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Oral pharmacological treatments for parasitic diseases of rainbow trout *Oncorhynchus mykiss*. III: *Ichthyobodo necator*

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ABSTRACT: A total of 32 drugs were evaluated as regards their efficacy for oral treatment of *Ichthyobodo necator* infestation of rainbow trout. In preliminary trials, all drugs were supplied to infected fish at 40 g per kg of feed for 10 d. The majority of the drugs tested (1,3-di-6-quinolylurea, aminosidine, amprolium, benznidazole, bithionol, chloroquine, diethylcarbamazine, dimetridazole, diminazene aceturate, febantel, flubendazole, ketoconazole, levamisole, mebendazole, netobimin, niclosamide, niridazole, nitroscanate, nitroxynil, oxibendazole, parbendazole, piperazine, praziquantel, ronidazole, sulphaquinoxaline, tetramisole, thiophanate, toltrazuril and trichlorfon) were ineffective. Metronidazole and secnidazole were 100% effective (unlike the other nitroimidazoles tested, namely dimetridazole, benznidazole and ronidazole). The non-carbamate benzimidazole triclabendazole was likewise 100% effective.

KEY WORDS: Ichthyobodosis · Rainbow trout · Treatment · Drugs

INTRODUCTION

One of the chief causes of mortality among intensively farmed fish is infestation with ectoparasites such as the flagellate *Ichthyobodo necator*. Ichthyobodosis is probably the most frequent external flagellate parasitosis among farmed fish, notably in young salmonids including rainbow trout. Heavily infected fish suffer high mortalities over a very short period due to rapid multiplication of the parasite and associated damage to the external epithelium. The most conspicuous early symptoms are excessive secretion of mucus and greyish white slime on the skin, while longer-term infestation may lead to extensive epidermal erosion and ulcer formation (Ghittino 1985, Stoskopf 1993).

In recent years, there has been little progress in the pharmacological treatment of ichthyobodosis, and only a small number of drugs have been shown to have anti-*Ichthyobodo* activity. In previous studies, we have screened a series of drugs for anti-*Ichthyobodo* efficacy in bath treatment. Of the 40 drugs tested, the only one that proved 100% effective was bithionol at 25 mg l^{-1}, administered for 3 h daily on 2 consecutive days (Tojo 1993, Tojo et al. 1994a, b, c).

There have been a number of reports of the efficacy of oral pharmacological treatments against ectoparasitoses. For example, malachite green in feed is highly effective for the treatment of the cutaneous ectoparasite *Ichthyophthirius multifiliis* in ornamental fish (Schmahl et al. 1992), and is easily administered in this form. Orally administered diflubenzuron has proved effective against infections of *Salmo salar* by the copepod *Lepeophtherius salmonis* ('salmon lice') (Wallace et al. 1997). Ivermectin is likewise widely used as an anti-lice treatment in Atlantic salmon farms, and its derivatives also show such activity (Kerry & Smith 1997).

Here, we report a screening study of possible oral pharmacological treatments of ichthyobodosis in rainbow trout. A total of 32 drugs were tested at high dosages (40 g per kg of feed, for 10 d). Oral treatments

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have a number of advantages, including ease of administration and the fact that there is no need to handle the fish.

MATERIALS AND METHODS

Fish. Rainbow trout *Oncorhynchus mykiss* Walbaum (weighing 16 g at the start of the experiments) were obtained from a local fish farm (Piscifactorías Coruñesas S.A., Carballo, A Coruña, Spain) and acclimatized for at least 36 h before assay in a 350 l tank with aeration and continuous flow of water (15 ± 3°C, pH 6.5 ± 0.5) from a nearby spring. The natural light-dark cycle was simulated (14–16 h light:8–10 h dark). Fish were fed daily with a commercial feed.

Infestation. At least 50% of the fish used in each assay showed high-intensity infestation by *Ichthyobodo necator* (Henneguy 1883), in some cases as a result of natural infestations contracted on the fish farm, in other cases following experimental infestation. Parasite-free fish were experimentally infested by holding them for 15 to 20 d in a 350 l tank that also contained fish showing high-intensity infestation (40 uninfected fish to 20 infected fish). Twenty fish were then sampled at random for determination of infestation intensity (see below). Since infestation intensity was in all cases high in less than 10 of the 20 fish, the experimental infestation period was extended by 7 d, after which time infestation intensity was determined again. This proved sufficient to achieve high-intensity infestation in at least 50% of fish.

Determination of infestation intensity. Fish were anaesthetized by bathing in MS-222 (Sandoz; 0.05 g l^{-1}) until respiration became weak. A mucus sample was then taken by gently scraping the body surface. The sample was mixed with 3 drops of water on a slide, coverslipped and examined with a light microscope (400×). Infestation intensity was recorded on a 5-point scale, after examination of a sample area of 24 × 32 mm, as follows: 'zero' (–), *Ichthyobodo necator* not detected in the sample; 'minimal' (+/–), only 1 individual of *I. necator* detected in the sample; 'low' (+), more than 1 individual of *I. necator* detected in the sample, the average number per microscope field being less than 10; 'moderate' (++), average number of individuals per microscope field 10 to 50; 'high' (+++), average number of individuals per microscope field more than 50.

Drugs and assay design. The drugs tested in the study are listed in Table 1. Each drug was assayed in 20 infected fish maintained in an 80 l tank with continous flow (5 l min^{-1}). A simultaneous control assay (also of 20 fish; identical treatment, but without drug) was performed for each drug. Tank conditions (water source, flow, aeration, pH, temperature, light/dark cycle) were identical to those during the acclimatization period. The treated fish received feed containing 40 g per kg of drug for 10 d. In all cases feed was supplied at 2% of total body weight per day. Throughout the assay period the fish were monitored regularly to ensure that they were eating the food, and to check for signs of toxicity. Twenty-four hours after the end of the assay (i.e. 11 d after the start), intensity of infestation of all fish was determined as above. Drugs found to be effective at the screening dosage were subsequently tested at lower dosages and/or duration (see 'Results').

RESULTS

Of the 32 drugs tested, 29 were not effective (i.e. did not completely eliminate infection); the results for these

Table 1. Drugs used in the present study, showing manufacturer, brand name and form of presentation. p.p.: pure product

Drug	Brand name	Form	Manufacturer
1,3-di-6-quinolylurea	p.p.	Powder	Bayer
Aminosidine	Gabbrocol	Injectable	Vetern S.P.A.
Amprolium	Prolsal	Powder	Iteve
Benznidazole	p.p.	Powder	Roche
Bithionol	p.p.	Powder	Syva
Chloroquine	p.p.	Powder	Cidan
Diethylcarbamazine	p.p.	Powder	Cidan
Dimetridazole	p.p.	Powder	Msd-Agvet
Diminacene	Berenil	Granulate	Hoechst
Febantel	p.p.	Powder	Bayer
Flubendazole	p.p.	Powder	Esteve
Ketoconazole	p.p.	Powder	Janssen
Levamisole	p.p.	Powder	Ovejero
Mebendazole	p.p.	Powder	Esteve
Metronidazole	Flagyl	Powder	Rhone Mérieux
Netobimin	p.p.	Powder	Ovejero
Niclosamide	Fugotenil	Pills	Uriach
Niridazole	Ambilhar	Pills	Janssen
Nitroscanate	Lopatol 500	Pills	Ciba-Geigy
Nitroxynil	p.p.	Powder	Ovejero
Oxibendazole	p.p.	Powder	Syva
Parbendazole	p.p.	Powder	Smith Kline
Piperazine	Pipersol	Granulate	Sobrino
Praziquantel	Droncit	Pills	Bayer
Ronidazole	p.p.	Powder	Sobrino
Secnidazole	p.p.	Powder	Rhone Mérieux
Sulphaquinoxaline	p.p.	Powder	Smith Kline
Tetramisole	p.p.	Powder	Ovejero
Thiophanate	p.p.	Powder	Uriach
Toltrazuril	p.p.	Powder	Bayer
Trichlorfon	Neguvón	Powder	Bayer
Triclabendazole	p.p.	Powder	Ciba-Geigy

Table 2. Results of the assays that proved ineffective. For each drug (in all cases administered at 40 g per kg of feed for 10 d), infection intensity 24 h post-treatment (as evaluated by examination of body scrapings; see text) is shown for each of the 20 fish included in the assay for that drug. Infection intensity: +++, high; ++, moderate; +, low; +/–, minimal; –, zero (i.e. no *Ichthyobodo necator* detected in body scrapings); nd: not determined

Drug (40 per kg feed for 10 d)	Trout number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1,3-di-6-quinolylurea	+++	+++	+++	+++	+++	++	+	+	–	–	–	–	–	–	–	–	–	–	–	–
Aminosidine	+++	+++	++	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Amprolium	+++	+++	+++	+++	+++	++	++	+	+	+	+	–	–	–	–	–	–	–	–	–
Benznidazole	+++	++	++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Bithionol	+++	+++	+++	++	++	++	++	++	++	+	–	–	–	–	–	–	–	–	–	–
Chloroquine	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	+	+	+
Diethylcarbamazine	+++	++	+	+/–	+/–	+/–	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–
Dimetridazole	+++	+++	+++	+++	+++	++	++	++	++	++	+	+	+	–	–	–	–	–	–	–
Diminazene	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	+	+	+	–	–	–	–	–
Febantel	+++	+++	+++	+++	+++	+++	+++	++	++	+	+	–	–	–	–	–	–	–	–	–
Flubendazole	+++	++	++	+	+	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ketoconazole	+++	+++	+++	+++	+++	+++	++	++	++	+	–	–	–	–	–	–	–	–	–	–
Levamisole	+++	+++	+++	++	++	+	+	+	+	+/–	+/–	+/–	–	–	–	–	–	–	–	–
Mebendazole	+++	+++	+++	+++	+++	+++	++	++	++	++	+	+	+/–	+/–	–	–	–	–	–	nd
Netobimin	+++	+++	+++	++	++	++	+	+	+	+	+	+	+	+/–	+/–	–	–	–	–	–
Niclosamide	+++	+++	+++	+++	+++	++	++	++	++	++	++	+	+	+	+	–	–	–	–	–
Niridazole	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	–	–	nd
Nitroscanate	+	+	+	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Nitroxynil	+++	+++	+++	+++	+++	++	++	++	+	+	+	–	–	–	–	–	–	–	nd	nd
Oxibendazole	+++	+++	+++	+++	++	++	+	+	+	–	–	–	–	–	–	–	–	–	–	–
Parbendazole	+++	+++	+/–	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Piperazine	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	nd	nd
Praziquentel	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	+	+	+	+	–	–	–	–	–
Ronidazole	+	+/–	+/–	+/–	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Sulphaquinoxaline	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	++	+	–	–	–	–	–	–	–
Tetramisole	++	++	+	+	+	+	+	+	+/–	–	–	–	–	–	–	–	–	–	–	–
Thiophanate	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	+	+	+	+	+	–
Toltrazuril	+++	+++	+++	+++	+++	+++	+++	+++	++	++	++	+	+	–	–	–	–	–	–	–
Trichlorfon	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+	–	–	–	–

drugs are listed in Table 2. The results for the 3 effective drugs (metronidazole, secnidazole and triclabendazole) are listed in Table 3; both eliminated infection in all 20 fish included in each assay. Metronidazole was effective only at the screening dosage (40 g per kg feed for 10 d), while secnidazole was effective at 20 g per kg feed for 2 d, and triclabendazole at 40 g per kg feed for 5 d (Table 3). None of the 3 effective drugs had evident toxic effects.

Table 3. Results of the assays for drugs that proved effective. For each drug and each dosage tested, infestation intensity 24 h after the end of the assay (as evaluated by examination of body scrapings; see text) is shown for each of the 20 fish included in each assay. Results of control assays are also shown. Infestation intensity: +++, high; ++, moderate; +, low; +/–; minimal; –, zero (i.e. no *Ichthyobodo necator* detected in body scrapings). The initial prevalence of infestation was at least 50% in all groups

Drugs	Doses (g kg^{-1})	Days	Trout number 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Metronidazole	40	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	+++	+++	++	++	++	++	++	++	+	+	+	–	–	–	–	–	–	–	–	–
	20	10	+++	+++	++	++	++	++	++	+	–	–	–	–	–	–	–	–	–	–	–	–
Secnidazole	40	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	+++	+++	+++	+++	+++	+++	++	++	++	+	+	+	+/–	–	–	–	–	–	–	–
	20	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	+++	+++	+++	+++	+++	+++	+++	++	++	++	+/–	–	–	–	–	–	–	–	–	–
	10	2	+++	++	++	+	+	+/–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Triclabendazole	40	10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+
	40	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	–	–	+++	+++	+++	+++	+++	+++	+++	+++	++	+	+	+/–	–	–	–	–	–	–	–	–

DISCUSSION

None of the 32 drugs tested showed evidence of toxicity to rainbow trout at the dosages used. Of the 32 drugs, 14 have been tested previously for efficacy against ichthyobodosis in bath treatment, while the remaining 18 have not been assayed previously against *Ichthyobodo necator* (though they have been assayed against other internal or external fish parasitoses). The 18 drugs not tested to date are diethylcarbamazine, dimetridazole, febantel, flubendazole, levamisole, mebendazole, netobimin, nitroscanate, nitroxynil, oxibendazole, parbendazole, piperazine, praziquantel, ronidazole, secnidazole, tetramisole, thiophanate and triclabendazole.

Of these 18 drugs, the only ones to show anti-*Ichthyobodo* activity in the present study were secnidazole and triclabendazole. Secnidazole was 100% effective at dosages as low as 20 g per kg of feed for 2 d, and triclabendazole at dosages as low as 40 g per kg of feed for 5 d. Both drugs have been tested previously against protozoan parasites of fish, and have been assayed by us in a study of possible oral pharmacological treatments of *Hexamita salmonis* infection of rainbow trout (Tojo & Santamarina 1998). In this latter study, secnidazole was 100% effective at doses as low as 2 g per kg of feed for 2 d; triclabendazole was very nearly 100% effective at the screening dosage (40 g per kg of feed for 10 d), a single parasite being detected in the intestine of one of the 20 fish included in the assay. The greater activity of secnidazole may be related to the fact that this drug has a longer half-life than other nitroimidazoles (Dupouy Camet 1994).

Triclabendazole is known to have anthelmintic activity, and is used as an anthelmintic in livestock; the results of the present study, indicating that it also shows antiprotozoal activity, require confirmation. Other drugs, such as albendazole, show both anthelmintic and antiprotozoal activity (Tojo & Santamarina 1998).

The present results also indicate that metronidazole is 100% effective against *Ichthyobodo necator*, although only at the screening dosage (40 g per kg of feed for 10 d). In our previous study of effectiveness against *Hexamita salmonis* (Tojo & Santamarina 1998), metronidazole was effective at doses as low as 5 g per kg of feed for 2 d (again lower activity than secnidazole). Another previous study found that bath treatment of *Octopus bimaculoides* with metronidazole (200 mg l^{-1} for 4 d) was not sufficient to eliminate *I. necator* infection (Forsyte et al. 1991).

In our previous study of possible bath treatments (Tojo et al. 1994c), neither trichlorfon and niridazole (200 mg l^{-1} for 3 h) nor niclosamide (3 mg l^{-1} for 3 h) were effective against *Ichthyobodo necator*. These drugs were likewise ineffective in the present study. Neither in this study nor in previous studies have we observed signs of toxicity of any of these drugs.

We have previously screened amprolium, chloroquine, ketoconazole, sulphaquinoxaline and toltrazuril (in all cases at 200 mg l^{-1}), together with 1,3-di-6-quinolylurea and diminazene aceturate (in both cases at 100 mg l^{-1}) and bithionol (at 35 mg l^{-1}), for efficacy in bath treatment (3 h) against *Ichthyobodo necator* (Tojo et al. 1994b). The only drug which was effective was bithionol (25 mg l^{-1} for 3 h on 2 consecutive days). In the present study, however, neither bithionol nor any of these drugs was effective. Note also that none of these drugs showed toxic effects in the present study, despite our previous finding (Tojo et al. 1994b) that both bithionol at 35 mg l^{-1} and diminazene aceturate at 100 mg l^{-1} have toxic, and in some cases lethal, effects.

In conclusion, most of drugs tested in the present study are clearly of no value for oral-route treatment of ichthyobodosis in rainbow trout, given that the screening dosage was already very high. The only drugs which were effective were metronidazole, secnidazole and triclabendazole. In all 3 cases, however, the required dose was too high to be economically viable, except possibly in situations in which bath treatment is not possible.

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