Oral treatment trials on telescope fish (Carassius auratus) experimentally infected with Ichthyophthirius multifiliis (Fouquet, 1876)

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Abstract
Ichthyophthirius multifiliis Fouquet, 1876 is a ciliate parasite causing white spot disease and may cause high mortality in fresh water fish species. Telescope fish (Carassius auratus) 1.97 (± 0.51) g in weight were experimentally infected with I. multifiliis at 23 ºC water temperature. After that, 2 different dosages (24 and 36 mg/kg body weight) metronidazole, secnidazole and ornidazole were added to feed and administered to fish for 10 days. One day later, skin scrapes were taken from fish and the slides were investigated under the microscope. At the end of the experiment, no parasite was found in groups which received 24mg/kg metronidazole and ornidazole. All three of the chemotherapeutants were also efficacious at 36 mg/kg dosages for treatment of the disease.

Introduction
Ichthyophthirius multifiliis Fouquet, 1876, is a cosmopolitan ciliate parasite of fresh water fish species. The infection has been reported from virtually all areas where fishes are cultured, ranging from the tropics to the subarctic, and in feral fish populations from most continents (8).

I. multifiliis causes white spot disease, and has three life stages. Briefly, the trophont stage feeds in the host epidermis. Reaching a size of 0.4-0.8 mm, the trophont escapes from the epidermis and reaches the tomont stage, which swims in the water. The tomont encysts by producing a gelatinous capsule and attaches to the substrate (pond and aquarium bottom or a stone, plant and so on). The tomocyst undergoes numerous cell divisions, producing up to 200-2000 tomites, depending on the temperature. The tomites escape from the tomocyst into the water and become theronts, which are free-swimming and search for a host. Theronts penetrate the skin and feed on epithelium cells and blood, and then enlarge and become trophonts (Buchmann et al., 2001). In severe cases of white spot disease, there are usually large numbers of spots, and ulcers develop on the skin. The fins become frayed due to loss of tissue between the fin rays. Mucus production is increased. Finally it leads to death.

To date, many chemotherapeutants have been tested in various ornamental and food-producing species like rainbow trout (Oncorhynchus mykiss) (Rapp, 1995; Tojo et al.,

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1994), cardinal tetras (*Paracheirodon axelrodi*), blue gouramis (*Trichogaster trichopterus*) and clown loach (*Botia macracantha*) (Schmahl *et al.*, 1992), swordtails (*Xiphophorus helleri*), black mollies (*Poecilia sphenops*), and black neons (*Hyphessobrycon herbertaxelrodi*) (Schmahl, 1996). Some oral treatment studies with malachite green (Schmahl *et al.*, 1992) quinine (Schmahl, 1996), clopidol, amprolium hydrochloride (Shinn *et al.*, 2003), metronidazole, secnidazole and levamizole (Tojo and Santamarina, 2001) have also been published.

Currently, the most commonly used bath treatment in ornamental fish farms in Turkey employ malachite green and formalin. Although these compounds are efficacious against free-living stages of the parasite, it is ineffective against penetrating stages, so therefore for completely elimination of parasite multiple bath treatments are necessary. This in turn means that much time and energy are spent, so there is a real need to find an alternative oral treatment. An additional advantage of the use of oral treatments is that they tend to have much less of an effect on water quality (pH, oxygen concentration, activity of organic loading and so on) than bath treatments.

In this research, three oral treatments have been attempted to counteract *I. multifiliis* infections on the experimentally infected telescope fish (*Carrasius auratus*). These chemotherapeutants (metronidazole, secnidazole and ornidazole) were administered in two different doses, with the aim of determining the most efficacious compound and its concentration.

### Materials and Methods

#### Experimental fish

Telescope fish were obtained from a local ornamental fish farm (Ordas, Bergama, Izmir, Turkey). The fish were 1.97 g (± 0.51) in weight. The water temperature was thermostatically adjusted to 23 °C. The fishes were treated for other external parasites with formalin (300 ppm 30 m) and trichlorofon baths (1 ppm 1 hour at 20 °C 3 times in 10 days) (Schaperclaus, 1992) before being transferred to laboratory aquaria. The fish were fed once a day with commercial gold fish floating pellets. Groups of 20 fish were maintained in each aquarium (50x30x19 cm) for 4 weeks until the commencement of trials. Identical heaters and filters were used in each aquarium. Two replicate tanks were maintained for each chemotherapeutic group.

#### Experimental Infection

Goldfish massively infected with white spot were bought from local pet shop and the parasite was isolated from those fish. Trophonts were transferred to an aquarium with 20 uninfected telescope fish. After 2 weeks, moribund telescope fish were killed using an overdose of 2-phenoxyethanol. Trophonts were collected from these fish, and then incubated 18 hours in 23 °C in petri dishes, until theronts were released from tomocysts. Then, 1000 theronts per fish (a total of 20,000) were isolated and added to each experimental aquaria.

#### Preparation of drugs-food mixture

Nitroimidazoles are highly soluble in oil but they are poorly soluble in water. Therefore, each of the three nitroimidazoles used in this study (metronidazole, secnidazole and
ornidazole) were dissolved in sunflower oil. Commercial gold fish floating pellets were crumbled in a mortar. An oil–drug mixture was sprayed onto crumbled food, then was air-dried. The drugs used in this study (Table 1) are forbidden in food fish, but can be used in ornamental fish.

**Oral Treatment Trials**

The fish were fed standard floating food (without drugs) until white spots appeared clearly. Four days after the initial challenge, trophonts were observed on the fish by naked eye, but they were still relatively small. When white spots were more clearly visible, each group was fed floating food twice a day which contained identical dosages of drugs.

When the application of medicated food was completed (after 10 days), the fish were killed using an overdose of 2-phenoxyethanol (2 ml/L). Tap water was put on a slide, and a mucus sample of each fish was taken by making a smear of the body surface, fins and gills, and these were then were mixed with a drop of aquarium water on the slide. The samples were examined under a light microscope (100x and 400x).

**Results**

Signs of irritation were observed within ten minutes of initial exposure to 1000 theronts per fish. The fish aggregated on the bottom of the aquarium. They were also lethargic and reactionless. These symptoms disappeared twelve minutes later. Trophonts were observable to the naked eye on the fourth day after exposure to theronts. The following day, white spots were clearly visible.

All of the drugs used were efficacious, leading to a decrease the number of the parasite per fish and lower mortality rate (Table 2). Theront and trophonts were observed on the skin of only one fish in one of the two secnidazole 24 mg/kg groups. Therefore, this dose rate could be considered ineffective for treatment.

Ornidazole and secnidazole were effective in a shorter time (2-3 days) than metronidazole (3-6 days).

None of the drugs caused any evident toxicity to the fish.

**Discussion**

*Ichthyophthirius multifiliis* Fouquet, 1876, is a common and important ciliate parasitizing fresh water fish species throughout the world, and causes serious mortality of fish in fresh water facilities. Therefore, many trials have been carried out on the treatment of white spot disease. Most of these effective studies consist of bath applications with different chemicals.

**Table 1. The drugs and their dosages.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Doses</th>
<th>Trade Name</th>
<th>Form</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>36 and 24mg/kg B.W. day</td>
<td>Omisid®</td>
<td>Pills</td>
<td>Abdi Ibrahim</td>
</tr>
<tr>
<td>Secnidazole</td>
<td>36 and 24mg/kg B.W. day</td>
<td>Flagyl®</td>
<td>Pills</td>
<td>Eczacibasi</td>
</tr>
<tr>
<td>Ornidazole</td>
<td>36 and 24mg/kg B.W. day</td>
<td>Flagentyl®</td>
<td>Pills</td>
<td>Eczacibasi</td>
</tr>
</tbody>
</table>
including formalin, chloramine-T, acriflavine, copper sulphate, potassium permanganate and so on (Cross, 1972; Ekanem et al., 2004; Farley and Heckmann, 1980).

Even though successful results have been reported, bath treatments of billions of fish are impractical and also stressful to the fish. As a result of these limitations, treatments are being developed that can be administered in feed.

Although malachite green in feed has been reported to be effective in the treatment of white spot disease (Schmahl et al, 1996), it was reported that this compound may be teratogenic, carcinogenic and mutagenic (CFS, 2003; DH, 1999). Similarly, metronidazole has also been reported to be mutagenic in bacteria and carcinogenic in mice and rats at high doses when administered over long periods. Nevertheless, it has been used in the treatment of various diseases in human and animals. This indicates that it can be used with more safely than malachite green for treatment of certain fish diseases.

Metronidazole and secnidazole from the nitroimidazole group of compounds were reported to have anti-protozoal activity against *Ichthyobodo necator* and *Hexamita salmonis* in rainbow trout (*O. mykiss*) (Tojo and Santamarina, 1998a; Tojo and Santamarina, 1998b). However, these chemotherapeutants were reported to be ineffective for the treatment of Ichthyophthiriasis, although it did decrease the number of fish which were infected with trophonts in skin scrapes (Tojo and Santamarina, 2001). Especially, most of the fish were free of trophonts in secnidazole administrated groups. Water temperature was reported at 15 °C in that study, but unfortunately, however, this report did not describe how the drugs and food mixture were prepared (Tojo and Santamarina, 2001).

**Table 2.** The efficacy of secnidazole, metronidazole ve ornidazole against experimentally infestation of *Ichthyophthirius multifiliis* in telescope fish.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage (mg/kg BWday)</th>
<th>Total period of treatment (days)</th>
<th>Total fish for each group (n)</th>
<th>Temp. (°C)</th>
<th>Mean number if trophonts at the end of the treatment</th>
<th>Total fish mortality at the end of the treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>10</td>
<td>20</td>
<td>23</td>
<td>59.4 (± 15.4)</td>
<td>8</td>
</tr>
<tr>
<td>Secnidazole</td>
<td>24</td>
<td>10</td>
<td>20</td>
<td>23</td>
<td>PF</td>
<td>-</td>
</tr>
<tr>
<td>Secnidazole</td>
<td>36</td>
<td>10</td>
<td>20</td>
<td>23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>24</td>
<td>10</td>
<td>20</td>
<td>23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>36</td>
<td>10</td>
<td>20</td>
<td>23</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ornidazole</td>
<td>24</td>
<td>10</td>
<td>20</td>
<td>23</td>
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</tr>
<tr>
<td>Ornidazole</td>
<td>36</td>
<td>10</td>
<td>20</td>
<td>23</td>
<td>-</td>
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</tr>
</tbody>
</table>

PF = Parasite free fish  
± = trophonts and/or seen theronts ≤10
Tojo and Santamarina (2001) tested 16 chemotherapeutic agents against *I. multifiliis* on rainbow trout and reported that diethylcarbamazine, ketoconazole, nitroscanate, piperazine, secnidazole and metronidazole were particularly effective.

Nitroimidazoles are metabolized by liver in mammals (Nigrelli *et al.*, 1976). Most fish are wholly poikilothermic animals, so their metabolism depends on water temperature. In this study, water temperature was 23 °C. The half life of metronidazole, ornidazole and secnidazole in human plasma is approximately 7.5, 12-14, and 20 hours, respectively (Kaya *et al.*, 2002; Sweetman, 2002). Because of this, we preferred to feed twice in one day.

In the present study, 3 anti-protozoal chemotherapeutic agents from the nitroimidazole group were tested against white spot disease in telescope fish (*C. auratus*) at two different dosages. All of the drugs were effective, although some trophonts and theronts were seen in some fish of the groups which was administrated 24 mg/kg Secnidazole. It means that, although 24 mg/kg BW secnidazole decreased number of parasites, it is ineffective for the eradication of Ichthiyophthiriasis. In contrast, ornidazole and metronidazole were effective as low as 24 mg/kg BW dosage. This appears to be the first time that ornidazole has been tested against Ichthyophthiriasis to date. Dimetridazole is also another of the nitroimidazoles, but its half life in human serum is shorter than secnidazole and ornidazole (Rapp, 1995; Kaya *et al.*, 2002; Sweetman, 2002). When dimetridazole was administrated orally at doses of 50 and 250 mg/kg BW, the elimination half-life from serum were reported to be 2.56h and 2.69h, after both oral doses respectively, in laying hens (Rapp, 1995).

Rapp (1995) reported that 28 mg/kg BW dimetridazole is effective for the oral treatment of Ichthyophthiriasis in rainbow trout at 18 °C. However, Tojo and Santamarina (2001) reported that dimetridazole isn’t sufficiently efficacious for oral treatment of the disease at 15 °C water temperature. Metrinadozole and secnidazole were more effective (decrease number of the parasite on slide from skin scrap) than dimetridazole at the same study (2001). It was reported that much more dimetridazole is stored in tissues, and that the elimination rate is slower at higher temperatures and presumably its absorption rate was increased (Rapp, 1995). Therefore, metrinadozole and secnidazole could have been more effective in the present trials because of the temperature difference from that of Rodriguez and Fernandez’s study (Tojo and Santamaria, 2001).

As a result of this study, three nitroimidazoles (secnidazole, ornidazole and metronidazole) were found to be effective against white spot disease in telescope fish (*C. auratus*) at 23 °C at 36 mg/kg body weight 2 times in day for 10 days. Furthermore, ornidazole and metronidazole were also found to be effective at lower dose as 24 mg/kg BW for treatment of Ichthiyophthiriasis.
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References


