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Absorption, distribution and excretion of the anthelmintic praziquantel (Droncit) in rainbow trout (*Salmo gairdneri* R.)

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Abstract. Praziquantel is an anthelmintic active against trematodes and cestodes. The absorption, distribution and excretion of the drug was studied in serum, muscles, liver, bile fluid and kidneys of rainbow trout at two temperatures, 12° C and 18° C, after a single oral dose of 500 mg/kg body wt. A bioassay, using cercaria larvae of the trematode *Diplostomum spathaceum* as the test organism, was employed to measure the drug levels in tissues of the fish. The cercariae were very sensitive to praziquantel; their mobility was significantly reduced within 20 min in a 0.01 µg/ml solution.

Praziquantel was readily absorbed from the gastrointestinal tract of the fish. Absorption was more rapid at 18° C than at 12° C. Only in the liver, however, did the peak values reach significantly higher levels at the higher temperature. The peak values in different tissues (10.2–31.8 µg/g) were reached 4–16 h after administration of the drug. The elimination of the drug from the tissues was less dependent on temperature than absorption. By 32 h p.a., 67%–96% of the maximum amounts had been eliminated from the tissues. Praziquantel was excreted partly with bile fluid and partly as water-soluble metabolites through the kidneys.

Praziquantel (Droncit) is a drug well-known for its broad spectrum of activity against trematodes and cestodes (Gönnert and Andrews 1977; Andrews et al. 1983; Groll 1984). Accumulated experience indicates that the drug also has a great potential for use against flatworm parasites in fish. It has been used with considerable success against parasites of the genera *Diplostomum* (Bylund and Sumari 1981), *Proteocephalus* (Andrews and Riley 1982), *Bothriocephalus* (Pool et al. 1984), *Dactylogyrus*, *Diplozoon* (Schmahl and Mehlhorn 1985), *Eubothrium* and *Bunodera* (Bylund, unpublished work).

The pharmacokinetics of praziquantel has been studied extensively in mammals (Andrews 1976; Diekmann and Bühring 1976; Steiner and Garbe 1976; Steiner et al. 1976; Leopold et al. 1978; Patzschke et al. 1979; Andrews et al. 1983; Groll 1984). The present tests were designed to clarify certain aspects of the pharmacokinetics of the drug in fish. The absorption, distribution and excretion of the drug in rainbow trout was tested at two temperatures, 12° C and 18° C. A bioassay determined the drug content in serum, bile fluid and tissues of the fish.

Materials and methods

The cercaria larvae of the trematode *Diplostomum spathaceum* (Rudolphi 1819), used as test organisms for bioassays of the drug levels, were obtained from infested freshwater snails of the genus *Lymnaea*, collected from natural waters. The effect of praziquantel on the cercariae was tested by exposing them to known concentrations of the drug.

Rainbow trout (*Salmo gairdneri* R.) weighing 150–500 g were obtained from a fish farm and kept in 200 l fibre-glass tanks supplied with dechlorinated and aerated tap water (pH 7.0–7.5). The water flow was 1 l/min. A photoperiod of 16 h light and 8 h dark was used, and the fish were allowed to acclimatise to laboratory conditions for at least 10 days prior to use. They were fed pelleted dry food at a rate of 2% body wt./day, except when starved 2 days before administration of the drug.

The fish were anaesthetised with MS-222 (Sandoz Ltd., Basel) after which 500 mg/kg body wt. praziquantel suspended in an 1% aqueous solution of Cremophor was administered orally via a stomach tube. A 1% Cremophor solution without praziquantel was administered to a control group kept at 12° C.

Five fish from both test groups (12° C and 18° C) and three from the control group were sampled at 0.5, 1, 2, 4, 8,

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16 and 32 h after administration. The fish were stunned by a blow on the head and blood was sampled by cardiac puncture. The bile fluid was collected and tissue samples were taken from the liver, the hind kidneys and the muscles. Blood serum, bile fluid and homogenised tissue samples were extracted with diisopropylether (E. Merck, Darmstadt) for 30 min with vigorous shaking. The ether aliquots from two extractions were combined and dried in a 40° C water bath with a jet of air.

The reduced tissue extracts were suspended in water and tested in successive 1:2 dilutions on cercariae of *D. spathaceum*. The normal, negative geotropic, swimming behaviour of cercariae was used to assess the effect of praziquantel. After 5, 20 and 60 min the numbers of immobilised and of normally swimming cercariae were counted under a stereo microscope. The results were compared with a standard graph for immobilisation of cercariae at different drug concentrations.

The efficacy of the extraction procedure was determined by testing the effect of the tissue residue on cercariae after ether extraction. The drug recovery by ether extraction was 95% ± 2% for blood serum ($n = 4 \pm$ SE), 85% ± 4% for muscles, 84% ± 5% for liver, 56% ± 6% for kidney and 95% ± 2% for bile fluid.

The excretion of anthelmintically active praziquantel by fish into the aquarium water was tested by keeping rainbow trout individually in small aquaria (vol. 4 l) with aerated water (temp. 18° C). Fish ranging from 55 g to 85 g were given 500 mg/kg body wt. praziquantel orally, and were allowed to swim in a basin for 10 min to rinse away any drug residue in the mouth and pharynx before being transferred to the test aquaria. Water samples were taken from the aquaria at 1, 2, 4, 8, 16, 24 and 32 h after administration and tested directly on cercariae of *D. spathaceum*.

Results

Effect on cercariae

The cercaria larvae of *D. spathaceum* proved very sensitive to praziquantel. Their mobility was significantly impaired ($P < 0.05$, *t*-test) by 0.01 µg/ml within 20 min with 10.6% ± 1.4% of the cercariae immobilised compared to 4.3% ± 1.4% in the control without praziquantel (Fig. 1). Higher drug concentrations had an almost complete and a rapid immobilising effect. At drug concentrations higher than 0.1 µg/ml the cercariae started shedding their tails. After 60 min incubation in 5 µg/ml, 99% of the cercariae had shed their tails. The cercariae also developed contractions and tegumental lesions after contact with praziquantel (Fig. 2).

Kinetics in fish

The absorption and elimination of praziquantel in serum, tissues and bile fluid of rainbow trout are shown in Fig. 3. Two different temperature-dependent patterns of uptake and elimination were observed. The drug was absorbed more rapidly at 18° C, when peak concentrations were reached 4–8 h after administration. At 12° C the peaks were reached within 8–16 h, except in liver tissue, where the uptake was equally rapid at both temperatures. In serum the peak level remained somewhat lower at 18° C (10.6 µg/ml) than at 12° C (16.1 µg/ml); in bile fluid the peak levels were

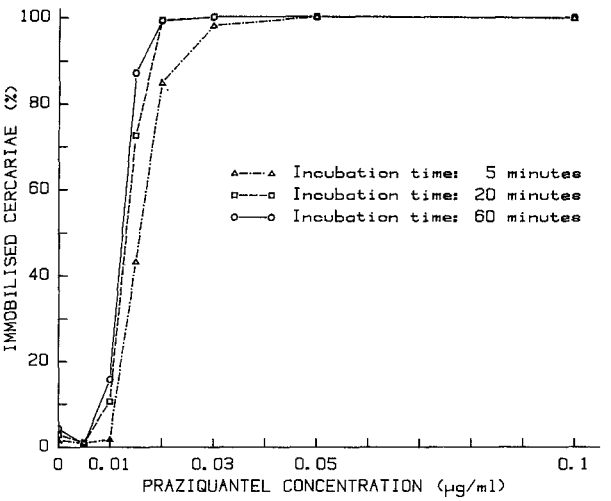

Fig. 1. The effect of praziquantel on the mobility of *Diplostomum spathaceum* cercariae. The total number of cercariae per drug concentration varies between 814 and 2,206

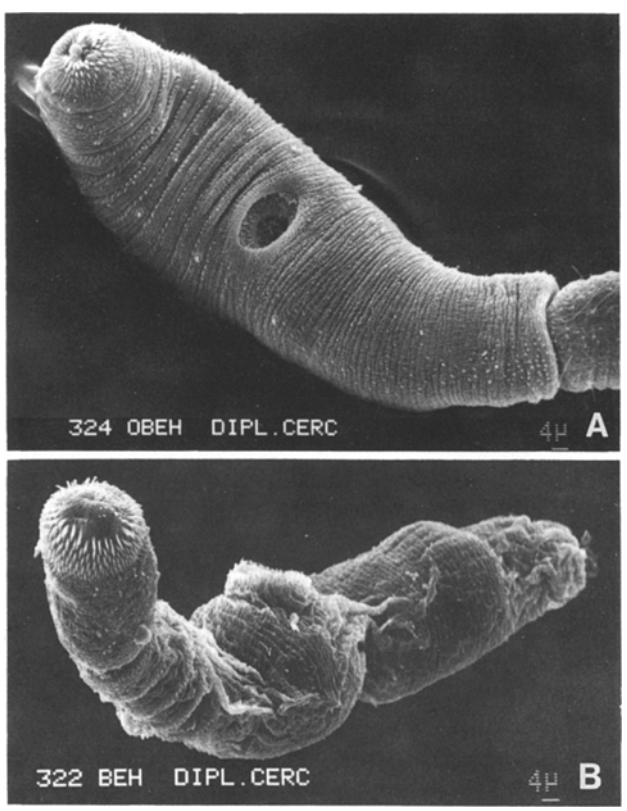

Fig. 2 A, B. Untreated (**A**) and praziquantel-treated (**B**) cercariae of *Diplostomum spathaceum*. The drug concentration was 0.2 µg/ml and the incubation time 1 h

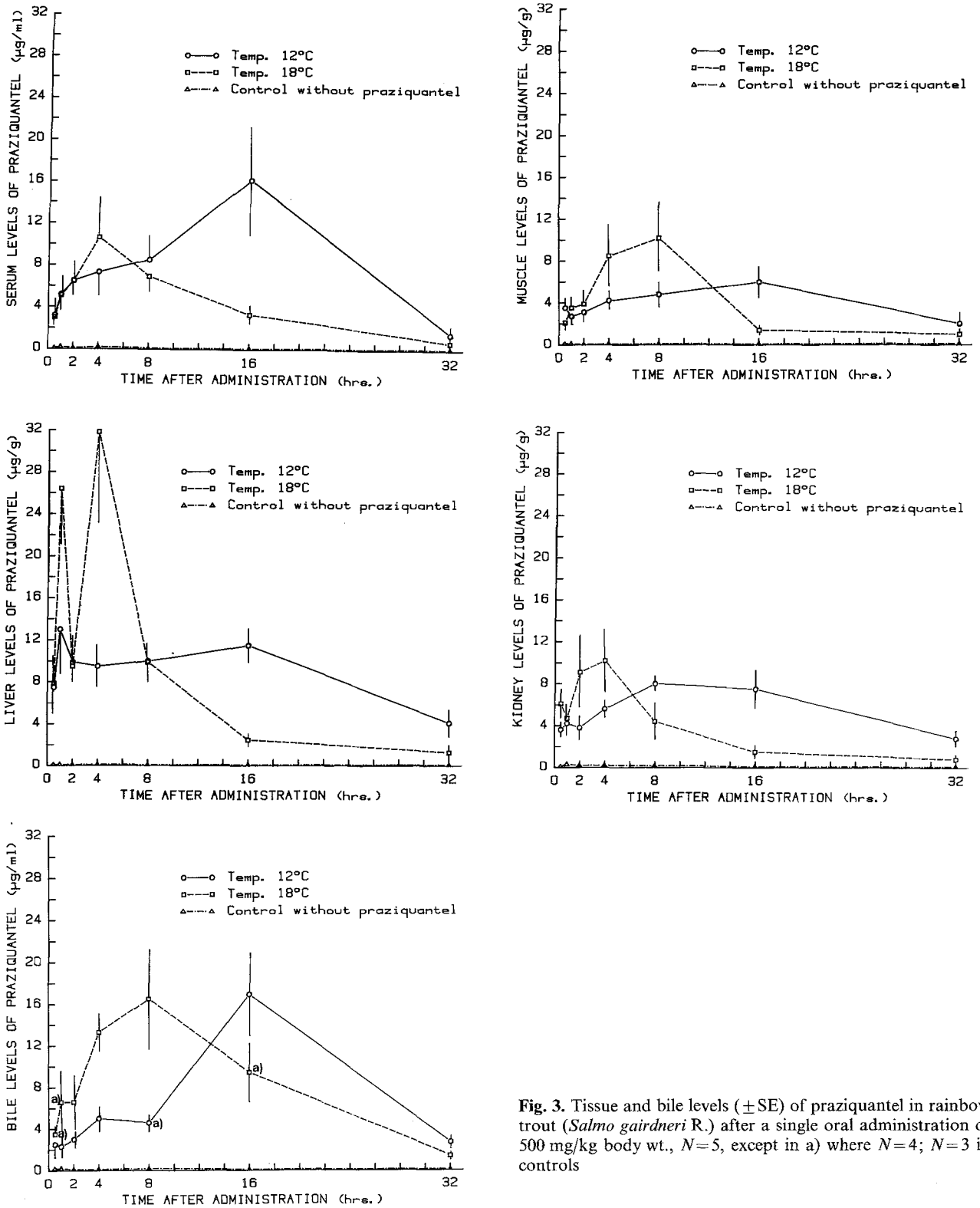

Fig. 3. Tissue and bile levels (±SE) of praziquantel in rainbow trout (*Salmo gairdneri* R.) after a single oral administration of 500 mg/kg body wt., $N=5$, except in a) where $N=4$; $N=3$ in controls

nearly identical at both temperatures (16.1 µg/ml at 18° C and 16.9 µg/ml at 12° C). In muscles, liver and kidneys the peaks seemed to reach a somewhat higher level at 18° C than at 12° C. Only in the liver, however, the peak values were significantly higher at the higher temperature ($P<0.05$). The highest level of the drug was recorded in liver (31.8 µg/g) at 18° C. The lowest peak concentra-

tions were found in muscles: 10.2 µg/g at 18° C and 6.0 µg/g at 12° C.

The drug levels in serum, bile fluid and tissues declined rapidly at both temperatures. By 32 h after administration 67%–96% of the maximum amounts had been excreted. Only in bile fluid and kidney tissue was there significantly more drug left at 12° C than at 18° C 32 h after administration ($P<0.05$).

Ether extracts from serum, tissues and bile fluid of control fish given a 1% aqueous Cremophor solution without praziquantel showed a minimal immobilising effect on the cercariae (Fig. 3).

Excretion from fish

At 32 h after administration the fish had excreted 15.4% of the drug dose in an anthelmintically active form. The excretion of active drug was most rapid immediately after administration, as 8.4% of the dose was excreted during the 1st h after administration.

Discussion

The absorption, distribution and excretion of praziquantel was studied in rainbow trout using a bioassay with parasitic cercariae as test organisms. The cercariae of *D. spathaceum* have not been used before as test organisms in biological assays. The cercariae proved a suitable test organism as they were easily obtained, easy to work with and very sensitive to praziquantel.

Effect on cercariae

The normal swimming behaviour of *D. spathaceum* cercariae was significantly impaired in a praziquantel concentration of only 0.01 µg/ml within 20 min. Complete immobility followed exposure to higher drug concentrations and many cercariae shed their tails. A very similar effect of praziquantel on cercariae of *Schistosoma mansoni* (Trematoda) was reported by Andrews (1978). Praziquantel also induced contractions and tegumental lesions in *D. spathaceum* cercariae, similar to those reported in other trematodes (Becker et al. 1980; Shaw and Erasmus 1983) and cestodes (Becker et al. 1981).

Bioassay

The bioassay proved a suitable method for measuring drug levels. The lowest measurable drug concentration in fish tissues was 0.1 µg/g. The method is simple and inexpensive, and measures only the anthelmintically active drug and/or its active metabolites in the tissues, thus giving good information on the anthelmintic potency of the drug. A disadvantage of the bioassay is its lower precision compared with high performance liquid chromatography (Xiao et al. 1983) and gas liquid chromatography (Diekmann 1979), for example.

Kinetics in fish

Rainbow trout tolerated the relatively high drug dose (500 mg/kg body wt.) well and none died during the experiments. Praziquantel was absorbed from the gastrointestinal tract of the fish and was distributed to serum, muscles, liver, bile fluid and hind kidneys without any pronounced tissue-specific accumulation. The absorption was temperature dependent, being more rapid at 18° C than at 12° C. In the liver the absorption of praziquantel at 18° C was divided into two peaks, at 1 and 4 h after administration, with a substantial decline in between. A similar, though weaker, pattern can be seen at 12° C (Fig. 3). Why does this double peak occur? The explanation could be that the first peak, 1 h after administration, represents a first-pass metabolism of the drug passing the liver. The second peak, 4 h after administration, could be the result of a reabsorption of active drug from bile fluid in the intestinal tract, leading to enterohepatic recirculation. This suggestion is supported by the high concentrations of the drug in bile fluid, with peak levels appearing somewhat later than in the liver.

Elimination of praziquantel from the tissues of fish was less dependent on temperature than absorption. At 32 h after administration 67%–96% of the maximum amounts had been eliminated from the tissues. As fairly large amounts of the drug were found in the bile fluid and hind kidneys it can be concluded that the drug is excreted partly with bile fluid and partly through the kidneys. Extraction efficiency with diisopropylether from blood serum, muscles, liver and bile fluid was between 84% and 95% but was only 56% from the kidneys. Thus only 56% of the drug was present in the organic ether phase and the rest was in the aquatic tissue phase. This indicates that praziquantel is partly excreted as water soluble metabolites through the kidneys.

Comparison with the kinetics in mammals

The pharmacokinetics of praziquantel has been extensively studied in mammals. The drug is rapidly

absorbed, showing peak concentrations within 1 h of administration, and almost completely excreted within 24 h in the mouse, rat, beagle dog, rhesus monkey, sheep and man. It shows a pronounced first-pass effect when passing the liver and is excreted mostly through the kidneys as water-soluble metabolites. Only traces of anthelmintic drug were present in faeces and urine, which shows that praziquantel in mammals is almost completely absorbed and metabolised before excretion (Diekmann and Bühring 1976; Steiner and Garbe 1976; Steiner et al. 1976; Leopold et al. 1978; Patzschke et al. 1979). In mouse the kinetic processes are extremely rapid. Only 15 min after an oral dose of 250 mg/kg body wt. Andrews (1976) measured drug concentrations as high as 87–507 µg/g in different tissues. Most of the active drug had been eliminated from the tissues 2 h after administration. The therapeutic dose of praziquantel in mammals is in most cases between 5 and 50 mg/kg body wt., administered either as a single dose or in subdoses.

The absorption, distribution and excretion of praziquantel in fish is largely similar to that in mammals. In fish, however, the pharmacokinetic processes are slower and less effective; absorption is slower, the maximum concentrations reached in the tissues are lower and more unmetabolised drug is excreted. This indicates that the drug dose used to treat fish should be higher than for mammals. However, results obtained so far show that praziquantel doses of 5–100 mg/kg body wt. also have a good curative effect on trematode and cestode infections in fish (Andrews and Riley 1982; Pool et al. 1984; Bylund, unpublished results). A plausible explanation might be that the slow drug metabolism in fish exposes the parasites to the active drug for longer time. No toxic effects of praziquantel on fish were found in this study.

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