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Challenge trials on the anthelmintic effect of drugs and natural agents against the monogenean *Heterobothrium okamotoi* in the tiger puffer *Takifugu rubripes*

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Abstract

In vitro and oral administration challenge trials were performed to search for effective agents derived from natural sources (natural agents) against infections by the monogenean *Heterobothrium okamotoi* in the tiger puffer *Takifugu rubripes*. First, four drugs (praziquantel, levamisole, pyrantel pamoate and antimony sodium tartarate) were screened for their anthelmintic efficacy against *H. okamotoi* to select the drug most suitable as an effective positive control for a challenge trial of natural agents. Of these, praziquantel showed anthelmintic efficacy against *H. okamotoi* in both in vitro and challenge trials and the in-feed praziquantel (4 g/kg basal diet) was chosen as the positive control. Next, four natural agents (caprylic acid, orange oil, peppermint oil and cinnamon oil) were screened. Of these, caprylic acid, peppermint oil and cinnamon oil had an efficacy against larvae of the *H. okamotoi* in in vitro trials. In the challenge trials, when each natural agent (2.5 g/kg basal diet) that was effective in vitro trials were also given to the fish in feed, caprylic acid and praziquantel prevented horizontal infection. Furthermore, the survival of groups treated with caprylic acid and praziquantel were significantly higher than the negative control (basal diet) and the other groups. Additionally, the number of matured parasites on the branchial cavity wall of fish, which was assumed to exist from the beginning of the challenge trials, decreased in the groups treated with caprylic acid and praziquantel. Our results show that

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caprylic acid has an anthelmintic efficacy against *H. okamotoi*.

Keywords: *Heterobothrium okamotoi*; Tiger puffer; Praziquantel; Essential oil; Caprylic acid; Anthelmintic efficacy

1. Introduction

The tiger puffer *Takifugu rubripes* is a high-value cultured fish in Japan (the market price is now around US$50/kg). Culture started in western Japan in the 1950s (Okamoto, 1963) and the annual production has exceeded 4,000 metric tons in recent years. They are cultured successfully in floating off-shore net pens, but many parasitic diseases have occurred often resulting in significant economic loss (Ogawa and Inouye, 1997a).

Among the parasites, the diclidophorid monogenean *Heterobothrium okamotoi* (Okamoto, 1963) is a problem because of its obvious pathogenicity and low susceptibility to chemicals. Ogawa and Inouye (1997c) described the infection cycle for this parasite. The free-swimming oncomiracidia emerge from eggs (6–10 days after spawning at 20°C), attach to the gill and grow on the gill filaments, then mature on the branchial cavity wall. Maturation of *H. okamotoi* takes approximately 49 days from attachment to the gill when the water temperature ranges between 16.8°C and 26.8°C, with an average of 21.1°C. To prevent infestation, cultured tiger puffers in a net pen have been treated routinely by bath treatments with formalin at intervals of about 20 days. However, this is considered to be harmful to the environment in general because used formalin is subsequently released into the sea. In recent years, the use of formalin for off-shore culture has been prohibited by law and bath treatment with hydrogen peroxide instead of formalin has become increasingly common. The impact on the environment when hydrogen peroxide is used and is released into the sea is thought to be relatively small. However, hydrogen peroxide treatment influences the viability of the fish in the summer season and bath treatment has limited effect against mature parasites embedded in the branchial cavity wall (Ogawa and Yokoyama, 1998). Also, the treatment requires additional labour and is stressful to the fish (Kim and Choi, 1998).

Parasiticides obtained from natural sources, which could be administered with the feed, would be tremendously convenient and safe for use. The best approach in determining the efficacy of any new drug or treatment is to compare the results obtained in trials with the new drug, a proprietary drug with known efficacy and untreated groups. To date, a drug effective against *H. okamotoi* has not been found. Among synthetic drugs, praziquantel and levamisole are parasiticidal against fish monogeneans (Schmahl and Mehlhorn, 1985; Schmahl and Taraschewski, 1987, respectively). Praziquantel and levamisole are generally used in human medicine: praziquantel to control trematode and cestode infection (Redman et al., 1996) and levamisole to control nematode infection (Robertson and Martin, 1993). Drugs used to control trematodes, cestodes and nematodes might also have an efficacy against monogeneans. In this study, these two synthetic drugs were tested for their efficacy against *H. okamotoi*. Addition-

ally, pyrantel pamoate and antimony sodium tartarate, which have an efficacy against nematodes (Klein et al., 1978) and trematodes (Sugiura, 1964) in humans, respectively, were also tested.

Two approaches exist when using natural agents to prevent parasites: strengthening the self-defense of fish by administering an agent as an immunostimulant (Kakuta and Kurokura, 1995) and using an agent that acts directly on the parasite. In this study, we made a screening test using the latter approach. Among natural agents, some essential oils, produced as steam distillates of plant materials, and caprylic acid, which is the constituent fatty acid of palm oil, butter and other edible oils, have been found to have a larvicidal activity against the dog-roundworm *Toxocara canis* (Nakamura et al., 1990; Kiuchi et al., 1987, respectively). In this study, three essential oils (orange oil, peppermint oil and cinnamon oil) and caprylic acid were tested for comparison with the synthetic drug (positive control) chosen above.

2. Materials and methods

2.1. Fish rearing method

Standard rearing methods with regards to flow rate, aeration and feeding rate for tiger puffer (Takii et al., 1995; Furuta et al., 1997) was employed for all the experiments. An aerated tank containing the tiger puffers were supplied with sand filtered and UV irradiated (approximately 50,000 $\mu W \cdot s/cm^2$; Flonlizer 4 L, Chiyoda Kohan, Japan) seawater (approximately 1.2 l/min/100 l volume of tank). The fish were fed the respective experimental dry pellet diet twice a day at a feeding rate of 1% body weight daily.

2.2. Source of fish and parasites

2.2.1. Uninfected fish

One-year-old tiger puffers (300 individuals), weighing 100–150 g, hatched at our laboratory were maintained in a 2-ton tank. The fish were fed a commercial expanded pellet diet (basal diet; Nippon Suisan, Japan). Gills, branchial cavity wall and skin surface of 10 fish sampled randomly were examined under a microscope to confirm that the fish were not infected by parasites before each experiment.

2.2.2. Parasite from infected fish

One-year-old tiger puffers (15 individuals), infected with *H. okamotoi*, were maintained in a 100-l polycarbonate tank. Uninfected fish with no record of previous infection with parasites were periodically mixed into the infected fish to maintain the parasites. The fish were fed the basal diet. Excised gills from these fish infected with parasite larvae (2–6 mm long) were used for in vitro studies. The oncomiracidia were obtained from eggs collected from the tank and were incubated in a 300-ml plastic beaker containing filtered seawater at 20°C for 8 days (Ogawa, 1998). The hatched oncomiracidia were used for challenge trials.

2.3. In vitro trials — anthelmintic drugs

The four drugs namely praziquantel (Bayer, Germany), levamisole (Aldrich, USA), pyrantel pamoate (Wako, Japan) and antimony sodium tartarate (Banyu, Japan) were tested in in vitro trials. Each drug was dissolved in filtered sea water at 20 mg l^{-1} and was made up to a volume of 50 ml. Excised gills infected with more than 30 parasites per gill arch were immersed with each 50 ml drug solution in a tissue culture dish at 20°C. The behavior and release of the larvae from the gills were observed every hour for 10 h under a microscope to determine the efficacy. Parasite-infected gills immersed in filtered seawater without the drug at the same temperature conditions acted as a control.

2.4. In vitro trials — natural agents

The four natural agents were tested by in vitro trials. Three of them are essential oils namely orange oil (Wako), peppermint oil (Kishida, Japan) and cinnamon oil (Kishida). The other is caprylic acid (Wako). Each agent was dissolved in filtered seawater at 80 mg l^{-1} and was made up to a volume of 50 ml. Excised gills infected with more than 30 parasites per gill arch were immersed with 50 ml of each natural agent solution in a tissue culture dish at 20°C. The behavior and the release of larvae from the gills were observed under a microscope for 1 h to determine the efficacy. As a control, excised gills were immersed in filtered seawater without the natural agent at the same temperature conditions.

2.5. Oral challenge trials — anthelmintic drugs

Praziquantel and levamisole, which were determined to be the most effective in in vitro trials were evaluated for their efficacy in in vivo challenge trials. Approximately 14,600 hatched oncomiracidia (body length: 200–300 μm) were put into a 100-l polycarbonate tank containing 24 1 seawater with aeration. Twenty uninfected fish weighing approximately 150 g were added. These fish were exposed to the oncomiracidia for 2 h at 20°C with no running seawater to complete the infection. After the exposure, fish were divided into five groups and were transferred to 100-l polycarbonate tanks.

Each group was fed the basal diet supplemented with the drugs at two doses, 2 g drug /kg basal diet and 4 g drug/kg basal diet throughout the trial. Each drug was mixed into the paste of basal diet and the pellet was formed by passing the paste through a disc pelleter (model F-5, Fuji Paudal, Japan). The diet pellets were dried at 40°C for 3 h and were stored at 4°C. A negative control group was fed the basal diet with no drug supplemented. Trials were made for 20 days. Water temperature ranged between 19.0°C and 21.0°C, with an average of 20.9°C, during the trial. The effectiveness of each treatment was determined by comparing the number and the growth (gain in body length of parasites) of the parasites on the gills.

2.6. Oral challenge trials — natural agents

2.6.1. Preparation of infected fish for the source of infection

Approximately 8,000 hatched oncomiracidia were put into a 100-1 polycarbonate tank containing 20 1 seawater with aeration. Forty uninfected fish weighing approxi-

mately 100 g were added. These fish were exposed to the oncomiracidia for 2 h at 18°C with no running seawater and then were transferred to a 500-1 polycarbonate tank where they were maintained for 63 days. Water temperature ranged between 17.3°C and 18.8°C with an average of 18.4°C. The fish were fed the basal diet. The parasites matured 50 days after infection and spawned parasite eggs were found attached to the drainpipe and the aeration tube. In order to prevent autoinfection of the next generation, these eggs were removed from the tank every 3 days. These infected fish were used for the source of infection and the assessment of therapeutic effect of the agents.

2.6.2. Oral therapy trials

Three agents (caprylic acid, peppermint oil and cinnamon oil) effective against *H. okamotoi* in in vitro trials were used in the challenge trials. Sixty parasite-free fish weighing approximately 122 g were divided into five groups and were maintained in a 100-1 polycarbonate tank. Fish were fed the experimental diets before the challenge trials. Each natural agent was adsorbed to the basal diet at 2.5 g agent/kg basal diet. The diet supplemented with 4 g praziquantel/kg basal diet was used as the positive control and the basal diet alone as the negative control. Seven days after starting to feed the experimental diets, six fish infected with mature *H. okamotoi* (114.6 ± 8.5 parasites per fish; mean $\pm$ S.E. from 10 fish randomly sampled) were added to each group. Each group was fed the respective diets continuously and the prevention of horizontal infection was assessed (prophylactic group) together with the assessment of the therapeutic effect on the newly added infected individuals (therapeutic group). The pectoral fin on the right side of the initially infected fish was removed to distinguish the therapeutic group.

Challenge trials were made for 72 days. Water temperature ranged between 16.6°C and 20.9°C, with an average of 18.3°C, during the trial. Four fish of each prophylactic group were randomly sampled and the number of infected parasites on the gill was counted 30 days after beginning the challenge. Mortalities were recorded daily and the parasites on the gills and branchial cavity wall of the dead fish were counted. At the end of the experiment, the number of parasites on the surviving fish was counted.

2.7. Statistical analysis

The number and the length of parasites on each fish were recorded. Results were analysed using the *t*-test and χ^2 analysis was used for the survival. A probability level of $P < 0.05$ was considered significant. All calculations were made using StatView statistical software (Abacus Concepts, USA).

3. Results

3.1. In vitro trials — anthelmintic drugs

A noticeable effect on parasite larvae was observed in media containing praziquantel and levamisole. The larvae treated with praziquantel contracted immediately (Fig. 1A)

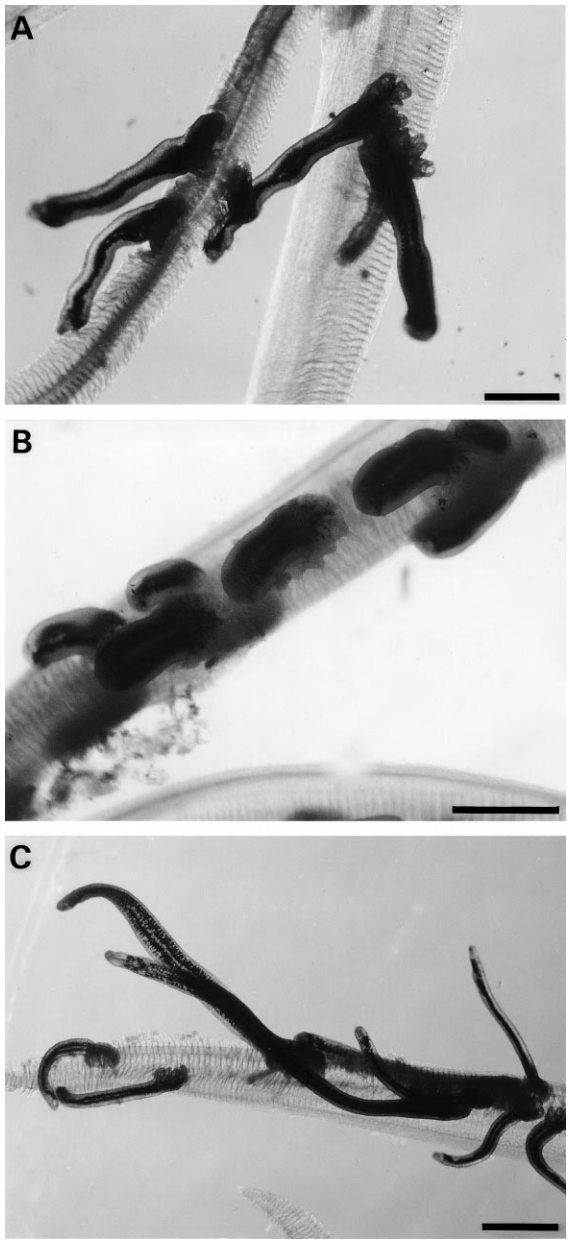

Fig. 1. In vitro incubation for 10 min of the larvae of *H. okamotoi* in seawater containing (A) 20 mg l^{-1} praziquantel, (B) 80 mg l^{-1} caprylic acid and (C) control. Contraction of the parasites in media containing praziquantel and caprylic acid occurred immediately. Scale bar = 1 mm.

and 100% of the larvae were released from the gills 1 h after starting the immersion. The larvae treated with levamisole contracted 5 min after starting immersion and 66.7% of

Table 1
Efficacy of dietary praziquantel and levamisole against *H. okamotoi* infection in tiger puffer ($n = 4$)
Fish were exposed to oncomiracidia and were used for the experiment.

Experimental group	Number of parasites per fish (Mean ± S.D.)	Parasite body length (mm) (Mean ± S.D.)
Negative control	317.3 ± 45.5	4.2 ± 0.7
2 g Praziquantel/kg basal diet	298.0 ± 105.0	2.1 ± 0.9 *
4 g Praziquantel/kg basal diet	104.0 ± 79.6 *	1.3 ± 0.1 *
2 g Levamisole/kg basal diet	296.8 ± 16.8	4.0 ± 0.9
4 g Levamisole/kg basal diet	302.5 ± 103.1	4.2 ± 0.9

* Significantly different from control at $P < 0.01$.

the larvae were released from the gills after 2 h and eventually 100% of the larvae dropped off from the gills after 3 h. Larvae in the control group (Fig. 1C) and in the media containing pyrantel pamoate or antimony sodium tartarate were not affected and did not drop off from the gills during the observation period.

3.2. Oral challenge trials — anthelmintic drugs

The number of parasites on the gills of fish fed with diet containing 4 g praziquantel/kg basal diet for 20 days was significantly fewer than the negative control (Table 1). The growth of parasites was also affected and the length of parasites from this group was significantly shorter than the negative control. No statistically significant differences in the number of parasites occurred between the 2 g praziquantel/kg basal diet group and the negative control group, but the length of parasites was significantly shorter. In contrast, no statistically significant differences occurred between the group treated with levamisole and the negative control group in either number or growth of the parasites.

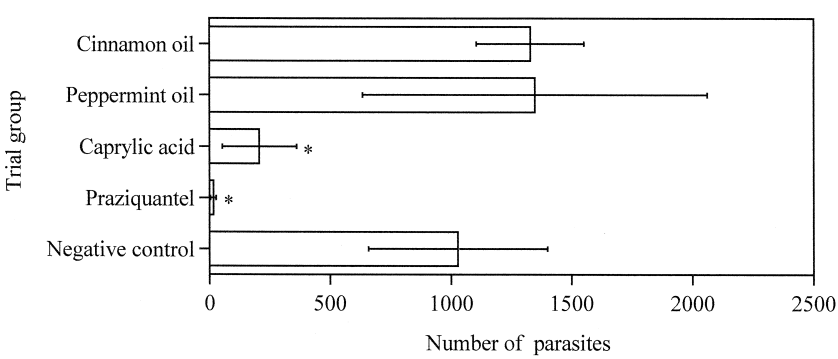

Fig. 2. Number of *H. okamotoi* per fish on challenged tiger puffer in each prophylactic group at 30 days after starting the challenge. Values are means and standard deviations of parasite larvae infected on the gills of four fish from each group. Significant differences from the negative control value are indicated by * ($P < 0.01$).

3.3. In vitro trials — natural agents

Affected parasite larvae were observed in media containing peppermint oil, cinnamon oil and caprylic acid. The larvae treated with these agents contracted immediately (Fig. 1B, caprylic acid group is shown) and 100% of the larvae were released from the gills 1 h after starting the immersion. Affected larvae were not observed in the control and media containing orange oil and the larvae of both groups did not drop off from the gills during the observation.

3.4. Oral therapy trials — natural agents

Thirty days after starting the challenge, the number of parasites was significantly fewer on the gills of fish examined to assess the prophylactic effect on the groups

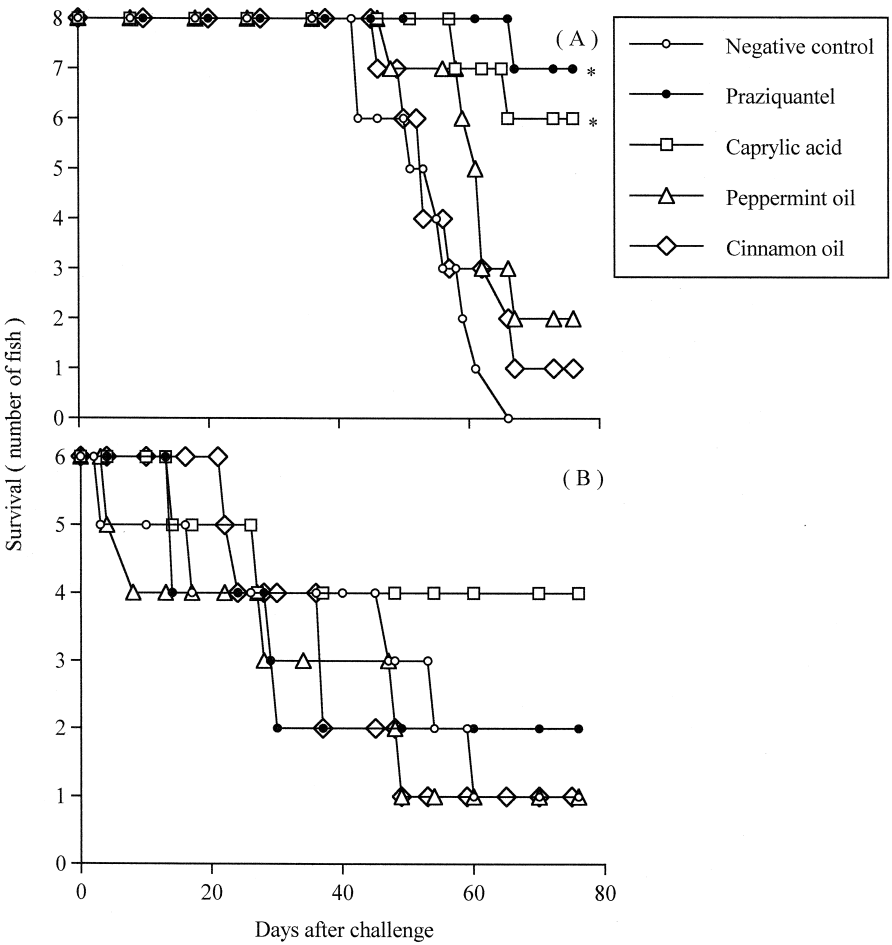

Fig. 3. Survival of (A) prophylactic group and (B) therapeutic group in challenged tiger puffer fed with a different diet. Significant differences from the negative control value are indicated by * ($P < 0.01$).

Table 2
Number of parasites infected on prophylactic group of each trial ($n = 8$)
Number of mature and larval parasites were counted separately. Values (per fish) are means and standard deviations.

Trial group	Mortalities			Surviving fish		
	N	Mature	Larvae	*N*	Mature	Larvae
Negative control	8	152.7 ± 93.1	2286.1 ± 866.9	0	–	–
Praziquantel	1	0	84	7	0	34.6 ± 44.0
Caprylic acid	2	119.0 ± 63.6	1190.5 ± 63.6	6	54.0 ± 18.1	352.0 ± 93.7
Peppermint oil	6	155.0 ± 98.0	1247.0 ± 375.9	2	189.5 ± 48.8	509.5 ± 12.0
Cinnamon oil	7	66.1 ± 50.9	2804.3 ± 1153.3	1	103	220

treated with caprylic acid and praziquantel than that in the negative control group (Fig. 2). However, no statistically significant difference was observed in groups treated with peppermint oil and cinnamon oil compared with the negative controls. No mature parasites were observed on the branchial cavity walls of fish examined.

The mortality of fish in the prophylactic group occurred earliest in the negative control group when 100% mortality was recorded by day 66 after starting the challenge (Fig. 3A). The survival of the groups treated with caprylic acid and praziquantel were significantly higher than the negative control. However, the differences in survival between the groups treated with essential oil and the negative control group were not statistically significant. Examination of the gills of fish that died revealed that the infection of parasite larvae was heavier in the negative control group and the group treated with cinnamon oil compared with the infection in the groups treated with caprylic acid, peppermint oil and praziquantel (Table 2). Infection of the surviving fish of the groups treated with caprylic acid and essential oil was low compared with the dead fish, but the number of mature parasites in the group treated with caprylic acid was apparently fewer than those in the groups treated with the essential oil. The group treated with praziquantel was not infected by any mature parasites and only a few larvae were found.

Table 3
Number of parasites infected on therapeuitic effect group of each trial ($n = 6$)
Number of mature and larval parasites were counted separately. Values (per fish) are means and standard deviations. The initial number of mature parasites per fish was 114.6 ± 8.5 (means ± S.E. from 10 fish randomly sampled).

Trial group	Mortalities			Surviving fish		
	N	Mature	Larvae	*N*	Mature	Larvae
Negative control	5	80.2 ± 23.5	2277.4 ± 1745.0	1	76	1463
Praziquantel	4	53.3 ± 13.2	9.3 ± 8.6	2	7.0 ± 4.2	3.0 ± 2.8
Caprylic acid	2	39.5 ± 20.5	299.0 ± 377.6	4	27.3 ± 19.2	203.5 ± 124.9
Peppermint oil	5	66.2 ± 14.7	809.4 ± 898.3	1	15	39
Cinnamon oil	5	49.0 ± 20.1	1876.6 ± 1394.8	1	1	38

In the therapeutic groups, the survival of group treated with caprylic acid was highest among the experimental groups (Fig. 3B). However, the difference between the group treated with caprylic acid and the negative control was not statistically significant ($P = 0.079$). As with the prophylactic groups, heavy infections were observed in dead negative control fish and in the group treated with cinnamon oil (Table 3). The number of mature parasites on the branchial cavity wall of the fish that survived in the groups treated with caprylic acid and praziquantel decreased from the initial infection rates and the difference between these groups and the initial infection rates was statistically significant ($P < 0.01$). Even though the survival of the groups treated with essential oil was as low as the negative control, there were few mature parasites on the branchial cavity wall of surviving fish in these groups. The number of larvae on the surviving fish of groups treated with caprylic acid, peppermint oil, cinnamon oil and praziquantel was less than on fish of the negative control.

4. Discussion

An effective agent against parasites, especially anthelmintic agents drived from natural sources for oral administration, has not been established in aquaculture. In net pen farms, as for the tiger puffer, an effective treatment with medicated feed would be much more practical than bath treatment. Moreover, agents from natural sources may be considered to be safe and ecoconscious. In making a screening experiment to discover new anthelmintic agents, using an effective positive control is necessary to ensure comparable and realistic results.

In this study, the anthelmintic efficacy of praziquantel against the diclidophorid monogenean *H. okamotoi* in tiger puffer was established and it was used as a positive control to compare the efficacy of natural anthelmintic agents in a challenge trial. Praziquantel is widely used to control trematode and cestode infections in mammals (reviewed by Martin et al., 1997) and the recommended oral dose of praziquantel is 20–40 mg/kg BW/day in humans (Kitahara, 1995). In fish, praziquantel has been used experimentally to treat monogenean disease by bath treatment (Schmahl and Mehlhorn, 1985; Buchmann et al., 1990). Recently, Kim et al. (1998) reported that the oral administration of praziquantel by intubation into the stomach of rockfish *Sebastes schlegeli* had an effect against *Microcotyle sebastis* (monogenea). In our experiment, the results clearly showed that feeding a diet containing praziquantel at a high dose to fish (40 mg drug/kg BW/day) had an anthelmintic efficacy against *H. okamotoi* larvae on gills. Also, the growth of the parasites was affected in both groups of high and low doses. Thus, the praziquantel in feed was chosen for the effective positive control.

In contrast, levamisole, which was apparently effective in in vitro trials, had no effect when administered in feed in our experiment. The recommended oral dose of levamisole is 2.5–5.0 mg/kg BW/day in humans to control gastrointestinal nematode infections (Miller et al., 1978). However, in this case, levamisole, which is hardly absorbed, may act directly on the parasites in the alimentary canal. Therefore, we tested levamisole at a high dose, the same dose as praziquantel, in this experiment so that the drug may reach the target. This drug has been also found to have parasiticidal activity against fish

monogeneans by in vitro assay (Schmahl and Taraschewski, 1987), but it has no efficacy by oral administration in rainbow trout *Oncorhynchus mykiss* (Tojo and Santamarina, 1998). *H. okamotoi* principally feeds on blood throughout its parasitic life (Ogawa and Inouye, 1997b). Although no work has been done on the pharmacokinetics of praziquantel and levamisole in fish, we assume that praziquantel reaches the parasites easier than levamisole via peripheral blood of the host and thus has an anthelmintic efficacy. Adams (1978) demonstrated that a single oral dose of [^{3}H]-levamisole of 150 mg/kg BW in humans produced a peak drug plasma level of 0.5 μg/ml after 2 h and unmetabolised drug represented only one-third of the total plasma radioactivity. Meanwhile, Andrews (1981) proved that a single oral dose of praziquantel of 46 mg/kg BW in humans produced a peak unchanged drug plasma level of 0.9 μg/ml after 2 h. The rate of unchanged drug in the plasma is apparently high for praziquantel compared with levamisole. These facts show that levamisole has more difficulty in reaching peripheral blood vessels compared with praziquantel in humans and the dose of levamisole may need to be increased if any therapeutic effect is to be seen.

Caprylic acid, cinnamon oil and peppermint oil had an effect against larvae of *H. okamotoi* by in vitro trials. The parasites contracted quickly and the degree of contraction was strong compared with the trials for the anthelmintic drugs, possibly due to the higher dose of the natural agents. Therefore, the in vitro trials for the natural agents were made for a short period compared with the trials for the anthelmintic drugs. In the groups treated with essential oil, fish survival was as low as in the negative control group and these oils had no clear effect in preventing horizontal infections in the challenge trials. However, the number of parasites on the surviving fish was fewer compared with those on the negative control fish. Higher doses of these essential oils may have a stronger effect but in previous experiments, the tiger puffers lost their appetite when the essential oils were absorbed at the rate of 3 g/kg diet (Hirazawa, unpublished observations). In mammals, the anthelmintic efficacy of essential oils against the larvae of the dog-roundworm has been investigated (Nakamura et al., 1990) and many oils, e.g., cinnamon oil, citronella oil, lemongrass oil, litsea cubeba oil and vetiver oil, were found to be nematocidal by in vitro assays. However, the anthelmintic effect by oral administration has not been investigated. These essential oils might also have an effect against fish parasites.

In the prophylactic group of caprylic acid in the challenge trial, the number of parasites on the gills was significantly fewer than the negative control at 30 days after starting the challenge. Moreover, the survival of the caprylic acid treated fish was higher than that of the negative control. Also, the number of the mature parasites on the surviving fish in the group treated with caprylic acid was nearly half of that in the groups treated with essential oil. Caprylic acid seemed to have an effect on the maturation of the parasites. The number of mature parasites, which were assumed to exist from the beginning of the challenge trials, decreased in the therapeutic group of the group treated with caprylic acid. The survival of this group was the highest and the number of the infected parasites on the surviving fish was fewer than those on fish in the negative control. These results showed that caprylic acid supplied orally has both a prophylactic and a therapeutic effect. The reduction in mortality of the therapeutic effect group suggests that caprylic acid had a higher effect in preventing mortalities compared

with praziquantel, although praziquantel was more effective in preventing horizontal infection. The oral administration of praziquantel at 40 mg/kg BW/day might have a toxic effect because the infection of the dead fish in this group was clearly low compared with the infection in other groups. The number of infected larvae on the surviving fish in the group treated with caprylic acid was not clearly reduced compared with the number in the groups treated with essential oil. Spawned parasite eggs were not removed in the oral challenge trials. These fish may have suffered autoinfection as the number of surviving fish per tank and the number of mature parasites per tank were more than for the groups treated with essential oil. We also assumed that the oral administration of both essential oils extended over a long period might have some effect in preventing autoinfection. Further studies with more fish are needed to determine the most effective dose in oral administration of caprylic acid. The larvicidal effect of caprylic acid has been investigated also for the dog-roundworm by in vitro assay (Kiuchi et al., 1987), but not in vivo. The effect of caprylic acid against parasites in fish has not been investigated. Therefore, this study is the first report of the anthelmintic efficacy of caprylic acid assessed by both in vitro and in vivo experiments in fish. No work has been done on the kinetics of caprylic acid in fish. Although medium-chain fatty acids, hydrolyzed from medium-chain triglycerides, appear in the peripheral blood of humans (Bach and Babayan, 1982), a study of the kinetics in fish is needed to clarify the reasons for the effect in vivo in fish.

In conclusion, our results show that caprylic acid has an anthelmintic efficacy against monogenean *H. okamotoi* infections in the tiger puffer. Caprylic acid might be effective against other fish monogeneans and if so, could be used widely in aquaculture as a safe and ecoconcious agent. Furthermore, our strategy to have an effective positive control and to determine both prophylactic and therapeutic effects has provided reliable results. This assay method may be used in general to discover new anthelmintic agents.

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