole at 10°C . Our results strongly suggest that oral administration of praziquantel and mebendazole can be used practically in netpen-culture of rockfish as an effective control measure against *M. sebastis* infestations without imposing handling-related stress to fish.

Key words: praziquantel, mebendazole, oral administration, *Microcotyle sebastis*, *Sebastes schlegeli*

Microcotyle sebastis is an important ectoparasite on the gills of rockfish, *Sebastes schlegeli*, reared in netpens in Korea. Because of lacking successful management and treatment strategies to prevent and control this parasite, extensive mortalities of juvenile rockfish have occurred every year.

Various chemicals including formalin, copper sulfate (Thoney, 1990), potassium permanganate (Chan and Wu, 1984), hydrogen peroxide (Kabata, 1985), trichlorfon (Imada and Muroga, 1979; Thoney, 1990) have been employed to control monogenean parasites. However, many of them were proved detrimental to fish and/or environment, and most were not 100% effective (Thoney and Hargis, 1991). Recently, praziquantel and mebendazole developed to treat various internal helminth infections in mammals have been used to treat monogenean diseases in aquaculture (Goven and Amend, 1982; Schmahl and Mehlhorn, 1985; Székely and Molnár, 1987, 1990; Buchmann, 1987, 1993; Buchmann and Bjerregaard, 1990; Buchmann *et al.*, 1990; Mellergaard, 1990).

To date, only bath treatments with those chemotherapeutants have been used to control monogenean diseases. The bath treatment of rockfish in netpens, however, requires additional labour and is stressful to the fish. Thus, other effective methods are needed to control *M. sebastis* of rockfish. Recently, Kim and Choi (1998) reported that oral administrations of mebendazole and bithionol were effective in treating *M. sebastis* infestations in cultured rockfish.

The aim of the present study was to determine the efficacy of praziquantel and mebendazole against *M. sebastis* infestation by oral administration, and to compare the efficacy of the both compounds at low (10°C) and high (20°C) water temperatures.

Materials and Methods

Fish

Netpen reared juvenile rockfish (body length: 8–13 cm) were obtained from a local producer in Tongyoung (endemic area of *M. sebastis*), Korea. The presence of *M. sebastis* on the gills was confirmed by examination of 10 fish. The prevalence was 100%, and the intensity was 54.2 ± 32.8 . A total of 150 juvenile rockfish

^{*3} Corresponding author
E-mail: khkim@dolphin.pknu.ac.kr

was randomly divided into a low water temperature ($10 \pm 1^\circ\text{C}$) and a high water temperature group ($20 \pm 1^\circ\text{C}$), then separated again into 5 groups of 15 fish in each temperature group. The fish were acclimated to $10 \pm 1^\circ\text{C}$ or $20 \pm 1^\circ\text{C}$ for 2 weeks prior to the experiment in a flow-through laboratory system. The water temperature was adjusted by means of cooling and heating devices. Fish were fed commercial pelleted rockfish food (1.0% of body weight per day) throughout the experiment. The volume of each experimental aquarium was 50 l, and flow rate was maintained at approximately 0.2 l/min under the condition of 16L: 8D. The seawater had a salinity of 33‰.

Treatment

Fish were anaesthetized with benzocaine and were intubated directly onto the stomach with varying concentrations of the test chemotherapeutants. In each temperature regime, one group was fed with 100 mg praziquantel (Shinpoong Pharm. Co. Ltd.)/kg B. W., and the second group 200 mg praziquantel/kg B. W. The third group 100 mg mebendazole (Sigma)/kg B. W., and the fourth group 200 mg mebendazole/kg B. W. The fish in the control group were given 0.8% saline (Table 1). The effectiveness of each treatment was confirmed by the comparison of the number of parasites in each treatment group with those in the control group at 7 days post-treatment. Abundance and prevalence were determined according to the methods given in Margolis *et al.* (1982).

Statistical analysis

All data were analyzed using Mann-Whitney's U-test

(SPSS 7.5 for Windows, SPSS Inc.).

Results

Oral administration of praziquantel or mebendazole resulted in significant reduction or completely extermination of *M. sebastis* infestations in juvenile rockfish, irrespective of the different dosages and water temperatures (Table 1). The treatment efficacy of praziquantel 200 mg/kg B. W. at both temperatures ($10 \pm 1^\circ\text{C}$ and $20 \pm 1^\circ\text{C}$) and mebendazole 200 mg/kg B. W. at $20 \pm 1^\circ\text{C}$ was 100%. No apparent abnormalities were noticed in the experimental fish during the experimental period.

At $10 \pm 1^\circ\text{C}$, statistically significant differences in treatment efficacy were revealed between praziquantel and mebendazole (Table 2). Praziquantel was more effective against *M. sebastis* than mebendazole at $10 \pm 1^\circ\text{C}$. At $20 \pm 1^\circ\text{C}$, however, there was no significant difference in treatment efficacy between praziquantel and mebendazole at each equal dosage.

Praziquantel was highly effective against *M. sebastis* at both temperatures. No statistically significant difference in treatment efficacy of praziquantel was revealed between 10°C and 20°C at each equal dosage (Table 2). On the other hand mebendazole showed a temperature-dependent exterminating property. The efficacy of mebendazole against *M. sebastis* was considerably higher at the higher water temperature.

There were significant differences in treatment efficacy between the two different dosages of praziquantel at both temperatures (Table 2). The efficacy of mebendazole was not significantly different between the two different dosages at $10 \pm 1^\circ\text{C}$, but was significantly

Table 1. Efficacy of oral administration of praziquantel and mebendazole against *Microcotyle sebastis* infestation in juvenile rockfish

Water Temp.	Experimental group	Dosages (mg/kg B. W.)	No. of rockfish examined	Prevalence (%)	Abundance (Mean $\pm$ S.D.)	Range of parasite number
$10 \pm 1^\circ\text{C}$	Control	Only saline	15	100.0	56.9 ± 39.3	3 – 136
		Praziquantel				
	Mebendazole	100	15	66.7	1.9 ± 2.3	0 – 6
		200	15	0	0	0
		100	15	100.0	16.7 ± 14.8	2 – 49
$20 \pm 1^\circ\text{C}$	Control	200	15	86.7	12.3 ± 10.8	0 – 38
		Only saline	15	100.0	58.7 ± 36.1	5 – 122
	Praziquantel	100	15	66.7	1.5 ± 1.8	0 – 7
		200	15	0	0	0
	Mebendazole	100	15	60.0	4.1 ± 6.4	0 – 24
		200	15	0	0	0

Table 2. The values of significance among experimental groups calculated using Mann-Whitney's U-test

Group	LC	LP1	LP2	LM1	LM2	HC	HP1	HP2	HM1	HM2
LC	S	0.000	0.000	0.002	0.000	0.901	0.000	0.000	0.000	0.000
LP1		S	0.000	0.000	0.001	0.000	0.863	0.000	0.579	0.000
LP2			S	0.000	0.000	0.000	0.003	S	0.001	S
LM1				S	0.467	0.001	0.000	0.000	0.001	0.000
LM2					S	0.000	0.000	0.000	0.007	0.000
HC						S	0.000	0.000	0.000	0.000
HP1							S	0.000	0.368	0.000
HP2								S	0.001	S
HM1									S	0.001

[LC: Control ($10 \pm 1^\circ\text{C}$), LP1: Praziquantel 100 mg/kg B. W. ($10 \pm 1^\circ\text{C}$), LP2: Praziquantel 200 mg/kg B. W. ($10 \pm 1^\circ\text{C}$), LM1: Mebendazole 100 mg/kg B. W. ($10 \pm 1^\circ\text{C}$), LM2: Mebendazole 200 mg/kg B. W. ($10 \pm 1^\circ\text{C}$), HC: Control ($20 \pm 1^\circ\text{C}$), HP1: Praziquantel 100 mg/kg B. W. ($20 \pm 1^\circ\text{C}$), HP2: Praziquantel 200 mg/kg B. W. ($20 \pm 1^\circ\text{C}$), HM1: Mebendazole 100 mg/kg B. W. ($20 \pm 1^\circ\text{C}$), HM2: Mebendazole 200 mg/kg B. W. ($20 \pm 1^\circ\text{C}$), S: Same data]

different ($p < 0.05$) at $20 \pm 1^\circ\text{C}$.

Discussion

The results clearly show that oral administrations of praziquantel or mebendazole are effective for controlling *Microcotyle sebastis* infesting the gills of cultured rockfish. Praziquantel has a very broad spectrum of activity against trematodes and cestodes infections in mammals (Andrew et al., 1983), and has recently been employed to control monogenean diseases in fish by bath treatment. Although praziquantel has been injected or given orally to eliminate endoparasitic helminths such as metacercariae of *Diplostomum spathaceum* infecting eye lenses of trout (Bylund and Sumari, 1981), *Clinostomum marginatum* infecting muscle of channel catfish (Lorio, 1989) and adult cestodes of both freshwater and marine fishes (Pool et al., 1984; Moser et al., 1986; Sanmartin Duran et al., 1989), it has never been used to treat monogenean diseases by oral administration. In the present study, we report for the first time that oral administration of praziquantel is a very effective method in treatment of infections with gill monogeneans. Mebendazole is a member of the benzimidazole group commonly used against nematodes infections in mammals (Roberson, 1982) but previously shown to be potent against fish monogeneans by bath treatment (Goven and Amend, 1982; Székely and Molnár, 1987; Buchmann, 1993; Buchmann and Bjerregaard, 1990; Møllergaard, 1990). According to the results of Kim and Choi (1998), oral administration of mebendazole at a dosage of 100 mg/kg B. W. ($23 \pm 1^\circ\text{C}$) significantly reduced the infestation level of *M. sebastis* in rockfish.

In the present experiment, mebendazole administered orally at a dose of 100 mg/kg B. W. ($20 \pm 1^\circ\text{C}$) significantly reduced the number of *M. sebastis*, also, and at a dose of 200 mg/kg B. W. ($20 \pm 1^\circ\text{C}$) completely eradicated *M. sebastis* from the gills of juvenile rockfish. Therefore it is indicated that oral administration of praziquantel and mebendazole can be used practically in netpen-culture of rockfish as an effective control measure for *M. sebastis* infestations without imposing handling related stress to fish.

The efficacy of praziquantel in treatment of *M. sebastis* was not significantly affected by water temperature but that of mebendazole was affected significantly. Elevated temperature apparently enhanced the efficacy of mebendazole against *M. sebastis* infestation. This was also shown by Buchmann et al. (1992) for gill monogeneans on European eel, *Anguilla anguilla*. Temperature-related variations in pharmacokinetic properties of antibiotics in fish have been previously demonstrated in several studies (Borgan et al., 1981), but the effects of temperature on the mode of action of praziquantel and mebendazole in fish administered orally are not known. Our results indicate that praziquantel can be used at both high and low water temperatures to orally treat *M. sebastis* infestation, and mebendazole can be used at a high water temperature to get high efficacy against *M. sebastis* infestation.

Experimental fish were infected not only with adult parasites, but also with immature ones. There was no big differences in the susceptibility of the parasite at different developmental stages to the chemotherapeutants tested (data not shown).

Buchmann et al. (1992) demonstrated that extended

exposures to subtherapeutic dosages of mebendazole resulted in drug resistant parasite populations and it was recommended by these authors to alternate between mebendazole and praziquantel treatments in order to delay the emergence of anthelmintic resistance. Indeed, our experiments suggest that development of the drug resistance in *M. sebastis* can be prevented or delayed by alternation in use between praziquantel and mebendazole.

Although Møllergaard *et al.* (1990) and Iosifidou *et al.* (1997) reported the residues of mebendazole in eel after bath treatment, no work has been done on the metabolism and excretion of mebendazole and praziquantel in fish administered orally. Regarding safety for the human consumption, therefore, it would be recommended that the oral administration of these chemotherapeutants should be limited to juveniles of rockfish until the residue depletion times of these compounds from flesh of fish are determined.

Although eradication of the parasite from the gills of rockfish was successful in the present experiments, we have the problem of reinfection in the field. We have to establish the retreatment scheme for net-pen cultured rockfish. Experiments on the determination of the retreatment interval is now in progress.

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