Anthelmintic activity of benzimidazoles against *Gyrodactylus* sp. infecting rainbow trout *Oncorhynchus mykiss*

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ABSTRACT: Various anthelmintics belonging to the pharmacological group of benzimidazoles (oxibendazole, thiabendazole, albendazole, oxfendazole, flubendazole, mebendazole, parbendazole, fenbendazole and triclabendazole) were tested and compared for their *in vitro* and *in vivo* activity against an infection of rainbow trout *Onchorhynchus mykiss* by *Gyrodactylus*. The trout were also observed for signs of toxic reaction to the drugs. Oxibendazole, albendazole, mebendazole, parbendazole, fenbendazole and triclabendazole, despite their insolubility, showed anti-*Gyrodactylus* activity *in vivo* that increased with time of exposure to the drug. Complete efficacy (100 % reduction) with no toxic effects was achieved only by fenbendazole (1.5 mg l^{-1}) and triclabendazole (25 mg l^{-1}) over 12 h. In our tests, thiabendazole, oxfendazole, and flubendazole were totally ineffective; oxibendazole and albendazole were less than 100 % effective and were toxic; mebendazole and parbendazole were non-toxic but less than totally effective.

INTRODUCTION

Infestations by homoxenous parasites of fish are favoured by intensive farming. Monogeneans of the genus *Gyrodactylus*, in particular, can by mass infestation cause serious pathogenies and even fish mortality [e.g. blindness by corneal infestation or asphyxia after bronchial infestation (Ghittino 1985)]. This parasite undergoes both sexual and asexual reproduction (Euzeby 1971, Harris 1989), which makes it difficult to treat, since a single remaining individual may lead again to massive infestation.

Certain commercial products such as formaline and malachite green, used heretofore in the control of this and other ectoparasites, are unsatisfactory due to their toxicity and low specificity. Various families of drugs have been tested for anthelmintic activity against several monogeneans infesting different species of fish. The activity of products such as toltralzuril (Schmahl & Melhorn 1988), praziquantel, trichlorphon, levamisol, and niclosamide (Schmahl &

Taraschewski 1987), and closantel, neguvon, bithionol, nitroscanate, and niclofolan (Santamarina et al. 1991), has already been proven. In the benzimidazole group, tests have been mostly limited to mebendazole, which despite its insolubility is of proven efficacy (Goven & Amend 1982). The present work reports the potential anti-*Gyrodactylus* activity of different benzimidazoles against infestations of rainbow trout.

MATERIALS AND METHODS

Tests were carried out on rainbow trout *Oncorhynchus mykiss* (from Piscifactorias Coruñesas, Carballo, La Coruña, Spain) infested with *Gyrodactylus* sp. Specimens taken from the infested rainbow trout were identified by G. Malmberg (University of Stockholm, Sweden) as *Gyrodactylus salaris* Malmberg, 1957 (pers. comm.). Prior to experimentation, the trout were acclimatised for at least 36 h in 10 l plastic tanks (Letica, Barcelona, Spain) with a constant flow of water (15 °C, pH 6.5) from a spring close to the laboratory. Oxygen from an air pump was bubbled

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Table 1. *Oncorhynchus mykiss*. Treatment against *Gyrodactylus* sp. with different anthelmintics. (+++) Very turbid; (++) moderately turbid; (+) slightly turbid; p.p.: pure compounds

Anthelmintic	Commercial name	Manufacturer	Presentation	Turbidity at 200 mg l^{-1}
Oxibendazole	p.p.*	Syva	Powder	++
Thiabendazole	Triasox®	Andreu	Suspension	+
Albendazole	Oversol®*	Ovejero	Suspension	++
Oxfendazole	p.p.*	Syntax	Powder	++
Flubendazole	p.p.*	Dr. Esteve	Powder	++
Mebendazole	p.p.*	Dr. Esteve	Powder	+++
Parbendazole	p.p.*	Smith Kline	Powder	++
Fenbendazole	Panacur®	Procida Ibérica	Powder	+++
Triclabendazole	Fasinex®*	Ciba-Geigy	Suspension	++

* Drugs donated by manufacturers

through the water. A suitable commercial feed was supplied daily. The anthelmintics studied are listed in Table 1.

In vitro and *in vivo* tests were carried out as previously described (Santamarina et al. 1991). For the *in vitro* test, groups of 8 parasites were obtained from rainbow trout, placed in Petri dishes with water containing different concentrations of anthelmintic, and observed 15, 30 and 60 min later to determine the number of dead (immobile) helminths. Control groups of 8 specimens per Petri dish in drug-free water were also studied for 60 min. For the *in vivo* test, groups of 5 *Gyrodactylus*-infested rainbow trout were tested for 3 and 12 h by bathing in 10 l of non-circulating water containing different concentrations of anthelmintic, after which the water was renewed. *Gyrodactylus*-infested control groups were kept in drug-free water under the same conditions. For both groups, pelvic fins were removed 24 h after treatment and viewed under a stereomicroscope to count the number of

parasites. Toxicity to the fish was described on a scale ranging from alteration of natatory movements to death. Significant differences between the groups were revealed using the Mann-Whitney-Wilcoxon test ($Z \leq 0.05$), and the percentage of reduction was calculated in each case.

RESULTS

Results for *in vitro* and *in vivo* treatments are summarized in Tables 2 & 3.

Oxibendazole: This was totally ineffective both *in vitro* and *in vivo* at concentrations up to 200 mg l^{-1} acting over 3 h, but over 12 h, 25 mg l^{-1} was 86.4 % effective *in vivo*. This dosage level was toxic to fish.

Thiabendazole: A higher concentration was possible *in vitro* due to the lower turbidity of this product compared to the other benzimidazoles; efficacy was 25 % at 300 mg l^{-1}. Nevertheless, no monogeneans were

Table 2. *Oncorhynchus mykiss*. Treatment against *Gyrodactylus* sp.: *in vitro* results for the studied drugs: Control lots were unaffected after 60 min in water free of drugs but otherwise under the same conditions as the treated lots

Drug	Dose (mg l^{-1})	Exposure time (min)	Percentage reduction	Number of *Gyrodactylus* sp. Initial	Dead
Oxibendazole	200	60	0	8	0
Thiabendazole	300	60	25	8	2
	150	60	12.5	8	1
Albendazole	200	60	0	8	0
Oxfendazole	200	60	0	8	0
Flubendazole	200	30	100	8	8
	50	60	100	8	8
Mebendazole	15	60	0	8	0
Parbendazole	200	60	0	8	0
Fenbendazole	12.5	60	0	8	0
Triclabendazole	4	15	100	8	8
	2	60	0	8	0

Table 3. *Oncorhynchus mykiss*. Treatment against *Gyrodactylus* sp.: *in vivo* results. Presence and absence of signs of toxicity indicated by + and − respectively

Drug	Dose (mg l^{-1})	Exposure time (h)	Percentage reduction[a]	Signs of toxicity
Oxibendazole	200	3	·	−
	25	12	86.36	+
Thiabendazole	100	3	·	+
	10	3	·	+
Albendazole	200	3	·	−
	25	12	95.45	+
Oxfendazole	200	3	·	−
	25	3	·	−
Flubendazole	200	3	·	−
	25	12	·	−
Mebendazole	100	3	·	−
	25	12	95.45	−
Parbendazole	200	3	·	−
	25	12	90.75	−
Fenbendazole	25	3	·	−
	25	12	100	+
	12.5	12	100	−
	6.2	12	100	−
	1.5	12	100	−
	0.77	12	·	−
Triclabendazole	25	3	·	−
	25	12	100	−
	12.5	12	96.66	−
	6.2	12	·	−

· No significant difference ($Z \leq 0.05$)
[a] Efficacy calculated as percentage of reduction = $100 (1 - N_t/N_c)$, where N_t and N_c = no. of *Gyrodactylus* host^{-1} counted on the fins of treated hosts (N_t) and control hosts (N_c) kept in water free of drugs but otherwise under the same conditions

killed *in vivo* and concentrations of 10 mg l^{-1} over 3 h were highly toxic to fish.

Albendazole: *In vitro* a concentration of 200 mg l^{-1} failed to kill any helminths in 3 h, and *in vivo* this concentration was also totally ineffective, but at a dosage level of 25 mg l^{-1} over 12 h efficacy was 95.45 %. Signs of toxicity to fish were observed.

Oxfendazole: No anthelmintic activity was observed either *in vitro* or *in vivo* at the maximum dose of 200 mg l^{-1}.

Flubendazole: Though all monogeneans were killed by 50 mg l^{-1} *in vitro*, this concentration was totally ineffective *in vivo* despite 12 h exposure to the drug.

Mebendazole: To enable observation of the monogeneans a lower concentration was required *in vitro* than *in vivo*, due to the high turbidity caused by the drug. Specifically, *in vitro* the maximum concentration possible of 15 mg l^{-1} was totally ineffective. *In vivo* exposure longer than 1 h is possible. At 100 mg l^{-1} over 3 h mebendazole was totally ineffective against *Gyrodactylus*, whereas 25 mg l^{-1} over 12 h killed 95.45 % of monogeneans with no apparent signs of toxicity. Hence for this drug exposure time rather than concentration determines efficacy.

Parbendazole: This was totally ineffective at 200 mg l^{-1} *in vitro*. *In vivo*, no monogeneans died after 3 h exposure to this concentration, but when dosage was reduced to 25 mg l^{-1} and exposure time was increased to 12 h efficacy was 90.8 %.

Fenbendazole: This, like mebendazole, makes water highly turbid. At the maximum concentration possible *in vitro* (12.5 mg l^{-1}) no anthelmintic activity was observed. Fenbendazole was nevertheless highly active *in vivo*, dosages down to 1.5 mg l^{-1} over 12 h eliminating all parasites without any apparent toxicity. The 100 % efficacy of 25 mg l^{-1} over 12 h, in contrast with the total lack of efficacy of the same dosage over 3 h, shows that exposure time is the determining factor.

Triclabendazole: Total efficacy was produced *in vitro* by exposure to 4 mg l^{-1} over only 15 min. At half the dosage there was a complete lack of anti-*Gyrodactylus* activity even over 60 min. *In vivo* a dosage of 25 mg l^{-1} over 3 h was totally ineffective against parasites, but 12.5 mg l^{-1} over 12 h yielded nearly 100 % reduction of the *Gyrodactylus* specimens. None of the tested concentrations were toxic to the rainbow trout specimens.

DISCUSSION

In fish farms the rapid reproduction of an infective *Gyrodactylus* species may cause problems when trying to find an effective treatment against this parasite.

Organophosphorus anthelmintics have proved ineffective against infection of fish by monogeneans. Trichlorfon, for example, though once thought effective for treatment of pseudodactylogyrosis in eels (Imada & Muroga 1979), was later reported not to be so (Szèkely & Molnár 1987). This inefficacy is attributed to the development of resistance (Goven et al. 1980).

Some monogeneans have also developed resistance to benzimidazoles (Kelly & Hall 1979). Recommendations for preventing or delaying the development of resistance include the sparing use of anthelmintics, the use of dosages that will kill the whole parasite population, and the use of anthelmintics of different groups in slow monthly rotation. In view of the rapid reproduction of *Gyrodactylus*, we believe, like Schmahl & Taraschewski (1987), that treatment of gyrodactylosis should be drastic enough to kill all the parasites. In our tests, thiabendazole, oxfendazole, and flubendazole were totally ineffective; oxibendazole and albendazole were less than 100 % effective and toxic; mebendazole and parbendazole were non-toxic but less totally effective. The only treatments that were 100 % effective were 12 h exposure to doses of 1.5 mg l^{-1} or more of fenbendazole, and 12 h exposure to 25 mg l^{-1} of triclabendazole.

Earlier studies with other anthelmintics (Santamarina et al. 1991) have resembled the present one in that *in vitro* results were not always an accurate indicator of *in vivo* activity. This may be due to *in vitro* tests' not reflecting the effect of the drug on parasite reproduction; this is particularly important for the benzimidazoles, whose anthelmintic activity against a number of parasites is attributed to their effects on reproduction. The efficacy of praziquantel against *Gyrodactylus* is reported as differing for young and mature parasites, the former being more resistent (Schmahl & Taraschewski 1987). Perhaps *Gyrodactylus* behaves similarly against benzimidazoles.

As in most other studies of treatments against ectoparasites, the anthelmintics were tested against natural infestations which may or may not be uniform. Therefore, our results for the *in vivo* efficacy of each treatment are only to be taken as indicative of the actual values.

In *in vivo* tests the drugs are in suspension, stirred up by the action of oxygenating the water and the movement of the fish. In practise, in large tanks or in tanks with few fish this stirring of the water might be insufficient to prevent the drug from forming a sediment.

During the treatments, water recycling was not performed, limiting the time fish could be exposed to the products to 12 h. Nevertheless, 100 % efficacy against *Gyrodactylus* was demonstrated by both fenbendazole and triclabendazole at non-toxic dosages. Although the highest dosage level of fenbendazole tested (25 mg l^{-1} over 12 h) was toxic, at dosages of 12.5, 6.2, and even 1.5 mg l^{-1} over 12 h there were no signs of toxicity. A 100 % effective dosage level of triclabendazole was found to be 25 mg l^{-1} over 12 h.

It is possible that the other benzimidazoles showing high efficacies (Table 3) may find use in other therapeutic applications such as repeated administration or combination with other anthelmintic drugs, e. g. mebendazole and trichlorfon (Goven & Amend 1982).

The literature reports anthelmintic activity against *Gyrodactylus* for drugs such as toltralzuril (Schmahl & Melhorn 1988), trichlorfon, levamisol, and niclosamide (Schmahl & Taraschewski 1987), and bithionol and nitroscanate (Santamarina et al. 1991). Depending on the product, these treatments were effective within 2 to 4 h, in contrast to which our results with benzimidazoles over 3 h were not generally sufficient, 12 h being required for fenbendazole and triclabendazole to kill all parasites. This seems to be a feature of these drugs, since to kill other species of monogeneans such as *Pseudactylogyrus* exposures over 24 h (Szèkely & Molnár 1987) and 72 h (Buchmann & Bjerregaard 1990) have been required.

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