

The Effects of Praziquantel on the Monogenean Gill Parasite *Pseudodactylogyrus Bini*

The anthelmintic Praziquantel is found to be effective against trematodes and cestodes (for a comprehensive review of various aspects of this drug, see *Andrews et al.* 1983). Praziquantel has been reported to affect fish cestodes (*Pool et al.* 1984, *Moser et al.* 1986, *Ward et al.* 1986), fish digeneans (*Bylund & Sumari* 1981) and fish monogeneans (*Schmahl & Mehlhorn* 1985). Therefore Praziquantel could be a potential drug against *Pseudodactylogyrus* spp. parasitizing the gills of eels. Infections with these monogeneans cause problems in eel farms (*Ogawa & Egusa* 1976, *Egusa* 1979, *Chan & Wu* 1984, *Mellergaard & Dalsgaard* 1986). *Köhler & Bachmann* (1978) tested the effect of Praziquantel on the activity of succinate dehydrogenase and NADH-diaphorase (NADH-oxidase) from *Ascaris suum* and reported that the latter enzyme was inhibited by Praziquantel.

The present study elucidates the influence of Praziquantel on the motility of *P. bini*. In addition by using histochemical methods the effect of this drug on succinate dehydrogenase and NADH-diaphorase in *P. bini* is determined.

Specimens of *P. bini* were removed from the gills of culture eels (*Anguilla anguilla*) and exposed in vitro to Praziquantel (No. of parasites, drug concentrations and exposure times are shown in Table 1).

The motility of the parasites was recorded with 60 min intervals (Table 1). After 8 h the parasites were rinsed in distilled water, frozen (30 min at -18°C) thawed and incubated as whole specimens (30 min at 36°C) to loca-

lize enzyme activity; Succinate dehydrogenase (SDH): a medium of sodium succinate (45 mg/ml) and nitroblue tetrazolium (NBT, Aldrich, 2 mg/ml) in 0.06 mol/l phosphate buffer was used. NADH-diaphorase (ND): NADH (Boehringer-Mannheim) (2 mg/ml) and NBT (2 mg/ml) were used in 0.06 mol/l phosphate buffer. For both processes controls were performed by omitting substrate and by pretreating parasites in 2.3% periodic acid for 10 min (to eliminate enzyme activity). After incubation parasites were mounted in glycerine jelly. To determine the tolerability of eels to the drug, eels (15-20 cm body length) were exposed for up to 15 h to various concentrations of Praziquantel (Table 2).

It is shown (Table 1) that concentrations of 1200 and 600 µg/ml of Praziquantel immediately killed all specimens of *P. bini*. After 8 h in 120 µg/ml all parasites were dead. However eels were paralysed within 60 s in Praziquantel concentrations of 1200 and 600 µg/ml and within 18 min in 120 µg/ml (Table 2).

In concentrations of 10 to 30 µg/ml about 80 to 90% of the monogeneans had lost motility totally after 8 h (Table 1). Eels survived these drug concentrations well for 15 h (Table 2). A concentration of 1 µg/ml seems to affect the parasites less. However more than 60% lost motility after 8 h.

Praziquantel did not inhibit the activity of SDH and ND in any of the drug concentrations used (Table 1). The activity of SDH and ND, detected histochemically, showed the same distribution as activity was found

Table 1. The effects of various Praziquantel solutions on the motility of *P. bini* and the activity of SDH and ND after 8 h in vitro drug exposure.

Drug conc. (µg/ml)	No. of para-sites	Activity level*								Activity of**				
		1 h	2 h	3 h	4 h	5 h	6 h	7 h	8 h	SDH	ND			
0	(a) 12	+	***	+	+	+	+	+	+	+	+	+	+	+
	(b) 14	++	++	++	++	++	++	++	++	++	++	++	++	++
1	9	+	+	+	+	+	+	+	+	+	+	+	+	+
10	12	+	+	+	+	+	+	+	+	+	+	+	+	+
20	11	+	+	+	+	+	+	+	+	+	+	+	+	+
30	14	+	+	+	+	+	+	+	+	+	+	+	+	+
120	15	+	+	+	+	+	+	+	+	+	+	+	+	+
600	12	all parasites paralysed immediately	-	-	-	-	-	-	-	-	-	-	-	-
1200	11													

(a): 2 and 4 per cent ethanol concentrations used in Praziquantel solutions of 600 and 1200 µg/ml.* : activity of motile** : enzyme activity:
 (b): 0.003-0.1 per cent ethanol concentrations used in Praziquantel solutions of 1-120 µg/ml. + : specimens . + + + : strong

+ : weak
 ++ : moderate
 +++ : strong
 *** : No. of parasites without motility

Table 2. Reaction of eels (*Anguilla anguilla*) exposed to various concentrations of Praziquantel. (Water temperature in aquaria 20°C).

	Praziquantel conc. ($\mu\text{g/ml}$)						
	1	10	20	30	120	600	1200
No. of eels	2	2	2	2	2	2	2
Reaction	no visible reaction	increased breathing rate for 1 h	increased breathing rate for 1 h	increased breathing rate for 1 h	eels paralysed paralysed within 18 min. increased breathing rate	eels paralysed within 60 sec.	eels paralysed within 60 sec.
	no visible reaction after 15 hrs.						

in all cells of the parasite. However, the pharynx and the cerebral ganglia stained particularly well. In all experimental groups some parasites were exposed to the drug while they were attached to pieces of gill filaments. None of these parasites were detached during exposure.

In vitro drug exposure of ectoparasitic monogeneans from fish is approximately similar to the exposure the parasites are experiencing on the host during a bath treatment with the drug. Thus this method seems to be suitable to determine the effect of drugs used in bath treatments. As a concentration of 10 $\mu\text{g/ml}$ proved to be approximately as effective as a concentration of 30 $\mu\text{g/ml}$ and the eels were not visibly affected by this concentration, a bath treatment in 10 $\mu\text{g/ml}$ of Praziquantel seems suitable for treatment of pseudodactylogyrosis in eels.

Schlifka (1986) (cited by Mehler 1987) reported that bath treatment of eels with Praziquantel concentrations of 10 $\mu\text{g/ml}$ for 36-48 h killed all gill parasites from eels. Thus bath treatment of pseudodactylogyrosis with this concentration of Praziquantel for up to 2 days seems to reduce the number of or eradicate the parasites. In addition the toxicity of Praziquantel to mammals was found to be very low (Andrews *et al.* 1983),

which will reduce the risk for the fish farmer.

Praziquantel has been reported to elicit a tetanic contraction of the musculature and a vacuolization of the tegument of cestodes and trematodes (Andrews *et al.* 1983). Also Schmahl & Mehlhorn (1985) found a vacuolization of the tegument of fish monogeneans after Praziquantel treatment.

Köhler & Bachmann (1978) reported that this drug inhibited the NADH-oxidase (ND) in muscle tissue from *Ascaris suum*. However as demonstrated in the present study even in similar and higher concentrations Praziquantel was not able to inhibit the activity of this enzyme (or SD) in *P. bini*. Thus the therapeutic effect against *P. bini* of the drug seems to be caused by other action mechanisms.

Acknowledgements

Laboratory facilities during this study was kindly provided by S. Møllergaard, Danish Institute for Fishery and Marine Research, Fish Disease Laboratory. J. Bjerregaard, Department of Pharmacy, Royal Veterinary and Agricultural University Denmark is thanked for valuable discussions.

Kurt Buchmann

Department of Hygiene and Microbiology, Royal Veterinary and Agricultural University, Denmark.

References

- Andrews P, Thomas H, Pholke R, Seubert J*: Praziquantel. *Med. Res. Rev.* 1983, 3, 147-200.
- Bylund G, Sumari O*: Laboratory tests with Droncit against Diplostomiasis in rainbow trout *Salmo gairdneri* Richardson. *J. Fish Dis.* 1981, 4, 259-264.
- Chan B, Wu B*: Studies on the pathogenicity, biology and treatment of *Pseudodactylogyrus* for the eels in fishfarms. *Acta zool. Sin.* 1984, 30, 173-180.
- Egusa S*: Notes on the culture on the European eel (*Anguilla anguilla* L.) in Japanese eel farming ponds. *Rapp. P. v. Reun. Cons. Int. Explor. Mer.* 1979, 174, 51-58.
- Köhler P, Bachmann R*: The effects of the antiparasitic drugs Levamisole, thiabendazole, praziquantel, and chloroquine on mitochondrial electron transport in muscle tissue from *Ascaris suum*. *Mol. Pharmacol.* 1978, 14, 155-163.
- Mehler J*: Neues auf dem Gebiet der Fischkrankheiten. (News from the area of fish diseases). *Fischer & Teichwirt.* 1987, 1, 8-11.
- Møllergaard S, Dalsgaard I*: Disease problems in Danish eel farms. *Proc. 2nd Intern. Colloq. Pathol. Marine Aquac.* 7-11 sept. Porto. 1986, p. 113.
- Moser M, Sakanari J, Heckmann R*: The effects of Praziquantel on various larval and adult parasites from freshwater and marine snails and fish. *J. Parasit.* 1986, 72, 175-176.
- Ogawa K, Egusa S*: Studies on eel *Pseudodactylogyrosis* - I. Morphology and classification of three dactylogyrids with a proposal of a new species, *Pseudodactylogyrus microrchis*. *Bull. Jap. Soc. Sci. Fish.* 1976, 42, 395-404.
- Pool D, Ryder K, Andrews C*: The control of *Bothriocephalus acheilognathi* in grass carp, *Ctenopomus idella*, using Praziquantel. *Fish. Mgmt.* 1984, 15, 31-33.
- Schmahl G, Mehlhorn H*: Treatment of fish parasites. 1. Praziquantel effective against *Monogenea* (*Dactylogyrus vastator*, *Dactylogyrus extensus*, *Diplozoon paradoxum*). *Z. Parasitenkd.* 1985, 71, 727-737.
- Ward S M, Allen J M, McKeer G*: Action of praziquantel on *Grillotia erinaceus* metacestodes (Cestoda: Trypanorhyncha) in vitro. *Parasitology* 1986, 93, 133-142.

Key words: *Pseudodactylogyrus bini*, Praziquantel, succinate dehydrogenase, NADH-diaphorase.

(Received May 21, 1987).

Reprints may be requested from: Department of Hygiene and Microbiology, Royal Veterinary and Agricultural University, 13 Bülowsvej, DK-1870 Frederiksberg C, Denmark.