Vol. 10: 39–43, 1991 | DISEASES OF AQUATIC ORGANISMS Dis. aquat. Org. | Published March 8

Anthelmintic treatment against *Gyrodactylus* sp. infecting rainbow trout *Oncorhynchus mykiss*

M. T. Santamarina, J. Tojo, F. M. Ubeira, P. Quinteiro, M. L. Sanmartín*

Departamento de Microbiología y Parasitología, Universidad de Santiago, Santiago de Compostela, Spain

ABSTRACT: Various anthelmintics belonging to different pharmacological groups (ivermectin, clorsulon, closantel, netobimin, febantel, praziquantel, niclofolan, bithionol, trichlorfon, levamisole hydrochloride, piperazine citrate and dihydrochloride, nitroscanate), all of proven efficacy against certain helminths, were tested and compared for their in vivo and in vitro activity on a natural infection of *Oncorhynchus mykiss* by *Gyrodactylus*, probably *G. salaris*. The trout were also observed for signs of toxic reaction to the drugs. Complete efficacy (100 % reduction) with no toxic effects was achieved only by bithionol (20 mg l^{-1}) and nitroscanate (0.07 mg l^{-1}).

INTRODUCTION

Antiparasitic drugs for fish are lately being more widely studied due to the need to keep farmed fish such as trout, salmon, carp and turbot free of infestations supported by intensive farming. Endoparasites have been successfully eliminated by oral administration of drugs in food or by catheters, but ectoparasite infested fish are normally treated by dipping in solutions of simple chemicals such as formalin or malachite green.

Among the most common fish parasites frequently causing serious problems, particularly in large-scale fish farms, are the monogeneans. According to Schäperclaus (1979), *Gyrodactylus* species are the most important helminths of farmed fish; their pathology and systematics have been widely studied (Bychowsky 1957, Bauer et al. 1969, Gläser 1969, Malmberg 1970, Mattheis & Gläser 1970). Their elimination is complicated by the fact that effective simple chemicals are frequently toxic to the infested fish, and the only anthelmintics tested hitherto have been praziquantel, niclosamide, levamisole hydrochloride, metriphonate and mebendazole (Schmahl & Taraschewski 1987), a mebendazole-trichlorfon combination (Goven & Amend 1982) and toltrazuril (Schmahl & Mehlhorn 1988, Schmahl et al. 1988). Praziquantel (Schmahl & Mehlhorn 1985), organophosphorates (Buchmann & Mellergaard 1988) and mebendazole (Buchmann & Bjerregaard 1990) have been tested on monogeneans other than *Gyrodactylus*.

This paper reports the potential anti-*Gyrodactylus* activity of various drugs that have previously proved effective against other helminth parasites.

MATERIALS AND METHODS

Tests were carried out on rainbow trout *Oncorhynchus mykiss* Richardson from Piscifactorias Coruñesas (Carballo, La Coruña, Spain), naturally infested with *Gyrodactylus* sp. Specimens from one sample obtained from infested rainbow trout were identified by G. Malmberg as *Gyrodactylus salaris* (pers. comm.). Prior to experimentation the rainbow 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 through the water. A suitable commercial feed was supplied daily. The anthelmintics studied are listed in Table 1.

In vitro test. Groups of 6 to 8 specimens of *Gyrodactylus* sp. were obtained from rainbow trout placed in Petri dishes containing different concentrations of anthelmintic, and observed 30 and 60 min later to determine the number of dead (immobile) helminths. Control groups of 8 specimens were also studied during 60 min under the same conditions.

* Addressee for correspondence: Dr Manuel L. Sanmartina, Laboratorio de Parasitología. Facultad de Farmacía, Universidad de Santiago, Santiago de Compostela, Spain.

0177-5103/91/0010/0039/$ 03.00

In vivo tests. Groups of 4 to 10 *Gyrodactylus*-infested rainbow trout were treated for 3 h in 10 l of non-circulating water containing different concentrations of anthelmintic, after which the water was renewed. A *Gyrodactylus*-infested control group was kept in drug-free water under the same conditions. Fish from both groups were anaesthetised 24 h after treatment by dosing each tank with 0.5 g of ethyl M-aminobenzoate (MS-222, Sandoz) per 10 l, and their pelvic fins were removed, placed in a Petri dish and viewed under a stereomicroscope to count the number of parasites.

Toxicity to the fish was considered as indicated by signs ranging from alteration of natatory movements to death.

RESULTS

Our results are summarized in Tables 2 and 3.

Ivermectin: At the concentration effective in vitro (0.031 mg l^{-1}), this synthetic drug proved to be highly toxic in tests in vivo , causing fish death within minutes. When ivermectin (I) was administered with clorsulon (C) (Ivomec F®) in vitro efficacy was total down to 6.25 mg l^{-1} I + 62.5 mg l^{-1} C; under in vivo conditions 0.05 mg l^{-1} I + 0.5 mg l^{-1} C was 100 % efficient against the helminth but still toxic to the host. Reducing the concentration to 0.025 mg l^{-1} I + 0.25 mg l^{-1} C avoided signs of toxicity but also decreased the efficacy to 59.3 %.

Closantel: This salicylanilide gave very similar results in in vitro and in vivo tests. Under in vitro conditions efficacy was total for concentrations down to 0.31 mg l^{-1}, and still high (83.3 %) at 0.15 mg l^{-1}. In vivo, 0.25 mg l^{-1} was 100 % effective but also slightly toxic. Reducing the concentration to 0.125 mg l^{-1} gave an efficacy of 99.6 % with no apparent toxicity.

Netobimin: No anthelmintic action was observed either in vitro or in vivo.

Febantel: This guanidinic derivative was totally effective in the in vitro test at a dosage of 1000 mg l^{-1}. In vivo, 4 trout were killed by 1 h exposure to 10 mg l^{-1}, which had no effect on the parasites.

Praziquantel: This was totally effective against the parasite in vitro at a concentration of 100 mg l^{-1}. A lower concentration was required in vivo due to the high turbidity caused by the drug, which is insoluble in water. At 10 mg l^{-1} the efficacy after a 3 h treatment was 97.7 %.

Phenolic derivatives: Treatment with niclofolan was totally effective in vitro down to 0.12 mg l^{-1}. In vivo, the drug was toxic at concentrations of 0.2 and 0.05 mg l^{-1}, killing all trout. These were apparently not affected by 0.025 mg l^{-1} which had an efficacy of 90.8 %, as compared to 100 % and 99.3 % at the higher concentrations.

Bithionol: In vitro efficacy was total at 12.5 mg l^{-1}, but concentrations of 20 mg l^{-1} or more were required to reproduce this effect in vivo. Efficacy dropped to 98.6 % at a concentration of 4 mg l^{-1}.

Trichlorfon: In vitro, all monogeneans were killed within 1 h by 100 mg l^{-1} of this organophosphate, and within 30 min by 200 mg l^{-1}. Under in vivo conditions, the latter concentration was only 97.7 % effective.

Levamisole hydrochloride: All parasites died in the in vitro test with 100 mg l^{-1}; however, in vivo this concentration was totally ineffective.

Table 1. *Oncorhynchus mykiss*. Treatment against *Gyrodactylus* sp. Listed are the anthelmintics studied

Anthelmintic	Commercial name	Manufacturer	Presentation	Solubility in water
Ivermectin	Ivomec^{R}•	Merck	Inject. sol.	Yes
Ivermectin + clorsulon	Ivomec-F^{R}•	Merck	Inject. sol.	Yes
Closantel	Flukiver^{R}•	Janssen	Inject. sol.	Yes
Netobimin	Hapasil^{R}•	Schering	Powder	No
Febantel	Rintal^{R}•	Bayer	Granulate	Yes
Praziquantel	Droncit^{R}•	Bayer	Pills	No
Niclofolan	Bilevon^{R}•	Bayer	Powder	Yes
Bithionol	p.p.•	Syva	Powder	No
Trichlorfon	Neguvon^{R}•	Bayer	Powder	No
Levamisol hydrochloride	Citarin-L^{R}•	Bayer	Granulate	No
Piperazine citrate	p.p.•	Syva	Powder	Yes
Piperazine dihydrochloride	p.p.•	Syva	Powder	Yes
Nitroscanate	Lopatol^{R}•	Ciba-Geigy	Pills	No

• Drugs donated by their manufacturers
p.p.: Pure compounds

Table 2. *Oncorhynchus mykiss*. Treatment against *Gyrodactylus* sp. Results obtained under in vitro conditions for the drugs examined. Doses expressed as mg l^{-1}; length of exposure, in min; percentage of reduction gives the number of live *Gyrodactylus* relative to the initial number; also listed are the number of *Gyrodactylus* initially tested and the number of dead worms counted at the end of the treatment

Drug	Dose (mg l^{-1})	Time of exposure (min)	Percentage of reduction	Nos. of *Gyrodactylus* sp. Total	Dead
Ivermectin	0.031	60	100	8	8
	0.031	30	50	8	4
Ivermectin + clorsulon	6.25 + 62.5	60	100	8	8
	25 + 250	30	100	8	8
Closantel	0.31	60	100	6	6
	0.15	60	83.3	6	5
	1.25	30	100	6	6
Netobimin	250	60	0	8	0
Febantel	1000	60	100	6	6
	1000	30	33.3	6	2
Praziquantel	100	60	100	6	6
	100	30	16.6	6	1
Niclofolan	0.12	60	100	8	8
	0.12	30	100	8	8
Bithionol	12.5	60	100	8	8
	25	30	100	8	8
Trichlorfon	200	30	100	8	8
	100	60	100	8	8
	100	30	87.5	8	7
Levamisole hydrochloride	100	60	100	8	8
	100	30	25	8	2
	50	60	63	8	5
Piperazine citrate	200	60	0	8	0
	200	30	0	8	0
Piperazine dihydrochloride	200	60	75	8	6
	200	30	0	8	0
Nitroscanate	156.25	60	50	8	4
	156.25	30	50	8	4

Control lots remained unaffected after 60 min in drug-free water under conditions identical to those in the treated lots

Piperazines: Piperazine citrate was totally ineffective both in vitro and in vivo. The dihydrochloride was 75 % effective in vitro, but ineffective in vivo.

Nitroscanate: In vitro, a concentration of 156.25 mg l^{-1} failed to kill all the helminths, but in vivo all concentrations equal to, or greater than, 0.07 mg l^{-1} were 100 % effective; at 0.04 mg l^{-1} the efficacy was 99.2 %.

DISCUSSION

In vitro tests like ours have been used to determine anthelmintic activity against the monogeneans *Dactylogyrus* and *Diplozoon* (Schmahl & Mehlhorn 1985) and *Gyrodactylus* and *Diplozoon* (Schmahl & Taraschewski 1987). In vitro effective exposure time increases as the concentration decreases; in most cases drugs active in vitro are also active in vivo. The usefulness of such tests is nevertheless limited by exposure time limitation (unless performed in suitable culture media) and by the difference between in vitro conditions and the parasite's in vivo environment. Tests in vivo are also necessary to determine drug toxicity towards the host.

In our in vivo tests, parasites were counted on fins rather than body scrapings because we found that parasite density (number of parasites cm^{-2}) is greater on the former (unpubl. own results). This makes it easier to find monogeneans on a fin rather than in scrapings when the infestation level is very low. Also, when scrapings are used, the areas examined are much less uniform than on fins.

Drugs administered by addition to tank water must obviously be non-toxic both for farmed fish and other animals with which these might come into contact after

Table 3. *Oncorhynchus mykiss*. Treatment against *Gyrodactylus* sp. Results obtained under in vivo conditions. Doses expressed as mg l^{-1}; length of treatment, in h; percentage of reduction gives the number of live *Gyrodactylus* sp. observed on pelvic fins relative to the controls; − or + indicate absence or presence of toxicity signs

Drug	Dose (mg l^{-1})	Time of exposure (h)	Percentage of reduction*	Signs of toxicity
Ivermectin + clorsulon	0.05 + 0.5	3	100	+
	0.025 + 0.25	3	59.3	−
Closantel	0.25	3	100	+
	0.125	3	99.6	−
Netobimin	2	3	0	−
Febantel	10	3	0	+
	2.5	3	0	−
Praziquantel	10	3	97.7	−
Niclofolan	0.2	3	100	+
	0.05	3	99.3	+
	0.025	3	90.8	−
Bithionol	60	3	100	+
	20	3	100	−
	4	3	98.6	−
Trichlorfon	200	3	97.7	−
Levamisole hydrochloride	100	3	0	−
Piperazine citrate	200	3	0	−
Piperazine dihydrochloride	200	3	0	−
Nitroscanate	156.25	3	100	−
	0.07	3	100	−
	0.04	3	99.2	−

* Efficacy calculated as percentage of reduction = $100(1 - N_t/N_c)$; where N_t and N_c = numbers of *Gyrodactylus* per host counted on fins of treated hosts (N_t); control hosts kept in drug-freewater under the same conditions (N_c)

the water has been returned to the environment. Anti-*Gyrodactylus* drugs must also be 100 % effective, since partial effectiveness may not prevent subsequent high levels of infestation, as was observed in the case of *G. bullatarudis* by Scott (1982). Ivermectin/clorsulon, closantel and niclofolan were toxic at concentrations at which they were 100 % effective but niclofolan and closantel might be used at smaller concentrations with a high effectivity and no toxicity. Trichlorfon and praziquantel were non-toxic but less than 100 % effective. Netobimin, levamisole hydrochloride, piperazine citrate and piperazine dihydrochloride were non-toxic but totally ineffective. Febantel was both highly toxic and totally ineffective.

Levamisole hydrochloride has been reported to be effective in vivo at a dosage of 50 mg l^{-1} against *Gyrodactylus aculeati*, obtained from *Gasterosteus aculeatus* and kept in rainbow trout (Schmahl & Taraschewski 1987). In our experiment this dosage was partially effective against *Gyrodactylus* sp. in vitro, but not in vivo. The only difference between the 2 experiments was the water temperature, which was 15 °C in our work and 20 °C in Schmahl & Taraschewski's. The difference of 5 °C may possibly affect the activity of the drug, although we have no data to support this hypothesis.

Of the drugs tested in this study the only ones fulfilling both these conditions were bithionol (100 % effective ≥ 20 mg l^{-1} over 3 h) and nitroscanate. A drawback for both drugs is their insolubility, which due to fish movements caused considerable turbidity even at the low dosages employed, although in the case of nitroscanate insolubility was associated with adherence to the fish surface; this may be the reason why dosages as low as 0.07 mg l^{-1} were effective. However, even in this case, drug insolubility might be a serious problem for use in large tanks, in which fish movement might be insufficient to prevent the drug from settling, and this would be unfavourable for adherence of nitroscanate to the fish.

Both praziquantel and trichlorfon were tested on *Gyrodactylus* species infesting *Sparus aurata*, trichlor-

fon having been reported to reveal no activity at a dosage level of 2 mg l^{-1} over 24 h (Goven & Amend 1982). In our experiments, 3 h exposure to 200 mg l^{-1} of this drug was 97.7 % effective. Praziquantel was observed by Schmahl & Mehlhorn (1985) to cause major vacuolization in the tegument of other species of monogeneans in vitro; Schmahl & Taraschewski (1987) have shown it to have in vivo activity against *Gyrodactylus* infesting *Gasterosteus aculeatus*. In the latter study, dosages higher than, or equal to, 10 mg l^{-1} (at which level young helminths were unaffected) caused toxic effects in the host (a tendency to lie upside down); however recovery could be achieved by transfer to drug-free water even after exposure to the effective dose of 50 mg l^{-1}. In our experiments, a concentration of 10 mg l^{-1} had no toxic effects on rainbow trout but reduced the number of helminths by 97.7 %; it is possible that, as in Schmahl & Taraschewski's work, the resistant 2.3 % represented young specimens.

Of the soluble anthelmintics we tested against *Gyrodactylus* sp., the best results were obtained with closantel which, at a dosage of 0.125 mg l^{-1}, was almost totally effective and produced no signs of toxicity. The piperazines were inactive.

Our results indicate that the most recommendable drugs for routine use in fish farms are those that gave a complete efficacy at non-toxic dosages: bithionol and nitroscanate; the latter is not toxic even at the highest dosage tested.

Acknowledgements. We thank Salvador Bonilla of Piscifactorías Coruñesas for donating the trout used in this study. This work was financed by the Comision Interdepartamental de Ciencias y Tecnología of the Xunta de Galicia-1987, Spain.

Responsible Subject Editor: Professor W. Körting, Hannover, Germany

LITERATURE CITED

Bauer, O. N., Musselius, M. A., Strelkov, J. A. (1969). Bolezni prudovych ryb. Izd Kolos, Moscow

Buchmann, K., Mellergaard, S. (1988). Histochemical demonstration of the inhibitory effect of Nuvan^R and Neguvon^R on cholinesterase activity in *Pseudodactylogyrus anguillae* (Monogenea). Acta vet. scand. 29: 51–55

Buchmann, K., Bjerregaard, J. (1990). Mebendazole treatment of Pseudodactylogyrosis in an intensive eel-culture system. Aquaculture 86: 139–153

Bychowsky, B. E. (1957). Monogenetic trematodes; their systematics and phylogeny. In: Hargis, W. J. (ed.) Institute of Biological Sciences, Washington, USA

Gläser, H. J. (1969). Die *Gyrodactylus*-Arten des Karpfens in der Deutschen Demokratischen Republik. Dt. Fischerei Ztg 16: 372–380

Goven, B. A., Amend, D. F. (1982). Mebendazole/trichlorfon combination: a new anthelmintic for removing monogenetic trematodes from fish. J. Fish Biol. 20 (4): 373–378

Malmberg, G. (1970). The excretory systems and the marginal hooks as a basis for the systematics of *Gyrodactylus* (Trematoda, Monogenea) Ark. Zool. 23: 1–235

Mattheis, T., Gläser, H. J. (1970). *Gyrodactylus sprostonae* LING MO-EN als Krankheitserreger beim Karpfen (*Cyprinus carpio*). Dt. Fischerei Ztg 17: 256–264

Schäperclaus, W. (1979). Fischkrankheiten. Vols. 1, 2. 4th edn. Akademie-Verlag, Berlin, GDR

Schmahl, G., Mehlhorn, H. (1985). Treatment of fish parasites. 1. Praziquantel effective against Monogenea *Dactylogyrus vastator, Dactylogyrus extensus, Diplozoon paradoxum*. Z. ParasitenKde 71: 727–737

Schmahl, G., Taraschewski, H. (1987). Treatment of fish parasites. 2. Effects of praziquantel, niclosamide, levamisole-HeL and metrifonate on Monogenea (*Gyrodactylus aculeati, Diplozoon paradoxum*) Parasitol. Res. 73 (4): 341–351

Schmahl, G., Mehlhorn, H. Haberkorn, A. (1988). Sym. triazinone (toltrazuril) effective against fish-parasitizing Monogenea. Parasitol. Res. 75: 67–68

Schmahl, G., Mehlhorn, H. (1988). Treatment of fish parasites. 4. Effects of sym. triazinone (toltrazuril) on Monogenea. Parasitol. Res. 75: 132–143

Scott, M. E. (1982). Reproductive potential of *Gyrodactylus bullatarudis* (Monogenea) on guppies (*Poecilia reticulata*). Parasitology 85: 217–236

Manuscript first received: July 17, 1990
Revised version accepted: October 29, 1990