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NOTE

Antihelminthic potential of quinacrine and oxyclozanide against gill parasite *Microcotyle sebastis* in black rockfish *Sebastes schlegeli*

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ABSTRACT: The aim of this study was to assess the treatment potential of quinacrine and oxyclozanide against *Microcotyle sebastis* (Monogenea: Polyopisthocotylea) infection in cultured black rockfish *Sebastes schlegeli*. The oral administration of quinacrine led to a reduction in the mean abundance of *M. sebastis* infection in all quinacrine-treated groups, and the groups of fish administered quinacrine at 50, 100, and 200 mg kg^{-1} for 3 consecutive days showed a parasite mean abundance that was 50 to 30% lower compared to that of the control group, suggesting that quinacrine has a therapeutic potential against *M. sebastis*. Although oxyclozanide showed a very high *in vitro* killing activity, in oral administration experiments, only the groups of fish administered 200 mg kg^{-1} showed less than 50% mean abundance of *M. sebastis* compared to the control groups, suggesting that the absorption efficiency of orally administered oxyclozanide might be low in black rockfish and/or that *M. sebastis* might be less sensitive to orally ingested oxyclozanide. As praziquantel has been the sole therapeutic against *M. sebastis* infection in Korea for a long time, a broadening of available control measures is advisable in order to reduce the possible emergence of praziquantel-resistant *M. sebastis*. In our study, although quinacrine and oxyclozanide showed a therapeutic potential against *M. sebastis*, the treatment efficacy was not high enough to replace praziquantel. Thus, after investigations on the pathological effects and pharmacodynamics, use of quinacrine or oxyclozanide in combination with praziquantel may be considered as a way to prevent praziquantel resistance in *M. sebastis*.

KEY WORDS: Gill monogenean · Antihelminthics · Oral administration · Aquaculture

INTRODUCTION

The gill monogenean *Microcotyle sebastis* is a major cause of parasitic diseases in cultured black rockfish *Sebastes schlegeli* in Korea. As polyopisthocotyleans including *M. sebastis* are sanguinivorous, fish heavily infected with polyopisthocotyleans show signs of pale gill anemia (Thoney 1986, Thoney & Hargis 1991). Oral administration of praziquantel is effective in treating *M. sebastis* infection, and praziquantel has been used as the main therapeutic for treatment of *M. sebastis* infection in Korea (Kim & Cho 2000, Kim et al. 2001).

Recently, the development of anthelminthic resistance due to the widespread use of anthelminthic drugs has become a major problem not only in veterinary medicine but also in human medicine (Geerts & Gryseels 2000). Similarly, although the resistance of *M. sebastis* against praziquantel has not been experimentally demonstrated, recently, some aquaculturists have indicated that the efficacy of praziquantel in the treatment of *M. sebastis* infection is weakened

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compared to the early period of praziquantel use. Considering the possible development of a praziquantel resistance in *M. sebastis* as a result of long-term use, it may be necessary to expand the available therapies against *M. sebastis* infection by alternative therapeutics.

The acridine derivative quinacrine has been used as a drug for treating human protozoal diseases such as malaria and giardiasis, and human tapeworm infections such as *Hymenolepis nana* and *Taenia saginata* (Beaver & Sodeman 1952, Koul et al. 2000, Ehsanian et al. 2011). Although quinacrine intercalates into double-stranded DNA, the antiparasitic mechanism of quinacrine has not been clearly elucidated. In fish, *in vitro* killing activity of quinacrine against several ciliate species such as *Tetrahymena pyrifirmis*, *Ichthyophthirius multifiliis*, and *Philasterides dicentrarchi* has been reported (Griffin 1989, Tojo et al. 1994, Iglesias et al. 2002). However, there are no reports on the use of quinacrine for the treatment of helminthic diseases in fish.

The salicilanilide compound oxyclozanide has been used as an anthelmintic especially against fasciolasis and paramphistomes in sheep, cattle, and goats (Mooney et al. 2009, Rojo-Vázquez et al. 2012). The research on the use of oxyclozanide for treatment of fish parasitic diseases is sparse, and only 1 paper has described *in vitro* scuticocidal activity of oxyclozanide (Iglesias et al. 2002). The anthelminthic effect of oxyclozanide against *Neobenedenia girellae* (Capsalidae) and *Zeuxapta japonica* (Heteraxinidae) parasitizing amberjack *Seriola dumerili* was described in a patent (Japanese patent number WO2012002379), in which the possible treatment of *Microcotyle sebastis* with oxyclozanide was briefly mentioned. Thus, as far as we know, no scientific report has investigated the effect of oxyclozanide against *M. sebastis*. The aim of the present study was to assess the treatment potential of quinacrine and oxyclozanide against *M. sebastis* infection in cultured black rockfish.

MATERIALS AND METHODS

Fish

Black rockfish fingerlings weighing 7 g on average obtained from a local net-pen farm were transferred into 500 l tanks (n = 4; 150 fish tank^{-1}), and seawater was changed once a day. Fish were fed with commercial dried pellets and acclimatized at 21–22°C for 2 to 4 wk before being treated. One day prior to the drug treatment, 10 fish were randomly sampled: infection of *Microcotyle sebastis* on the gills was examined, and 100% prevalence was verified.

In vitro killing activity of quinacrine and oxyclozanide

Fish were killed by severing the spinal cord, and all gill arches were isolated. Individuals of *M. sebastis* on the gill filaments were carefully detached under a microscope, and kept in filtered seawater. Quinacrine was dissolved in filtered seawater, and oxyclozanide (Sigma) was dissolved in ethanol (stock solution 20 000 ppm) and then diluted with filtered seawater. The isolated *M. sebastis* were placed on 24-well plates with 10 worms well^{-1}, to which 50, 100, and 200 ppm of quinacrine or serially diluted oxyclozanide ranging from 25 to 0.4 ppm were added. In the case of oxyclozanide, the control wells contained ethanol at the same concentration as in the wells with 25 ppm oxyclozanide, or filtered seawater alone. The mortality of worms was examined at 30 min, 1 h, and 2 h post-exposure to each drug. Worms showing no response to a needle stimulation were considered dead.

Oral administration of quinacrine

The acclimatized fish were randomly divided into 10 groups (n = 12 fish group^{-1}) and were orally intubated using gastric tubes. Quinacrine dihydrochloride (Sigma) was dissolved in phosphate buffered saline (PBS), and the fish were intubated individually with 50 µl of quinacrine (50, 100, or 200 mg kg^{-1}) in PBS once on the first day (3 groups), or once a day for 2 consecutive days (3 groups), or once a day for 3 consecutive days (3 groups). The fish in the control group were intubated with 50 µl of PBS alone. Two days after the last administration, all fish in each group were sacrificed and *M. sebastis* on the gills were counted under a dissecting microscope.

Oral administration of oxyclozanide

In Experiment (Expt) 1, 48 fish were divided into 4 groups (n = 12 fish group^{-1}) and were orally intubated with 50, 100, or 200 mg of oxyclozanide (suspended in PBS) per kg of fish in a volume of 50 µl. In Expt 2, fish were divided into 4 groups and fed 200 mg of oxyclozanide per kg of fish once, twice over 2 d, or 3 times over 3 d. The fish in the control groups of both experiments were intubated with

50 µl PBS alone. *M. sebastis* on the gills were counted 2 d after the last administration.

Statistical analysis

Statistical significance was analyzed using SPSS for Windows. Data were analyzed using 1-way ANOVA followed by Tukey HSD post hoc test, and $p < 0.05$ was considered statistically significant.

RESULTS

In vitro killing activity of quinacrine and oxyclozanide

Quinacrine showed a low *in vitro* killing activity, and only 20% of *Microcotyle sebastis* were killed by exposure to 200 ppm of quinacrine for 2 h. On the other hand, *M. sebastis* was very sensitive to oxyclozanide, where 100% of the worms were killed within 30 min of exposure to 3.12 ppm, and within 1 h of exposure to 1.56 ppm.

Treatment efficacy of quinacrine

The prevalence of *M. sebastis* infection was 100% in the control group, 91.7% in the group administered quinacrine (50 mg kg^{-1}) once, 83.3% in the groups administered 100 mg kg^{-1} quinacrine twice, 200 mg kg^{-1} twice, and 50 mg kg^{-1} 3 times, and 100% in the other quinacrine-administered groups. All groups given quinacrine through an oral route with the various schemes showed reduced mean abundances of *M. sebastis* infection. However, only groups that were administered quinacrine at a dose of 50, 100, and 200 mg kg^{-1} once a day for 3 consecutive days showed less than a half of *M. sebastis* mean abundance compared to the control group. The group administered quinacrine at a dose of 200 mg kg^{-1} 3 times showed significantly lower mean abundance of *M. sebastis* infection compared to the control group (Fig. 1). A few fish in the groups fed 200 mg kg^{-1} for 2 or 3 consecutive days showed lethargy and black discoloration.

Treatment efficacy of oxyclozanide

In a preliminary experiment, fish orally administered with oxyclozanide at 15 or 30 mg kg^{-1} of fish showed very low treatment efficacy. Even fish administered 30 mg kg^{-1} once a day for 3 consecutive days showed only 10 to 20% reduction in the mean abundance of *M. sebastis*. Thus, we increased the dose in these experiments. In Expt 1, the prevalence of *M. sebastis* in the control, 50, and 100 mg kg^{-1} groups at the examination point was 100%, and 91.7% in the group administered 200 mg kg^{-1}. The mean number of *M. sebastis* in the groups administered various doses of oxyclozanide was reduced, and the group administered oxyclozanide at 200 mg kg^{-1} showed more than 50% reduction in *M. sebastis* abundance, which was statistically significant (Fig. 2A). In Expt 2, the prevalence of *M. sebastis* was 100% in the control group, 83.3% in the groups administered oxyclozanide (200 mg kg^{-1}) once and twice, and 66.7% in the group administered oxyclozanide 3 times. All groups administered oxyclozanide at 200 mg kg^{-1} once, twice, or 3 times showed significantly lower *M. sebastis* abundance than the control group; however, we found no significant differences among oxyclozanide-administered groups (Fig. 2B). Fish fed oxyclozanide (200 mg kg^{-1}) 3 times over 3 d showed no morphological or behavioral changes.

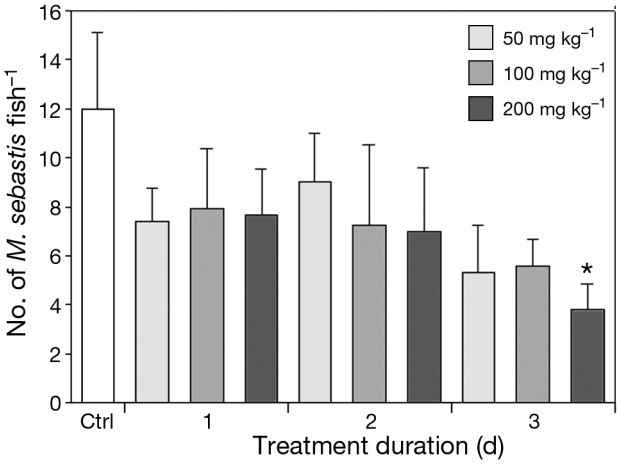

Fig. 1. Mean (+SE) abundance of *Microcotyle sebastis* on the gills of black rockfish *Sebastes schlegeli* fingerlings (n = 12 fish group^{-1}). Fish were orally intubated with 50 µl of quinacrine (50, 100, or 200 mg kg^{-1}) in phosphate buffered saline (PBS) once on the first day, or once a day for 2 or 3 consecutive days. Fish in the control group (Ctrl) were intubated with 50 µl of PBS alone. *Significant ($p < 0.05$) difference from the control group

DISCUSSION

Historically, quinacrine was primarily used for the control of malarial infection in humans, but was later

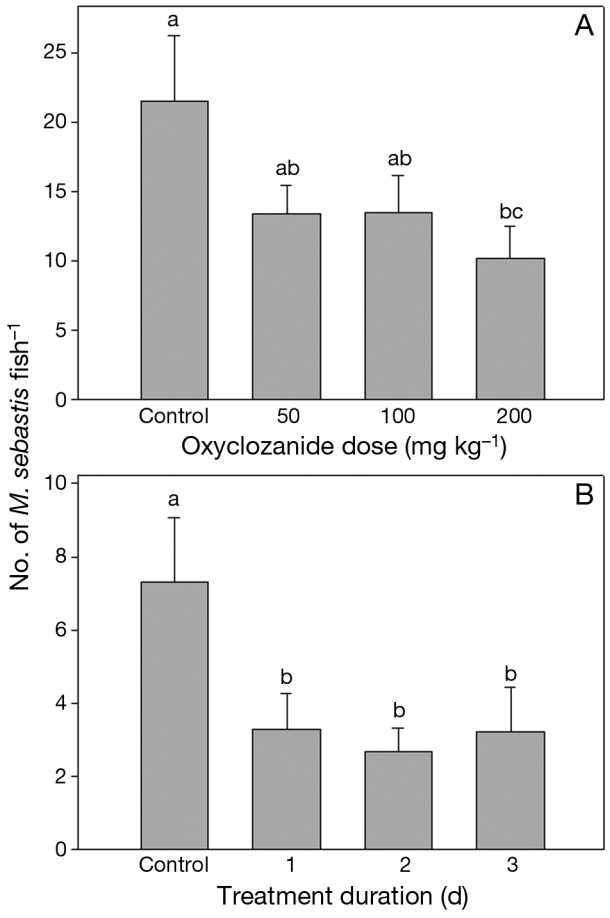

Fig. 2. Mean (+SE) abundance of *Microcotyle sebastis* on the gills of black rockfish *Sebastes schlegeli* fingerlings (n = 12 fish group^{-1}). Fish were administered (A) 50 µl of oxyclozanide (50, 100, or 200 mg kg^{-1}) in phosphate buffered saline (PBS) via an oral route, or (B) 50 µl of oxyclozanide (200 mg kg^{-1}) once on the first day, or once a day for 2 or 3 consecutive days. Fish in the control groups were intubated with 50 µl of PBS alone. Different letters over the bars represent significant differences ($p < 0.05$)

superseded by chloroquine. Although the treatment of cestode infections in mammals using quinacrine has been reported (Beaver & Sodeman 1952, Koul et al. 2000), to our knowledge, this is the first report on the use of quinacrine for the treatment of a fish helminthic disease. In the present study, although *in vitro* killing activity of quinacrine against *Microcotyle sebastis* was low, the oral administration of quinacrine led to the reduction of the mean abundance of *M. sebastis* infection in all quinacrine-treated groups, and the groups of fish administered quinacrine at 50, 100, and 200 mg kg^{-1} for 3 consecutive days reduced the mean abundance to 50 to 30% compared to that of the control group, suggesting that quinacrine has therapeutic potential against *M. sebastis* infection in black rockfish. However, 2 or 3 fish in the groups administered quinacrine at 200 mg kg^{-1} for 2 or 3 consecutive days also showed lethargy and black discoloration, suggesting that orally administered quinacrine at 200 mg kg^{-1} can induce adverse reactions in black rockfish fingerlings. Thus, further investigations on the safety level of quinacrine are needed, before the application of quinacrine for treatment of infectious diseases in black rockfish can be recommended.

Oxyclozanide showed a very high *in vitro* killing activity against *M. sebastis*; however, in *in vivo* oral administration experiments, only the groups of fish administered 200 mg kg^{-1} showed less than 50% mean abundance of *M. sebastis* compared to the control groups, suggesting that the absorption efficiency of orally administered oxyclozanide might be low in black rockfish and/or that *M. sebastis* might be less sensitive to orally ingested oxyclozanide.

Long-term use of a drug can lead to the emergence of drug-resistant parasites (Goven et al. 1980, Aaen et al. 2015) with resultant severe losses in productivity of aquaculture operations. Drug combinations can be a way to reduce the development of resistance (White 1998, Mitchison 2012, Shanks et al. 2015). As praziquantel has been the sole therapeutic against *M. sebastis* infection in Korea for a long time, a broadening of available control measures is advisable in order to reduce the possible emergence of praziquantel-resistant *M. sebastis*. In our study, although quinacrine and oxyclozanide showed therapeutic potential against *M. sebastis*, the treatment efficacy was not high enough to replace praziquantel. Thus, after investigations on the pathological effects and pharmacodynamics, use of quinacrine or oxyclozanide in combination with praziquantel could be considered as a way to prevent or delay an emergence of praziquantel-resistant *M. sebastis*.

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