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ORIGINAL PAPER

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Treatment of fish parasites. 11. Effects of different benzimidazole derivatives (albendazole, mebendazole, fenbendazole) on *Glugea anomala*, Moniez, 1887 (Microsporidia): ultrastructural aspects and efficacy studies

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Abstract Three different benzimidazole derivatives, albendazole [methyl-5-(propylthio)-2-benzimidazolcarbamate], mebendazole (methyl-5-benzoyl-2-benzimidazolcarbamatic acid methyl ester), and fenbendazole [methyl-5-(phenylthio)-2-benzimidazolcarbamate] were tested in vivo against *Glugea anomala* parasitizing the connective tissue of sticklebacks (*Gasterosteus aculeatus*). Naturally infected sticklebacks were incubated in aerated plastic aquaria (10 l) at 22 °C in water containing 0, 1, 5, 10, or 50 μg of either albendazole, mebendazole or febendazole for 2 or 6 h. For intermittent treatment, 2 μg substance was administered three times for 6 h at intervals of 36 h. At the ultrastructural level, at all developmental stages of *G. anomala* there were no significant differences in the kind of damage caused by either albendazole, mebendazole, or febendazole. Starting with a dose of 1 μg/ml for 2 h, each of the drugs irreversibly damaged uni- and multinucleate meronts, sporogonial plasmodia, and sporoblasts. Disorganized spores were also observed. Treatment with higher doses (10 μg/ml, 2 or 6 h) caused malformations of the merogonic and the sporogonic stages, a significant reduction in the number of ribosomes, and disruptions of the nuclear membranes. The first recognizable treatment effect was an enlargement of the smooth endoplasmic reticulum. In the sporogonial plasmodia, the membranes of the sporophorous vesicle envelopes were lumpy or even completely destroyed. After incubation with the highest dose (50 μg/ml, 6 h), microtubules were apparent within the karyoplasm of the uninucleate meronts. After interval treatment, all forms of damage were intensified, especially in the mature spores. When treatment was done three times at low doses (3 × 2 μg/ml, 6 h, 36-h intervals), spore infectivity was drastically lowered. Therefore, it seems likely that an intermittent regimen of medicinal baths can be successfully applied against susceptible Microsporidia in fish.

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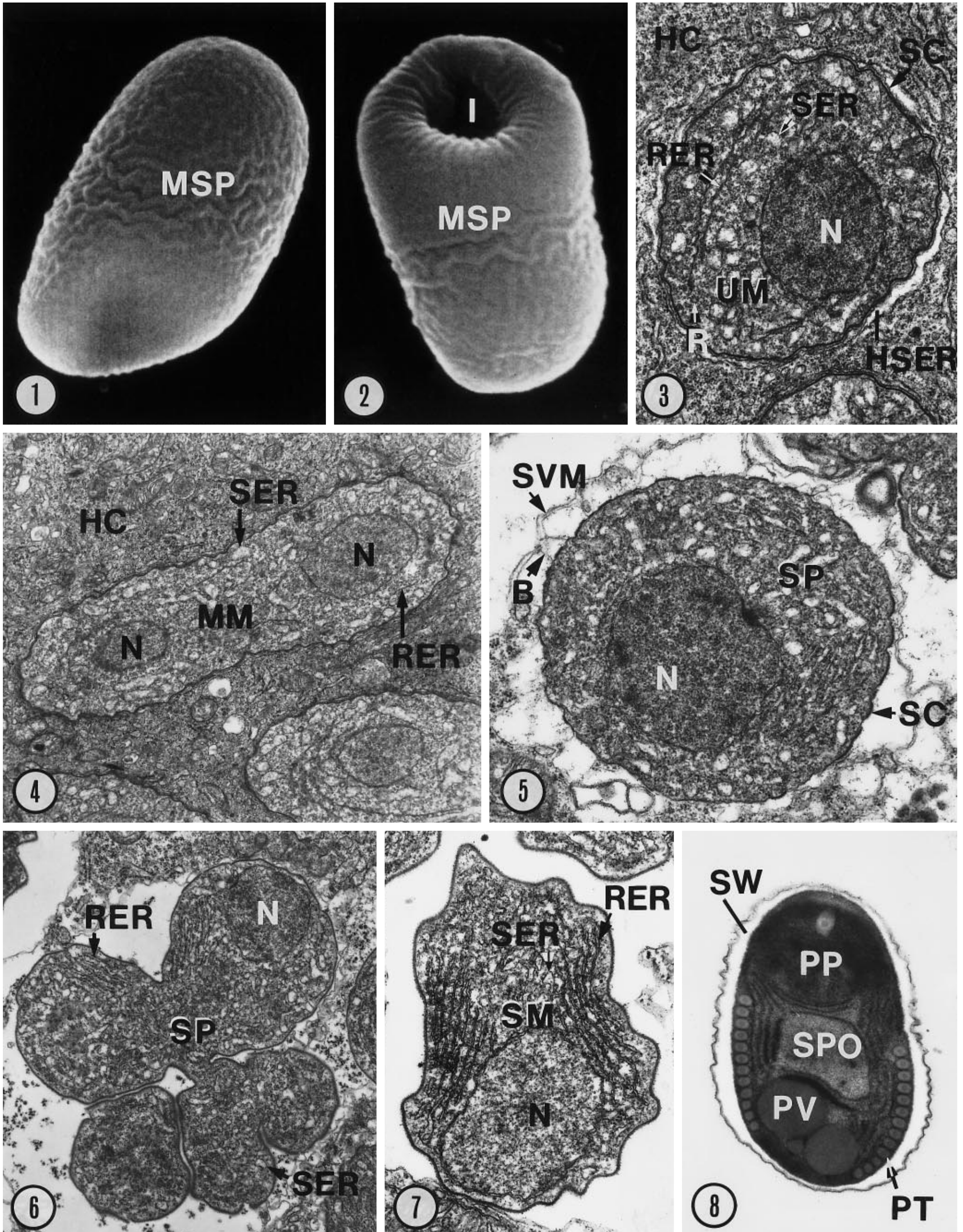
Introduction

Microsporidia are strictly intracellular parasites of most animal groups, including fish. Microsporidian infections in fish are always detrimental and endanger the lives of their hosts and, under conditions favoring transmission, especially in hatcheries, outbreaks can reach epizootic proportions (McKenzie et al. 1976; Kabata 1985; Canning and Lom 1986; Schmahl et al. 1989; Lom and Dykova 1992).

Regarding treatment, it has been reported that an antibiotic, fumagillin, acts against *Glugea plecoglossi* in *Plecoglossus altivelis*, but the mortality of treated fish has sometimes been higher than of untreated fish (Takahashi and Egusa 1976). Previous contributions have reported the severe effects of a symmetric triazinone, toltrazuril, and an asymmetric triazinone, HOE 092 V, on the microsporidian *G. anomala* as well as on other fish and crayfish parasites (Mehlhorn et al. 1988; Schmahl and Mehlhorn 1989; Schmahl et al. 1990, 1992; Cauphey 1991; Schmahl and Senaud 1996).

In human beings infected with *Enterocytozoon bieneusi*, oral administration of the benzimidazole derivative albendazole (400 mg/day for 4 weeks) has proved an effective treatment (Hingh et al. 1992; Mehlhorn et al. 1995). Clearance of disseminated microsporidiosis due to *Septata intestinalis* in HIV-infected patients was also achieved by oral treatment with albendazole (Dore et al. 1995).

Actually, there are no reports on the effects of albendazole or other benzimidazole derivatives in fish-parasitizing Microsporidia. In the present study, three-spined sticklebacks (*Gasterosteus aculeatus*) naturally infected with *G. anomala*, Moniez, 1887 were chosen as a model for treatment. The fish were incubated in different solutions of either albendazole [methyl-5-(propylthio)-2-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benzimidazolcarbamate] or mebendazole (methyl-5-benzoyl-2-benzimidazolcarbamatic acid methyl ester). A third series of experiments was done using solutions of fenbendazole [methyl-5-(phenylthio)-2-benzimidazolcarbamate]. Infectivity experiments were carried out by feeding treated spores to uninfected fish via water fleas in a food chain. Finally, the parasites were studied using light and electron microscopy to evaluate the drug-derived effects.

Materials and methods

Drugs

Albendazole and mebendazole were obtained from Sigma (Deisenhofen, Germany). Fenbendazole was delivered from Hoechst, Frankfurt, Germany. For final use, the drugs were dissolved in 1 ml dimethylsulfoxide (DMSO) for all concentrations tested.

Parasites and hosts

Young and adult three-spined sticklebacks (*G. aculeatus*) naturally infected with *G. anomala* were captured in the ponds of the Botanical Garden of the Ruhr University, Bochum. The fish measured 15–77 mm in total length, their weight ranged from 0.2 to 2.15 g. In a preexamination, 1–14 *Glugea* xenomas measuring up to 5 mm in diameter were counted for each fish.

Experimental design

Before starting the experiments, the fish were maintained in constantly aerated tanks containing 600 l water 22 °C. The pH of the water was 7.0–7.5; its NO_2 content was zero, and the NO_3 content was 10 mg/l. Fish were fed twice a week with frozen larvae of *Chironomus* spp. (Redeo Frost). A total of 180 sticklebacks were incubated in groups of 10 in aerated 10-l plastic aquaria in water containing 0, 1, 5, 10, or 50 μg/ml of either albendazole or mebendazole. For additional experiments, fenbendazole was used at the same concentrations. Incubations were for 2 or 6 h. Intermittent treatment was done with 2 mg substance/ml for 6 h, three times at 36-h intervals. In control runs, the pure solvent (1 ml/10 l water) was checked for eventual effects on parasites and hosts. The fish were sacrificed 24 or 28 h after the end of the incubation, and the xenomas were processed for light and electron microscopy. For tolerance tests, separate groups of 10 sticklebacks each were incubated for 100 h in water containing 1, 5, 10, 50, or 100 μg/ml of either albendazole, mebendazole, or fenbendazole.

Infectivity tests

Spores for infectivity tests were harvested from the xenomas of either untreated or treated sticklebacks. A single treatment was done with either albendazole, mebendazole, or fenbendazole, with 10 μg/ml and 6 h exposure. Intermittent treatment was given in accordance to the schedule described above. The xenoma contents were washed several times in distilled water followed by repeated centrifugation and resuspension steps. Before use, the spores were stored in tap water for 3 weeks. Finally, they were given to non-infected sticklebacks via water fleas (*Daphnia* spp.) in a food chain. For each series of tests, a total of ten sticklebacks was used.

Light and transmission electron microscopy

The processing of the parasites for light and transmission electron microscopy was carried out as described by Schmahl and Mehlhorn (1989).

Scanning electron microscopy

The xenomas were pierced with a thin needle, the content of the xenomas was centrifuged for 15 min at 400 *g* on round glass object slides. The pellets were then washed twice with cacodylate buffer (2 × 30 min). The spore suspension was centrifuged again and post-fixed in 2% OsO_4 for 10 min. After washing with distilled water (2 × 30 min), the specimens were dehydrated in graded ethanol, followed by acetone. The dried samples were mounted on metal stubs and sputtered with gold in a scanning electron microscope coating unit E 5100 (Polaron Equipment). Examination was done with a Zeiss DSM 950 scanning electron microscope.

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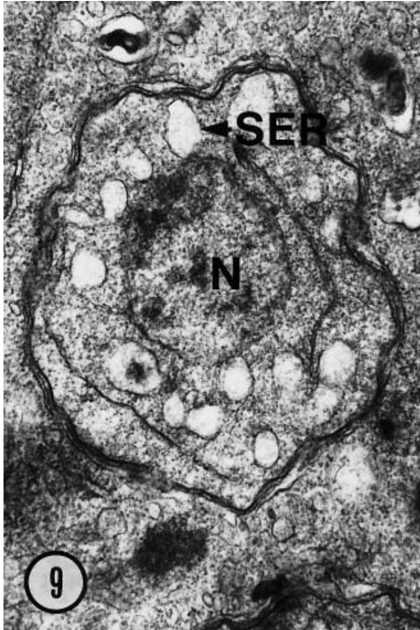
Figs. 1–8 Scanning and transmission electron micrographs of *Glugea anomala* (*B* blister, *HC* host cell cytoplasm, *HSER* host all smooth endoplasmic reticulum, *MM* multinucleate meront, *MSP* mature spore, *N* nucleus, *PP* polaroplast, *PT* polar tube, *PV* primary vacuole (later fusing), *R* ribosome, *RER* rough endoplasmic reticulum, *SC* surface coat, *SER* smooth endoplasmic reticulum; *SP* sporogonial plasmodium, *SPO* sporoplasm, *SVM* sporophorous vesicle membrane, *SW* spore wall, *UM* uninucleate meront). **Fig. 1** Control. Scanning micrograph of a mature spore. Note the crenated surface. ×23 000. **Fig. 2** Treated specimen. Scanning micrograph of a mature spore after interval treatment with fenbendazole (3 × 2 μg/l, 6 h, at 36-h intervals). Note the deep invagination at the posterior pole. ×23 000. **Fig. 3** Control. Uninucleate meront surrounded by a cisterna of host cell smooth endoplasmic reticulum. Note the rough and smooth endoplasmic reticulum, and also the numerous ribosomes within the meront cytoplasm. ×15 000. **Fig. 4** Control. Multinucleate meront. Note the lacunas of the smooth and the rough endoplasmic reticulum. ×9900. **Fig. 5** Control. Sporogonial plasmodium with its characteristic layer of blisters during formation of the sporophorous vesicle membrane. ×19 500. **Fig. 6** Control. Sporogonial plasmodium. The daughter cells of the plasmodium were held together by cytoplasmic bridges. ×13 100. **Fig. 7** Control. Sporoblast mother cell. Note the well-developed smooth and rough endoplasmic reticulum. ×25 000. **Fig. 8** Control. Nearly mature spore. Note the polaroplast and the polar tube. The spore wall has been formed. ×19 200

Results

General remarks

As confirmed by means of scanning and transmission electron microscopy, each of the benzimidazole derivatives tested – albendazole, mebendazole, and fenbendazole – irreversibly damaged all developmental stages of *G. anomala* including the majority of the mature spores. The merogonic and the sporogonic stages, which were typical either in the untreated controls or in control runs containing DMSO only, were enlarged and/or misshapen. On treatment, the membranous borders of the parasitophorous vacuoles were grossly thickened in comparison to that of the controls or, in many cases, they were no longer detectable by transmission electron microscope. In nearly all cases, the mature spores had malformations due to drug treatment.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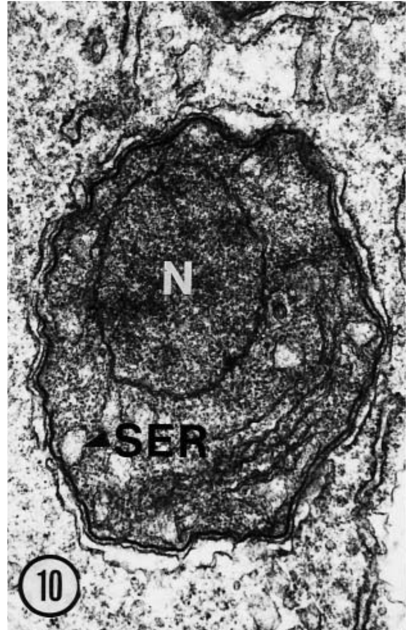
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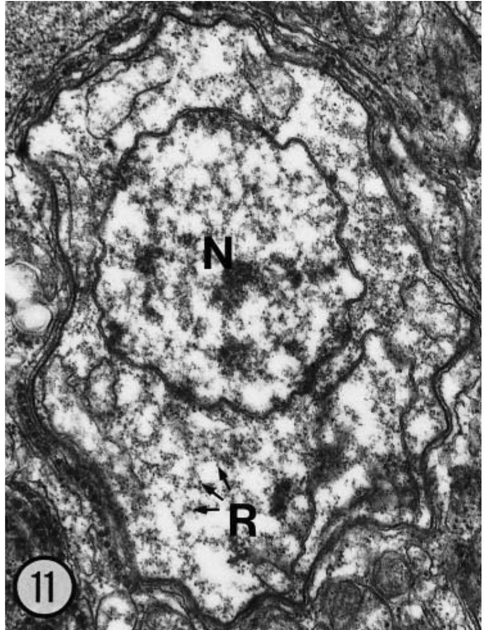
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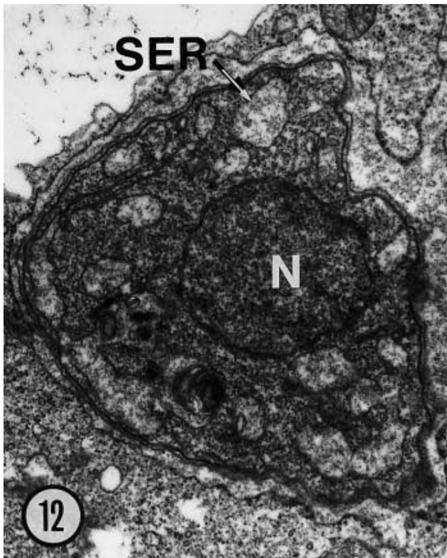
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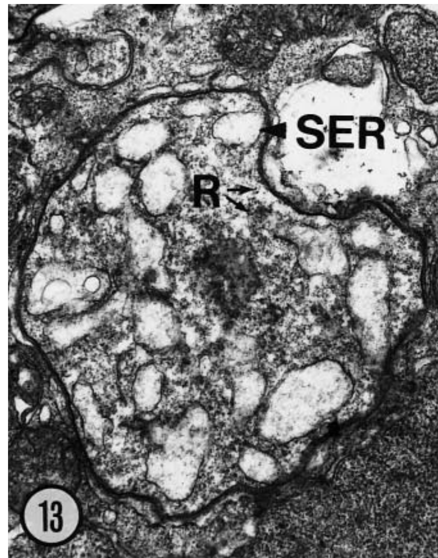
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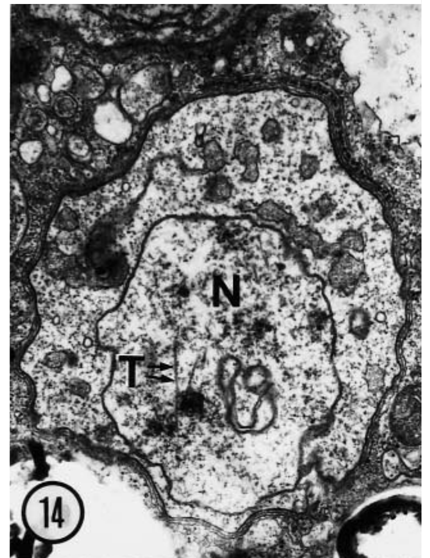
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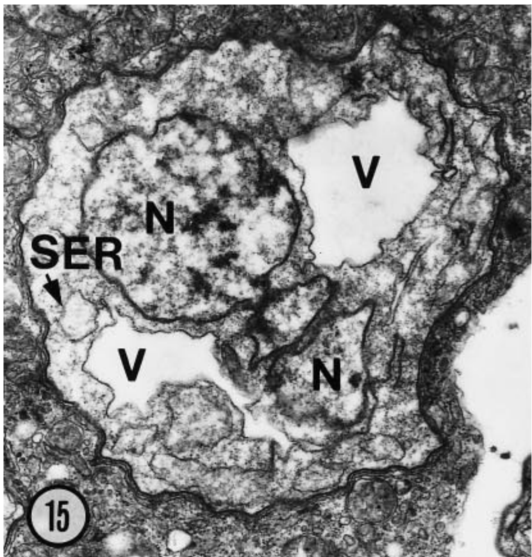
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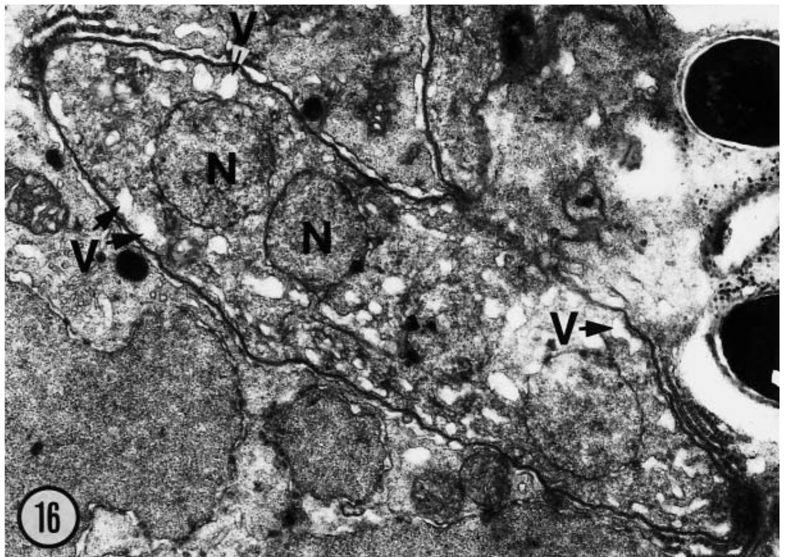
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Scanning electron microscopy

Untreated controls

Non-medicated mature spores of *G. anomala* had an oval outline and were slightly broader posteriorly. The surface of the spore was finely corrugated (Fig. 1).

Spores after treatment

After incubation with one of the benzimidazole derivatives, and especially when treatment was done at intervals, deep invaginations at the interior or the posterior end of the spores became obvious (Fig. 2).

Transmission electron microscopy

Untreated controls

All merogony stages of *G. anomala* were surrounded by cisternae of the smooth endoplasmic reticulum (SER) of their host cells. The cytoplasm of the uni- and the multinucleate meronts contained numerous ribosomes as well as some cisternae of the rough endoplasmic reticulum (RER) and SER (Figs. 3, 4). Sporogonial plasmodia were multinucleate stages. During the development of the sporogonial plasmodium, blisters were produced external to the plasmalemma as the first stage of the sporophorous vesicle membrane. These blisters contacted some places on the surface of the sporogonial plasmodium. The membrane between these points was thickened by the newly produced surface coat. A conspicuous kinetic center was present in a shallow depression of the nuclear envelope (Fig. 5). The SER and RER of these stages were larger than those of the meronts (Fig. 5). The daughter cells of the plasmodia were held together by narrow cytoplasmic constrictions (Fig. 6). In the newly formed, uninucleate sporoblast mother cells, the smooth and rough endoplasmic reticulum was abundant. The sporoblast was bordered by a dense coat (exospore layer) covering the plasmalemma (Fig. 7).

Mature spores were elongate-ovoid with a size of about 1.9–2.5 × 3–4 μm (Fig. 8). The spore wall consisted of a characteristic dense outer envelope and an electron-light inner zone. The spore extrusion apparatus was composed of the polar sac, the polar tube, and the polaroblast. The polar tube was coiled in the peripheral cytoplasm of the spore. Three pre-stage vacuoles were located at the posterior end of the immature spore.

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Figs. 9–16 Transmission electron micrographs of uni- and multinucleate meronts of *G. anomala* after treatment with different benzimidazole derivatives (*N* nucleus, *R* ribosome, *SER* smooth endoplasmic reticulum, *T* microtubulus, *V* vacuole caused by treatment). **Fig. 9** Uninucleate meront (1 μg albendazole/ml, 2 h). Note the enlargement of the smooth endoplasmic reticulum. ×18 200. **Fig. 10** Uninucleate meront (5 μg mebendazole/ml, 2 h). Note the enlargement of the smooth endoplasmic reticulum comparable to that observed after albendazole treatment. ×18 700. **Fig. 11** Uninucleate meront (10 μg mebendazole/ml, 2 h). Note the decrease in the number of ribosomes, and the bleaching of the cyto- and the karyoplasm, ×19 000. **Fig. 12** Uninucleate meront (5 μg fenbendazole/ml, 2 h). Note the dilatation of the smooth endoplasmic reticulum. ×17 600. **Fig. 13** Uninucleate meront (10 μg fenbendazole/ml, 6 h). Note the paucity of ribosomes, the enlargement of the smooth endoplasmic reticulum and the disintegration of the nuclear envelope. ×17 500. **Fig. 14** Uninucleate meront (50 μg fenbendazole/ml, 6 h). Note the microtubules within the karyoplasm. ×13 000. **Fig. 15** Multinucleate meront (10 μg mebendazole/ml, 6 h). Note the vacuolization of the cytoplasm, and the enlargement of the smooth endoplasmic reticulum. ×8800. **Fig. 16** Multinucleate meront (10 μg fenbendazole/ml, 6 h). Note the numerous small vacuoles close to the cell membrane. × 9400

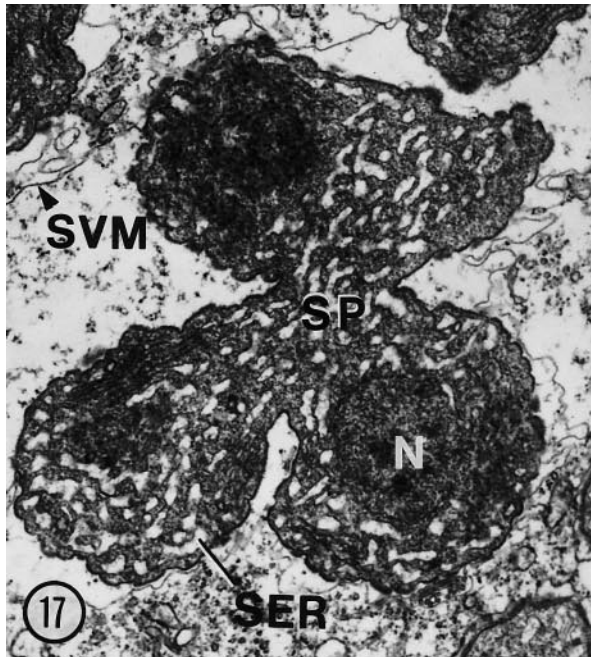
Parasites after treatment

Uninucleate meronts

On treatment with 1 mg/l albendazole for 2 h, the SER in the uninucleate meronts was significantly enlarged compared to that of the untreated controls (Fig. 9). An enlargement of SER was also observed when treatment was done with 1 or 5 mg/l mebendazole for 2 h (Fig. 10). Treatment with higher doses, regardless of whether albendazole, mebendazole, or fenbendazole was used, and also prolonged exposure periods caused additional damage in the uninucleate meronts. Most of them were swollen, and their outer contour was misshapen (Fig. 11). The number of ribosomes was significantly lowered compared to that of the non-medicated controls, and the karyoplasm of the uninucleate meronts was leached (Fig. 11). Incubation with fenbendazole (5 μg/ml, 2 h) led to comparable disorders in the uninucleate meronts as already described for albendazole and mebendazole (Fig. 12). In some cases, the uninucleate meronts showed not only a paucity of ribosomes and a gross enlargement of the SER, but also a complete disintegration of the nuclear structures (Fig. 13). When treatment was done with the highest dose (50 μg/ml, 6 h) of one of the benzimidazole derivatives, microtubules (25 μm in diameter) were detectable within the karyoplasm (Fig. 14).

Multinucleate meronts

Starting with a concentration of 10 μg/ml mebendazole for 6 h, the structures of multinucleate meronts were drastically changed in comparison to non-medicated controls. The cytoplasm was highly vacuolated (Fig. 15). The number of ribosomes was reduced, and the SER was considerably swollen. Treatment with 10 μg/ml fenbendazole for 6 h caused comparable damage, as seen after incubation with mebendazole but, in addition, numerous small vacuoles in the cytoplasm close to the limiting membrane were detected (Fig. 16).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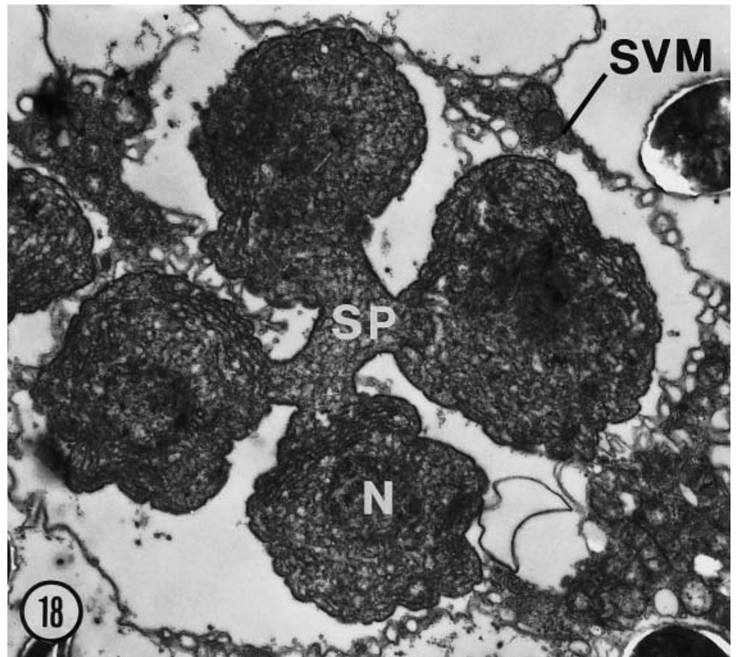
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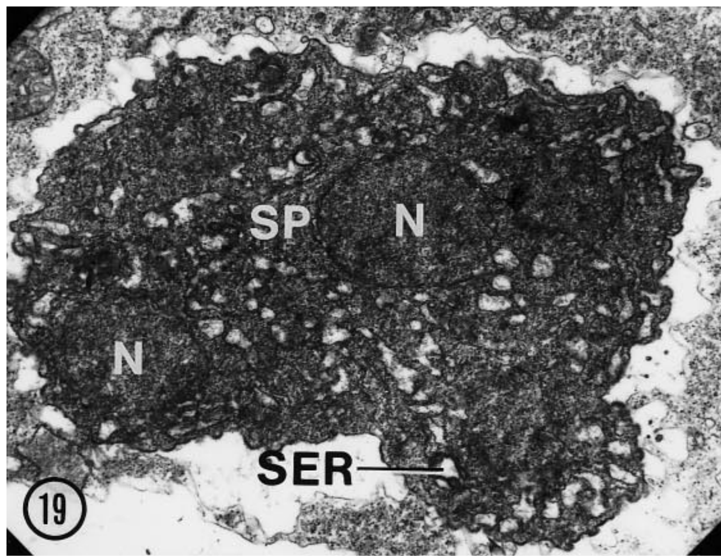
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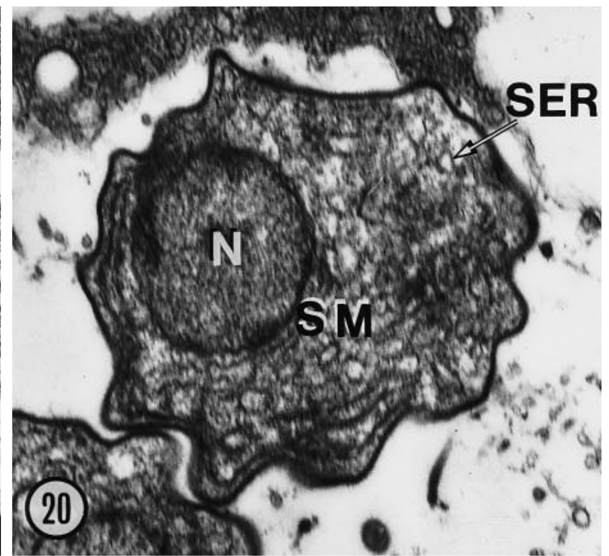
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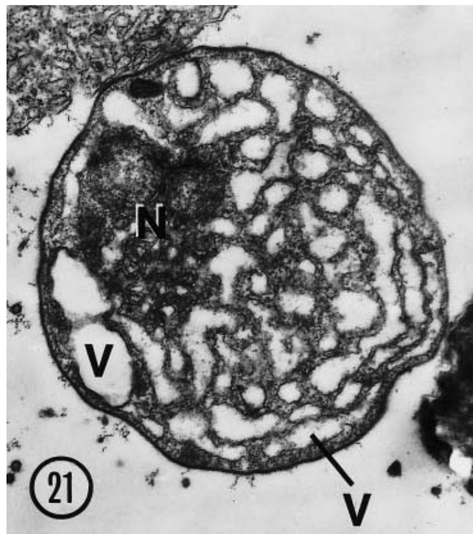
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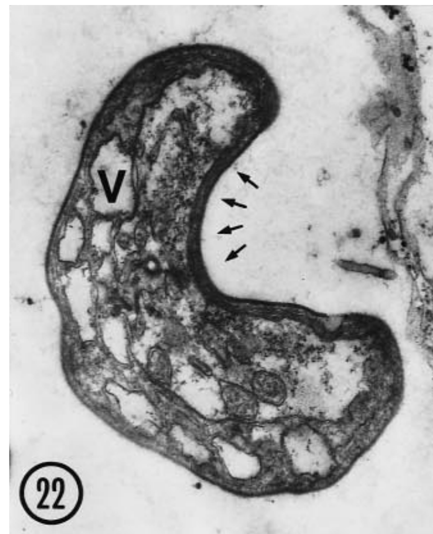
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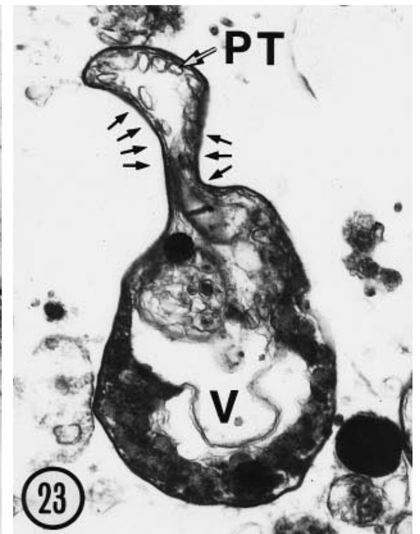
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Sporogonial plasmodia

When treatment with albendazole was done at the highest concentration (50 μg/ml, 6 h), only remnants of the sporophorous vesicle membrane were detectable in the sporogonial plasmodia (Fig. 17). In many cases, the sporogonial plasmodia showed bizarre shapes. Their SER was enlarged, and often the nuclei of the future sporoblasts were disintegrated (Fig. 17).

Treatment with mebendazole (10 μg/ml, 6 h) caused the structure of the sporophorous vesicle membrane which was formed by the sporogonial plasmodia to appear lumpy (Fig. 18). In many specimens investigated, the nuclear membranes were either no longer recognizable, or the nuclei were completely disintegrated. After initial treatment with fenbendazole (5 μg/ml, 2 h), the sporogonial plasmodia were misshapen (Fig. 19). The sporophorous vesicle membrane was largely destroyed. Similar to the multinucleate meronts, small vacuoles were apparent beneath the limiting cell membrane of the sporogonial plasmodia, and their SER was enlarged (Fig. 19).

Sporoblastic stages

Sporoblasts reacted very sensitively to incubation with either albendazole, mebendazole, or fenbendazole, and they often lay in sporophorous vesicles with discontinuous membrane borders. As shown with albendazole, even the lowest concentration (1 μg/ml, 2 h) caused dilatation of the SER in early sporoblasts (Fig. 20). Treatment with higher doses (10 μg/ml, 6 h) led to a gross vacuolization of the sporoblast cytoplasm (Fig. 21). In many specimens, the nuclear membranes were no longer detectable at the ultrastructural level, and within the karyoplasma, no discernible structures were seen (Fig. 21).

Figs. 17–23 Transmission electron micrographs of sporogonic stages of *G. anomala* after treatment with different benzimidazole derivatives (*N* nucleus, *PT* polar tube, *SER* smooth endoplasmic reticulum, *SM* sporoblast mother cell, *SP* sporogonial plasmodium, *SVM* sporophorous vesicle membrane). **Fig. 17** Sporogonial plasmodium (50 μg/ml albendazole, 6 h). The sporophorous vesicle membrane is destroyed in places. ×13 900. **Fig. 18** Sporogonial plasmodium (10 μg/ml mebendazole, 6 h). Note the lumpy appearance of the sporophorous vesicles membrane. ×12 200. **Fig. 19** Sporogonial plasmodium (5 μg/ml fenbendazole, 2 h). The sporophorous vesicle membrane is disrupted. Note the enlargement of the smooth endoplasmic reticulum. ×14 100. **Fig. 20** Sporoblast (1 μg/ml albendazole, 2 h). Note the dilatation of the smooth endoplasmic reticulum. ×15 500. **Fig. 21** Sporoblast (10 μg/ml mebendazole, 6 h). Note the gross vacuolization of the cytoplasm. ×15 200. **Fig. 22** Prespore (1 μg/ml mebendazole, 2 h). Due to the treatment, the prespore is malformed (*arrows*). Its cytoplasm is vacuolized. × 20 300. **Fig. 23** Prespore (1 μg/ml fenbendazole, 2 h). Note the malformation (arrows) and the thoroughly vacuolized cytoplasm. ×17 600

Prespore stages

Abnormal development of late sporoblastic stages was detected in places in the unmedicated controls, but it was nearly the rule after medication. Even the lowest dose of one of the benzimidazole derivatives caused malformation in the majority of prespores (Fig. 22, arrows). Their cytoplasm was extensively vacuolated, and in numerous sections, nuclei were not detectable. Incubation with fenbendazole (1 mg/l, 2 h) also led to malformation of the prespores (Fig. 23, arrows). In contrast to the controls, the precursors of the polar tubes were completely electron lucent without any discernible internal structures. The prespores were vacuolized throughout, with some depleted membranes. Nuclei were not detectable.

Mature spores

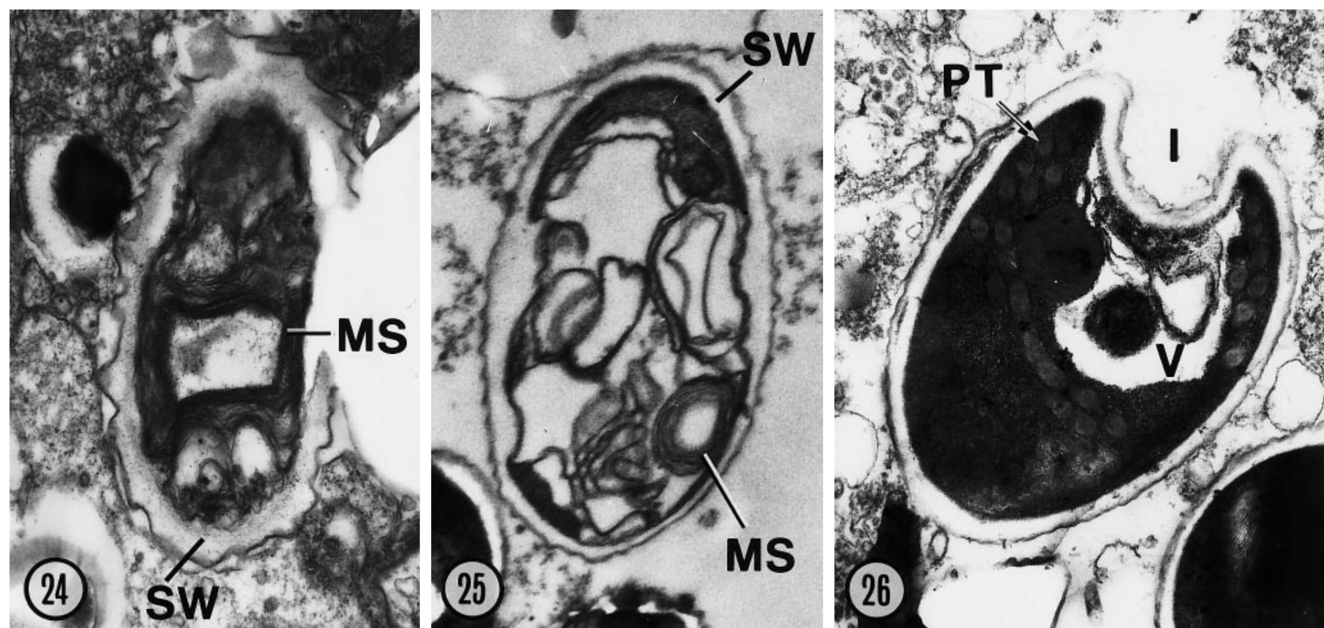
In contrast to the untreated controls, where normally developed spores were frequent, in the treated xenomas, abnormal spores were common. This was especially the case when treatment was achieved either with higher concentrations of one of the drugs tested (10 μg/ml; 2 or 6 h), or treatment was done at 36-h intervals with low concentrations (3 × 2 μg/ml, 6 h). After single treatment with albendazole (10 μg/ml, 2 h), the majority of the spores contained stacks of concentrically arranged membranes within the cytoplasm (Fig. 24). Comparable damage was observed after single treatment with 50 μg/ml fenbendazole for 6 h (Fig. 25). Intermittent treatment with fenbendazole caused deep invaginations in the anterior or posterior pole of mature spores (Fig. 26). Their polaroplast was frequently completely destroyed.

Drug tolerance of sticklebacks

Sticklebacks showed no visible adverse effects during bath treatment with either albendazole, mebendazole, or fenbendazole (1, 5, 10, 50, or 100 μg/ml, 100 h). After incubation in drug-containing water, the sticklebacks were monitored under normal rearing conditions for 20 days. No signs of toxic or other negative effects on the vital functions or on behavior were observed except after incubation with 100 μg/ml albendazole for 100 h, where after nine of the ten fish died within 16 days after the end of the test.

Infectivity of spores before and after treatment

When previously non-infected sticklebacks were fed with untreated spores via water fleas, eight of ten fish developed clear signs of *Glugea* infection starting with day 20 post-infection. After single treatment with 10 μg/ml of either albendazole, mebendazole or fenbendazole for 6 h and subsequent infectivity tests, five to seven fishes ($n = 10$ for each group) were infected with *G. anomala*. However,



Figs. 24–26 Transmission electron micrographs of mature spores of *G. anomala* after treatment with different benzimidazole derivatives (*I* invagination caused by treatment, *MS* membrane stack, *PT* polar tube, *SW* spore wall, *V* vacuole caused by treatment). **Fig. 24** Mature spore (10 μg/ml albendazole, 2 h). Note the concentrically arranged membrane stacks. ×17 000. **Fig. 25** Mature spore (50 μg/ml fenbendazole, 6 h). Note the membrane whirls caused by the treatment. ×17 400. **Fig. 26** Mature spore (3 × 2 μg/ml fenbendazole, 6 h, at 36-h intervals). Note the deep invagination at the anterior pole. ×21 400

when intermittently treated with either albendazole, mebendazole or fenbendazole (3 × 2 μg/ml, 6 h, at 36-h intervals), infection trials were successful in only one or two of the fishes ($n = 10$) for each group tested.

Discussion

The present study clearly indicates that each of the three benzimidazole derivates tested – albendazole, mebendazole, and fenbendazole – has significant deleterious effects on uni- and multinucleate meronts, sporogonial plasmodia, sporoblasts, and later sporogonic stages of the microsporidian species *G. anomala*. In addition, the formation of the sporophorous vesicle membrane was severely disturbed, and the membranous borders of already existing sporophorous vesicles were depleted.

In general, there were no significant differences in the kind of damage to the merogonic and the sporogonic stages of *G. anomala* caused by either albendazole, mebendazole or fenbendazole. A prominent drug-derived effect was the malformation of the merogonic and the sporogonic stages and of the majority of the mature spores. When studied at the cytological level, the effects of the different benzimidazole derivatives tested here comprised a decrease in the number of ribosomes, gross enlargement of the SER, vacuolization of the cytoplasm and, as observed in numerous samples, complete disintegration of the nuclear structures. When treatment was done with high doses (50 μg/ml), microtubules within the nuclei of the uninucleate meronts were apparent.

As seen in in vitro experiments with solutions of albendazole against *Encephalitozoon cuniculi*, the growth of meronts and sporonts continued in the absence of division so that grossly enlarged and distorted stages developed (Colbourn et al. 1994). The authors state that the mode of action of albendazole, and of related benzimidazole derivatives, is to a large extent the prevention of microtubule assembly, which in the case of susceptible microsporidian species will inhibit the formation of the intranuclear spindle, the only known case of microtubule formation in microsporidians.

The distorted and leached cytoplasm observed in the merogonic and sporogonic stages of *G. anomala* after medication was also reported for *E. cuniculi* as an effect following albendazole treatment (Colbourn et al. 1994). The authors argue that this effect in *E. cuniculi*, and also the paucity of ribosomes, is likely to result from the loss of cytoplasm from disrupted merogonic and sporogonic stages rather than prevention of ribosomal synthesis, since ribosomes were abundant in other samples which had other damage.

In the silkworm parasite, *Nosema bombycis*, clumping of chromatin in the nuclei, inhibition of spindle formation and also malformation of spores after exposure to albendazole has been shown by transmission electron microscopy (Haque et al. 1993). In addition, an enlargement of their nuclei and disruption of the nuclear membranes was also observed. An antimicrosporidial activity has also been demonstrated for another benzimidazole compound, benomyl (Hsiao and Hsiao 1973).

The antibiotic fumagillin, which has antimicrosporidial activity, also causes a depletion in the number of ribosomes in *E. cuniculi* (Shadduck 1980). The mode of action of fumagillin is believed to be by inhibition of DNA or RNA synthesis, which will immediately lead to a reduction in the number of ribosomes.

As revealed by ultrastructural investigations, the symmetric triazine derivative toltrazuril has deleterious effects on the merogonic and the sporogonic stages of *G. anomala*. The effects of toltrazuril comprised a decrease in the number of ribosomes, enlargement of the SER, depletion of the nuclear membrane, and destruction of the nuclear structures. In meronts just prior to entering the sporogony stage, the membrane of the sporophorous vesicle was disrupted (Schmahl and Mehlhorn 1989). The destruction of the dividing nuclei in the sporoblast mother cells indicated an inhibitory effect of the drug on nuclear division (Schmahl et al. 1990). In biochemical experiments, the influence of toltrazuril on two enzymes involved in pyrimidine synthesis and nuclear division was demonstrated by Harder and Haberkorn (1989). Similar effects against *G. anomala* were produced by another triazine derivative, HOE 092 V (Schmahl and Senaud 1996).

In the present study it was evident that each of the benzimidazole derivatives, albendazole, mebendazole, and fenbendazole, disturbs the intracellular development of the microsporid *G. anomala* by severely damaging its merogonic, sporogonic, and prespore stages as well as the mature spores. All the forms of damage were more pronounced after intermittent treatment. Since spore infectivity after interval treatment is far less than after single treatment, it seems likely that medicinal baths at 36-h intervals (3×2 µg/ml of either albendazole, mebendazole, or fenbendazole for 6 h) may reduce or even eliminate infections with susceptible microsporidian species in fish.

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