Effects of orally administered chemotherapeutics (quinine, salinomycin) against Henneguya sp. Thelohan, 1892 (Myxozoa: Myxobolidae), a gill parasite in the tapir fish Gnathonemus petersii Günther, 1862 (Teleostei)

Abstract When given orally, quinine or salinomycin cause irreversible damage to the plasmodial developmental stages of Henneguya sp., a gill parasite in the tapir fish Gnathonemus petersii. Naturally infected tapir fish measured 75–169 mm in total length and their total weight ranged over 4.3–11.7 g. The fish bore 7–77 plasmodia in their gill arches. Medicinal food containing either quinine (5 g/1000 g food) or salinomycin (0.075 g/1000 g food) was given once a day to naturally infected fish in a food chain via water fleas (Daphnia spp) for a period of 3, 6, or 9 days. From the monitored feeding of the tapir fish and weight determinations of the water fleas, it was calculated that gross uptake was 18.5 μg/kg body weight daily for pure salinomycin and was 1.25 mg/kg body weight daily for quinine. After the end of the experiments, the fish were sacrificed and the plasmodia were carefully prepared from the gill arches and processed for transmission electron microscopy. As seen by ultrastructure investigations, for both substances the grade of damage in the parasites correlated positively with the period of application. When quinine was given for a 3-day period, the trophozoite ecto- and endoplasm exerted numerous vacuoles, caused by the drug, and the presporogenous and the pansporoblastic stages were malformed. Following a 6-day period, numerous abortive polar capsules were found in the trophozoite cytoplasm. To a large extent, the limiting membranes of the polaroblasts and valvogenic cells were destroyed. In addition, deep clefts between the polaroblasts, the valvogenic cells and between the two sporoblasts were observed. Following a 9-day treatment, all damage increased and, in addition, generative cells and two-cell stages were no longer detectable. As a first sign for the effects of salinomycin, following a 3-day treatment, a shrinking of the whole plasmodia occurred and the sutures in the pansporoblasts were enlarged. The polar capsules were malformed and the zonar structures of the polar filament were no longer detectable. The sporoplasmosomes were more electron-pale than those of the control samples. After a 9-day treatment, the pansporoblasts were completely destroyed. Under the experimental conditions chosen, both compounds were very well tolerated by the fishes.

Introduction

More than 120 species of the genus Henneguya Thelohan, 1892 have been described from freshwater and marine fish, representing one of the largest groups of the family Myxobolidae (Lom and Dyková 1992). Diseases caused by Myxoporea are considered as an increasing economic problem in the culture of commercially important fishes; and the list of pathogenic species is constantly expanding (Azevedo and Matos 1996).

During the past 15 years, some progress has been achieved in treating diseases caused by Myxozoa. When given orally, the efficacy of an antibiotic, fumagillin, against Sphaerospora renicola in common carp, Myxidium giardii in European eel, Hoferellus carassii parasitising goldfish and Myxobolus cerebralis in rainbow trout has been reported (Molnár et al. 1987; Székely et al. 1988; Yokoyama et al. 1990; El-Matbouli and Hoffmann 1990, 1991; El-Matbouli et al. 1992). A decade ago, it was found that a symmetric triazine derivative, toltrazuril, and an asymmetric triazine derivative, HOE 092 V, have significant deleterious
effects when dissolved in medicinal baths against the gill-parasitic *Henneguya* sp. in tapir fish (*Gnathonemus petersii*) and against the skin-parasitic *H. laterocapsulata* in hybrids of clariid catfish (*Clarias gariepinus × Heterobranchus bidorsalis*), as demonstrated by Schmahl et al. (1991, 1993).

In more recent investigations, it was shown that medicinal baths containing quinine had negative effects against the plasmodial developmental stages of *Henneguya* sp. in tapir fish (Zegula 1997). Salinomycin \{ε-ethyl-6-[5-ethyl-9-(2-furanyl)-2,6-dihydroxy-1,3,7-trimethyl-4-oxo-decyl] tetrahydro-5-methyl-2H-pyran-2-acetic acid\} is an antibiotic belonging to the ionophorous polyethers (Kinashi et al. 1973; Miyazaki et al. 1974; Pressman and Fahim 1982; Mehlhorn et al. 1983; Glatzer et al. 1992; Birch and Robinson 1995). Salinomycin has been described as a product of fermentation from *Streptomyces albus*. It reveals a strong activity against Gram-positive bacteria, mycobacteria, fungi and against protozoans, especially coccidia (Kinashi et al. 1973; Miyazaki et al. 1974; Pressman and Fahim 1982; Mehlhorn et al. 1983; Hoefft 1993; Birch and Robinson 1995; Gerhardt et al. 1995).

Considering the facts presented above, we had the idea of testing two different chemotherapeutics (quinine, salinomycin) against the plasmodial stages of *Henneguya* sp. in tapir fish (*G. petersii*). From both the economic and the ecological point of view, it is regarded as a clear advantage to try therapy in diseased fish orally rather than in medicinal baths.

### Materials and methods

#### Drugs

Medicinal fish-food containing 0.075 g salinomycin/1,000 g was obtained from W. Raether (Dreieich, Germany). Medicinal food flakes containing 5 g quinine/1,000 g food in a non-water-soluble formulation was obtained from Tetra Werke Dr. rer. nat. Ulrich Baensch, Melle, Germany.

#### Parasites and hosts

Tapir fish naturally infected with *Henneguya* sp. were obtained from various pet shops. Specimens measured 75–169 mm in total length and total weight ranged over 4.3–11.7 g. The fish were imported from Nigeria to Germany during November–December 1997. Under laboratory conditions, single fish were kept in aerated glass aquaria at 25 °C and a light-dark cycle of 16:8 h. The fish were fed three times a week with living water fleas (*Daphnia* spp). During examination, 7–77 pale spherical to ellipsoidal plasmodia were fed immediately to the tapir fish. Dependent on its size, each tapir fish took up about 30–70 water fleas/day. From dry weight determinations for the water fleas before and after ingestion of the medicated food suspensions, it was calculated that 2.25–5.25 mg medicinal food were administered to each fish per single administration. Therefore, the oral dose of salinomycin was 18.5 μg/kg body weight/day and was 1.25 mg/kg body weight/day for quinine.

At the end of the tests, the fish were sacrificed. Pieces of the gill filaments were immediately cut off and processed for microscopy.

#### Light and transmission electron microscopy

Plasmodia containing all developmental stages were fixed in 5% glutaraldehyde buffered with 0.1 M sodium cacodylate, pH 7.4, at 4 °C for 24 h. Postfixation was done in 2% OsO4 in the same buffer. For light and transmission electron microscopy, the specimens were dehydrated in graded ethanol, transferred to propylene oxide and embedded in Araldite. Semithin sections were stained with methylene blue and Azur A. Ultrathin sections were contrasted with uranyl acetate and lead citrate and examined in a Zeiss 9-S2 electron microscope.

#### Results

#### Light microscopy

Infected fish examined following treatment contained up to 77 plasmodia, which were even visible with the naked eye. The plasmodia predominantly occurred towards the distal end of the primary gill filaments. In treated fish, the diameter of the plasmodia was reduced from 0.3–0.9 mm to 0.16–0.21 mm and the contacts between the plasmodia and the gill tissue were weakened after treatment with either quinine or salinomycin. The kind and the degree of damage, however, could only be evaluated when using electron microscopy.

#### Electron microscopy – untreated controls

**Plasmodium wall and cytoplasm**

The plasmodia were limited by a single membrane which was continuous with pinocytic canals extending for various lengths into the trophozoite ectoplasm. The plasmodium (= trophozoite) membrane was covered by a smooth, granular coat preventing direct contact between the plasmodium and the host tissues (Fig. 1). Beneath the zone of pinocytic canals, there was an endoplasmic zone containing mitochondria with tubular cristae (Fig. 2). The endoplasm contained the vegetative nuclei, the uni- and bicellular stages and the early pansporoblasts. The immature and the mature spores were located in the central zone of the endoplasm.

For the oral administration of medicinal food containing either salinomycin or quinine, the flakes were suspended in water. Then, starved water fleas (*Daphnia* spp) were put into these suspensions. After a 60-min period, the digestive tracts of the water fleas were filled with medicinal food particles. Then the water fleas were fed immediately to the tapir fish. Dependent on its size, each tapir fish took up about 30–70 water fleas/day. From dry weight determinations for the water fleas before and after ingestion of the medicated food suspensions, it was calculated that 2.25–5.25 mg medicinal food were administered to each fish per single administration. Therefore, the oral dose of salinomycin was 18.5 μg/kg body weight/day and was 1.25 mg/kg body weight/day for quinine.

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