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ORIGINAL PAPER

In vitro and in vivo effects of flubendazole on *Echinococcus granulosus* metacestodes

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Abstract The aim of the present work was to determine the efficacy of flubendazole (FLBZ) against *Echinococcus granulosus* metacestodes by using in vitro and in vivo models. Groups of 50 microcysts developed in vitro, and groups of 10 peritoneal cysts were obtained from Balb C mice with experimental secondary infections of 8 months. The cysts were placed in Leighton tubes containing 10 ml of culture medium. FLBZ was added to the medium resulting in final concentrations of 5 and 1 μg/ml for mycrocysts treatment and 10, 5, and 1 μg/ml for murine cysts treatment. In vivo treatment was performed on 20 mice that developed an experimental secondary hydatid disease over a period of 11 months. FLBZ was given (1.5 mg/kg) by the oral route once a day for 50 days. A loss of turgidity was detected in all in vitro drug treated cysts irrespective of the drug concentration or parasite origin. Inspection of treated cysts by scanning electron microscopy (SEM) revealed that the germinal layer lost it characteristic multicelular structure. These results were confirmed on the ultrastructural level by transmission electron microscopy (TEM), treated metacestodes had undergone considerable degenerative changes after the in vitro treatment. The results obtained after the in vivo treatment with FLBZ showed no significant difference between the control and treated groups related to the weight of cyst masses. However, the ultrastructural study at TEM of cysts that developed in mice from the treated group revealed alterations in the germinal layer with the presence of numerous vacuoles. With regard to the ultrastructural study at SEM, only cellular debris of the germinal layer could be seen. In conclusion, the data obtained clearly demonstrate that in vitro and in vivo treatment with FLBZ is effective against *E. granulosus* metacestodes.

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Introduction

Human cystic echinococcosis (CE) is a zoonosis caused by the larval or metacestode stage of the tapeworm *Echinococcus granulosus*. The treatment of the disease can be looked at in two ways: surgical and chemotherapeutic. The drugs commonly used for anti-hydatid cysts treatment are benzimidazole carbamate derivatives (BZD), such as mebendazole (MBZ) and albendazole (ABZ). These anthelmintic drugs exert their action by binding to β-tubulin (Borgers and De Nollin 1975; Lacey 1988; Lubega and Prichard 1991), thereby inhibiting the polymerization of microtubules (Friedman and Platzer 1978). This induces degenerative alterations in the endoplasmic reticulum and mitochondria of the cells in the germinal layer, resulting in a blockage of glucose absorption, a glycogen depletion, and an increase in lysosomes with consequent cellular autolysis (Campbell and Blair 1974; Heath and Chevis 1974; Verheyen 1982; Luider et al. 1985; Pérez-Serrano et al. 2001). These BZD's have limited solubility and, therefore,

poor absorption after oral administration (Da Silva et al. 1997). Despite this, they are extensively used in hydatid disease, although long-term treatments are usually required, and adverse side effects with failed results may occur (El-On 2003).

Flubendazole (FLBZ) is a broad-spectrum anthelmintic available for use in human and veterinary medicine. Low in vivo efficacy of FLBZ against CE has been reported by different authors (Recco et al. 1984; Davis et al. 1986). Therefore, FLBZ is not considered to be a suitable drug for treatment of this disease. However, the rate of absorption, metabolism, and excretion of BZD anthelmintics fluctuate from drug to drug, and probably, adjusting the dose rate and/or the interval between doses may improve the clinical efficacy of FLBZ against CE.

MBZ, ABZ, and its metabolite albendazole sulphoxide (ABZSO) showed to be active against protoscoleces of *E. granulosus* in in vitro culture systems (Chinnery and Morris 1986; Morris et al. 1987; Pérez-Serrano et al. 1994). Casado et al. (1996) have developed a chemotherapeutic model for the in vitro screening of drugs against *E. granulosus* cysts, mainly studying the efficacy of ABZ and ABZSO. Moreover, (Pérez-Serrano et al. 1997) reported a high correlation between the results obtained for in vitro models and those obtained from in vivo models as well as in the experimental treatment of secondary echinococcosis in mice.

In a previous work, we reported the in vitro effect of FLBZ on *E. granulosus* protoscoleces (Elissondo et al. 2006). The aim of the present work was to determine the efficacy of FLBZ against *E. granulosus* metacestodes by using in vitro and in vivo models.

Materials and methods

Protoscoleces collection and culture

Protoscoleces of *E. granulosus* were collected aseptically from liver and lung hydatid cysts of infected cattle slaughtered in two abattoirs located in the southeast of Buenos Aires Province, Argentina. Vitality was assessed by muscular movements (evaluated under light microscope), motility of flame cells, and by the methylene blue exclusion test (Casado et al. 1986). The culture protocols were carried out as described previously (Elissondo et al. 2004) using medium 199 (Gibco) supplemented with 100 IU penicillin, 100 μg/ml streptomycin, 4 mg/ml glucose, and 20% (v/v) fetal calf serum. Cultures were maintained at 37°C, and the medium was changed every 3–4 days. After 50–60 days, microcysts developed under in vitro conditions were recovered and transferred to Leighton tubes for incubation with FLBZ.

Mouse infection

Balb C mice (body weight 25±5 g) were infected by intraperitoneal infection of 1,500 protoscoleces in 0.5 ml of medium 199 to produce experimental secondary hydatid disease. Animals were housed in a temperature-controlled (22±1°C), light-cycled (12-h light/dark cycle) room.

In vitro incubation procedures

(1) Groups of 50 microcysts developed in vitro were placed in Leighton tubes containing 10 ml of medium 199. (2) Groups of 10 peritoneal cysts obtained from Balb C mice with experimental secondary infections of 8 months standing were also placed in Leighton tubes containing 10 ml of culture medium. Tubes were maintained at 37°C without changes of medium during the entire drug incubation period (Casado et al. 1989). FLBZ (kindly provided by Janssen Animal Health, Beerse, Belgium) was dissolved in dimethyl sulphoxide (DMSO) at a drug concentration of 10 mg/ml and added to the medium, resulting in final concentrations of 5 and 1 μg/ml for microcysts treatment and 10, 5, and 1 μg/ml for murine cysts treatment. Cysts and microcysts incubated with a culture medium alone and with a culture medium containing DMSO were used as controls. Each experiment was repeated three times. Culture tubes were followed macro- and microscopically every day. Samples of cysts and microcysts from each of the dosing groups and the controls were taken every 8–10 days for up to 30 days and then fixed for electron microscopy. The criteria for cysts vitality assessment included the loss of turgidity, the collapse of cysts, and the ultrastructural observation of the germinal layer as described by Casado et al. (1996).

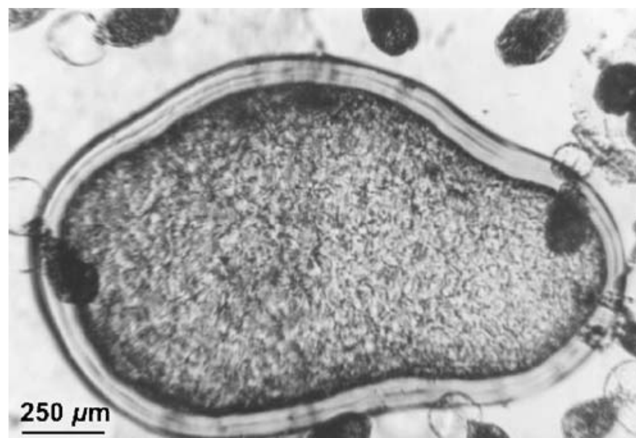

Fig. 1 Light microscopy of *E. granulosus* microcysts incubated in vitro with FLBZ. Control microcysts (×40)

Table 1 Time of appearance (postincubation days) of different indicators of tissue damage on *E. granulosus* murine cysts, after its incubation with FLBZ, under in vitro conditions

	Postincubation days			
	FLBZ concentrations (μg/ml)			
Parameters of the study	10	5	1	0
Loss of cyst turgidity	2	2	9	–
Appearance of collapsed cysts	6	6	10	–
Ultrastructural damage (MEB)	6	6	13	–

In vivo experiment

The treatment experiment was performed on 20 mice that developed an experimental secondary hydatid disease over a period of 11 months and were randomly divided into two groups of ten mice each (control group and FLBZ-treated group). FLBZ (Janssen Animal Health) was dissolved in 100 ml of acidified water (pH 1.5) and given by gavage for 50 days at a dose of 1.5 mg kg^{-1} day^{-1}. A solution of acidified water (pH 1.5) was given to control mice at the same time and dose. At the end of each treatment, mice were killed by cervical dislocation under ether anesthesia, and their peritoneal cavity was opened for removal of the hydatid cysts. Cysts from control and treated animals were separated and weighted and compared statistically by means of Student's *t*-test. Samples of parasite tissue were taken and fixed for electron microscopy.

Electron microscopy

Ultrastructure studies with scanning and transmission electron microscopy (SEM and TEM) were performed. For ultrastructure studies, samples were fixed with 3% glutaraldehyde in sodium cacodylate buffer for 72 h at 4°C. Then, several washes in cacodylate buffer were made. For SEM analysis, the specimens were dehydrated by sequential incubations in increasing concentrations of ethanol (50–100%) and were finally immersed in hexamethyl disilazane for 5 min, 1 h, and then, overnight. They were then sputter-coated with gold (100-Å thickness) and inspected on a JEOL JSM-6460 LV scanning electron microscope operating at 15 kV. For TEM analysis, samples were postfixed in 2% OsO4 in cacodylate buffer, followed by several washes in water. They were dehydrated in a graded acetone series and subsequently embedded in Spurr's resin. Polymerization of the resin was carried out at 70°C overnight. Sections 700-Å thick were cut off on a LKB ultramicrotome with a diamond knife, stained with uranyl acetate saturated solution (45 min) and lead citrate (20 min), and examined with a JEOL 100 CXII transmission electron microscope at 80 kV.

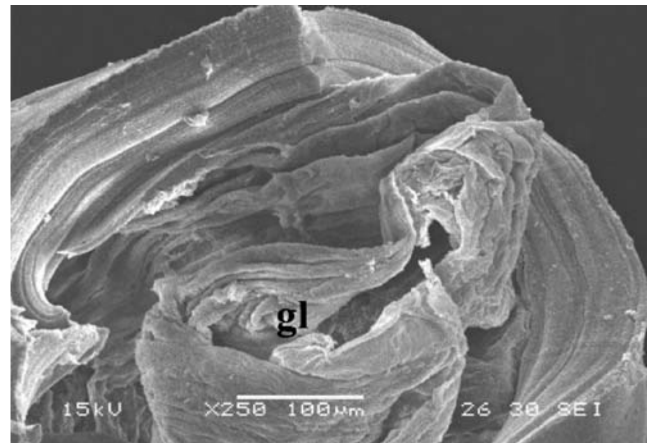

Fig. 3 SEM of *E. granulosus* metacestodes incubated in vitro with FLBZ. Murine cyst after 6 days postincubation (5 μg/ml). Germinal layer detached from laminated layer (*gl* germinal layer; ×250)

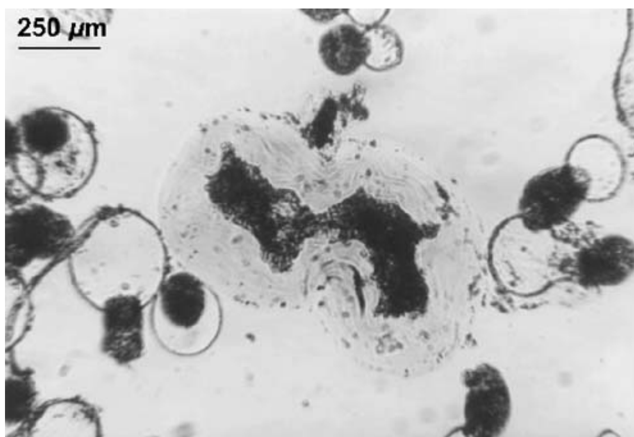

Fig. 2 Light microscopy of *E. granulosus* microcysts incubated in vitro with FLBZ. Altered microcyst after 3 days postincubation (1 μg/ml; ×40)

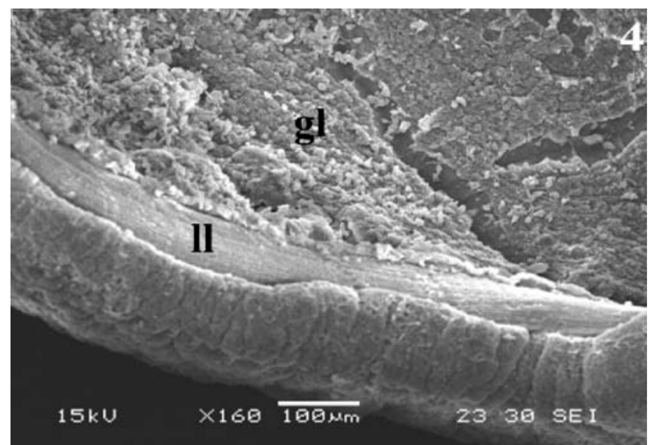

Fig. 4 SEM of *E. granulosus* metacestodes incubated in vitro with FLBZ. Control murine cyst with an intact germinal layer (*gl* germinal layer, *ll* laminar layer; ×160)

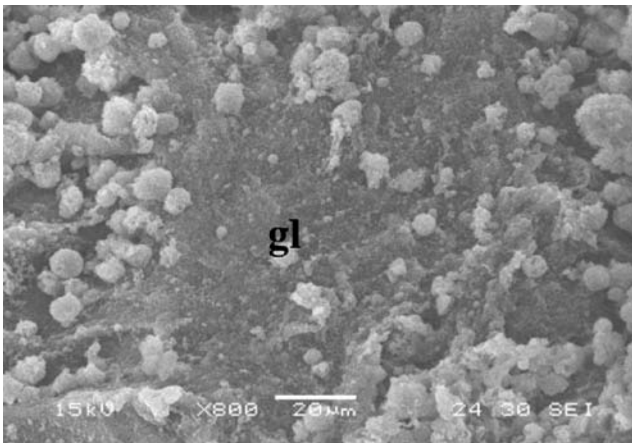

Fig. 5 SEM of *E. granulosus* metacestodes incubated in vitro with FLBZ. Murine cyst (13 days p.i., 1 μg/ml). The germinal layer is altered (*gl* germinal layer; ×800)

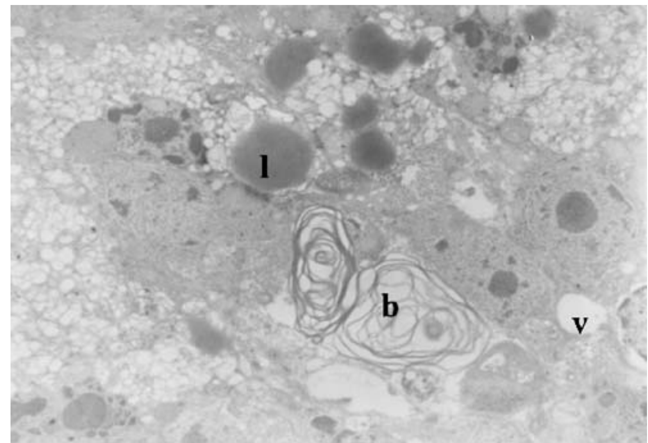

Fig. 7 TEM of *E. granulosus* metacestodes incubated in vitro with FLBZ. Treated microcyst (15 days p.i., 1 μg /ml). Note the presence of vacuoles (*v*), lipid droplets (*l*) and residual lamellar bodies (*b*; ×5,000)

Results

All control cysts (microcysts and murine cysts) appeared turgid with no observable collapse of the germinal membrane over the course of the in vitro experiment (Fig. 1). In contrast, a loss of turgidity was detected in all drug-treated cysts irrespective of the drug concentration or parasite origin. This effect was more rapidly detected in microcysts than in cysts obtained from mice. The in vitro results of the FLBZ treatment of hydatid murine cysts are shown in Table 1. Although damage was homogeneous within a single cyst, the process progressed at different rates in the different cysts. Table 1 indicates the first day on which a loss of cyst turgidity was optically detected in any of the cysts being incubated at the indicated drug concentration.

With the progress of degeneration, the germinal membrane detached from the laminated layer, and a necrotic central mass could be seen in both murine and cultured cysts (Figs. 2 and 3).

Inspection of control cysts by SEM revealed that they exhibited typical features of *E. granulosus* metacestodes, with a distinct acellular outer laminated layer and an intact germinal layer comprised of a multitude of different, morphologically intact, cell types (Fig. 4).

In contrast, after 6 days postincubation (p.i.) at the concentrations of 5 and 10 μg/ml, the germinal layer of murine cysts lost the characteristic multicellular structure clearly showing disintegrated areas. At 13 days p.i., less cells were observed in the germinal membrane of cysts incubated at 1 μg/ml (Fig. 5).

These results were confirmed on the ultrastructural level by TEM. Control and DMSO-treated cultures exhibited no ultrastructural alterations in parasite tissue during the whole incubation period (Fig. 6). In contrast, FLBZ-treated

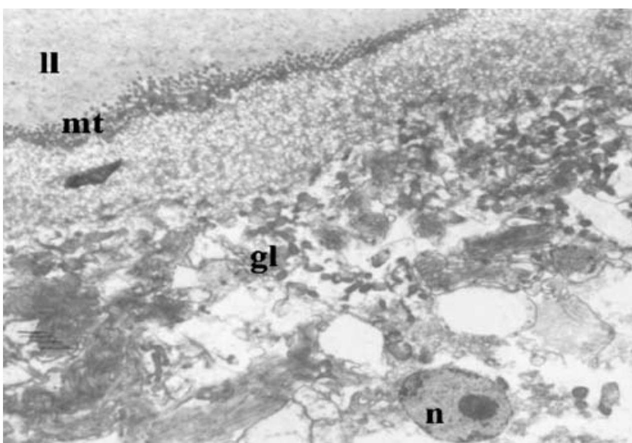

Fig. 6 TEM of *E. granulosus* metacestodes incubated in vitro with FLBZ. Control cyst (*ll* laminar layer, *mt* microtrichies, *gl* germinal layer, *n* nucleus; ×7,500)

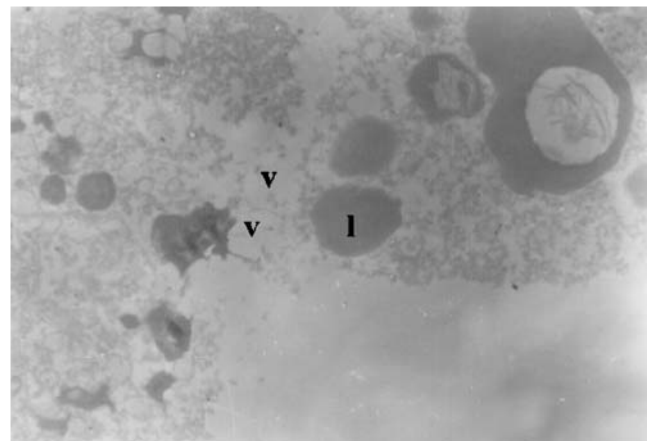

Fig. 8 TEM of *E. granulosus* metacestodes incubated in vitro with FLBZ. Treated murine cyst (25 days p.i., 10 μg/ml). The germinal layer is altered with the presence of lipid droplets and vacuoles (*v* vacuoles, *l* lipid droplets; ×5,000)

Table 2 Effect of FLBZ treatment on secondary *E. granulosus* infection with drug treatment starting on the 11-month postinfection

	Mice (n=20)	Wet weight (g) of cysts (mean±SD)	t-Test
Control	10	7.89±8.91	P>0.05
FLBZ	7	4.97±5.75	P>0.05

metacestodes underwent considerable degenerative changes after in vitro treatment. At 15 days p.i., the germinal membrane of microcysts was extensively distorted upon drug treatment with 5 and 1 μg/ml of FLBZ, showing vacuolated areas, numerous lipid droplets, and residual lamellar bodies (Fig. 7). The loss of microtrichies was also evident.

In murine cysts, after 13 days p.i., the presence of numerous vacuoles was observed. At 25 days p.i., the germinal layer was completely altered with the presence of lipid droplets and vacuoles (Fig. 8).

The results obtained using treatment with FLBZ at 1.5 mg kg^{-1} day^{-1} for 50 days are shown in Table 2. No significant difference between the control and treated groups was found related to the weight of cyst masses (P>0.05). Three mice from the treated group did not present cysts at the necropsy.

All cysts in the samples removed from control mice appeared turgid, showing no observable collapse of the germinal layer, in which no alteration in ultrastructure was detected (Fig. 9). However, the ultrastructural study at TEM of cysts that had developed in mice from the treated group revealed alterations in the germinal layer with the presence of numerous vacuoles (Fig. 10). Regarding with the ultrastructural study at SEM, only cellular debris of the germinal layer was observed (Fig. 11).

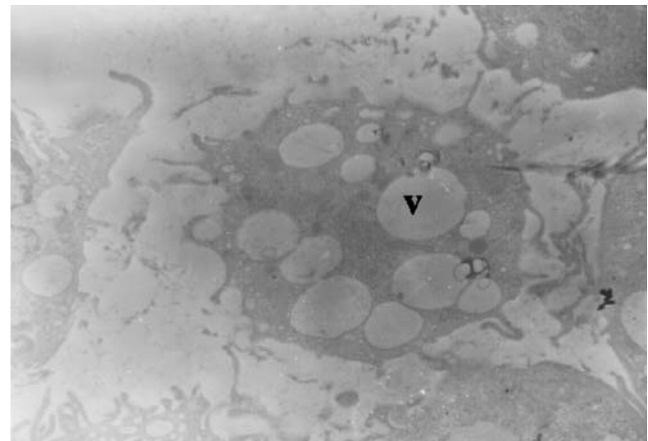

Fig. 10 Electron microscopy of *E. granulosus* cysts treated in vivo with FLBZ. Treated cyst (50 days p.i., 1.5 mg kg^{-1} day^{-1}). Note the presence of numerous vacuoles (v; ×8,000)

Discussion

In a previous study (Elissondo et al. 2006), the in vitro protoscolicidal effect of FLBZ on cultured *E. granulosus* has been established. This work describes the effect of FLBZ on *E. granulosus* metacestodes in in vitro and in vivo models.

The results of the in vitro treatment with FLBZ were similar in both microcysts and secondary murine cysts. The observed changes were identical to those reported by Casado et al. (1996). In contrast, they observed loss of turgidity at day 15 in microcysts incubated with 10 μg/ml of ABZ+ABZSO. In the present study, these changes were observed between the third and fifth day.

The employment of SEM and TEM allowed us to examine, at an ultrastructural level, the effects induced by

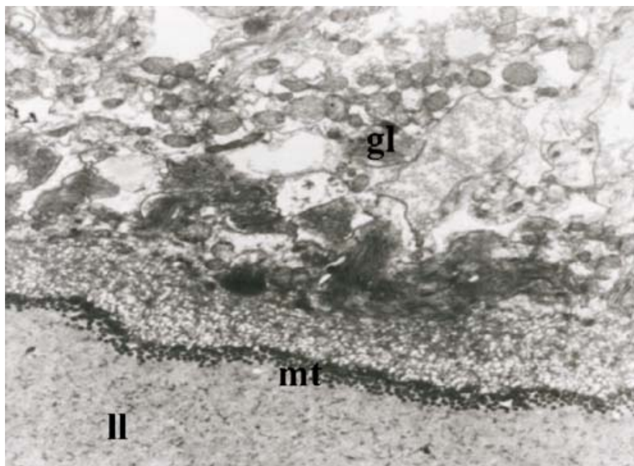

Fig. 9 Electron microscopy of *E. granulosus* cysts treated in vivo with FLBZ. Control cyst (11-month postinfection; *ll* laminar layer, *mt* microtrichies, *gl* germinal layer, *n* nucleus; ×5,000)

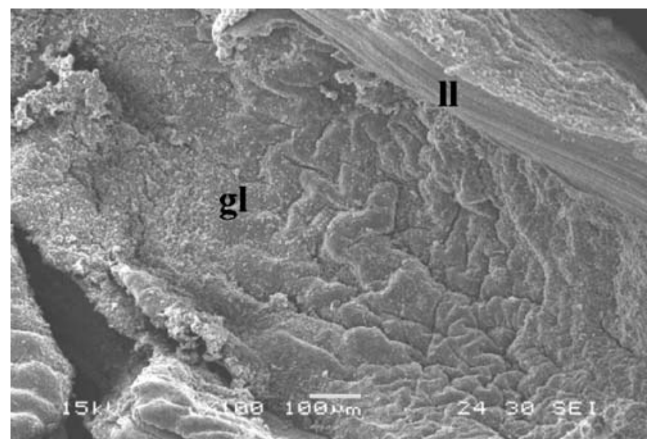

Fig. 11 Electron microscopy of *E. granulosus* cysts treated in vivo with FLBZ. Treated cyst (1.5 mg kg^{-1} day^{-1}, 50 days). Germinal layer is altered (*gl* germinal layer, *ll* laminar layer; ×100)

FLBZ on *E. granulosus* metacestodes. Thus, we demonstrated that identical ultrastructural changes were induced in each of the three incubation conditions. However, at the minimal used concentration (1 μg/ml), the alterations were detected later.

Our results are consistent with those reported by Casado et al. (1996), Urrea-Paris (1999), and Walker et al. (2004) working with other anthelmintic drugs. At the ultrastructural level, it was noted that the tissue damage induced after in vitro treatment occurred in the germinal membrane. Additionally, the damage was identical in both microcysts and murine cysts. The ultrastructural changes observed by SEM included the loss of the characteristic multicellular appearance of the germinal membrane. The changes observed by TEM were increased vacuolation of the distal cytoplasm, damage on germinal membrane, the presence of lipid droplets, and lamellar residual bodies. (Pérez-Serrano et al. 1994) suggested that the vacuolation of cyst tissue could reflect general tissue stress because it appears after incubation with different drugs.

The treatment with FLBZ produces loss of turgidity in the cultured cysts in less time than in murine cysts. This could be explained by the fact that the cultured cysts are smaller and the laminated layer is thinner than in murine cysts (Casado et al. 1992; 1996). Moreover murine cysts have a fibrous layer being another obstacle to drug diffusion. The biggest concern in in vitro drug assessment is the criterion of cyst viability. The criterion used in this work was the loss of cyst turgidity, which has been widely used by other investigators as a marker of cyst nonviability (Heath et al. 1975; Gemmell and Parmenter 1983; Richards et al. 1988; Casado et al. 1996; Urrea-París et al. 1999, 2000). In the current report, that criterion was corroborated by the marked ultrastructural tissue damage detected by electron microscopy.

In vivo treatment with FLBZ did not exhibit a statistically relevant effect with regard to parasite weight reduction in secondary hydatidosis. However, all murine cysts treated showed ultrastructural alterations in the germinal layer similar to those detected in the cysts treated in vitro with FLBZ. This finding has also been described by other authors working with praziquantel (Gemmell and Parmenter 1983; Xiao et al. 1988; Urrea-París et al. 1999, 2002).

In conclusion, the data obtained clearly demonstrated that the in vitro and in vivo treatment with FLBZ is effective against *E. granulosus* metacestodes. More exhaustive evaluation of FLBZ chemotherapeutic effect in vitro at a lower concentration than 1 μg/ml should be undertaken. Moreover, further in vivo (pharmacokinetics and efficacy) experiments will be required to evaluate the potential of FLBZ as a useful anthelmintic for the treatment of human CE.

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References

Borgers M, De Nollin S (1975) Ultrastructural changes in *Ascaris suum* intestine after mebendazole treatment in vivo. J Parasitol 61:110–122

Campbell WC, Blair LS (1974) Treatment of the cystic stage of *Taenia crassiceps* and *Echinococcus multilocularis* in laboratory animals. J Parasitol 60:1053–1054

Casado N, Rodríguez-Caabeiro F, Hernández S (1986) In vitro survival of *Echinococcusgranulosus* protoescolices in several media, at 4°C and 37°C. Z Parasitenkd 72:273–278

Casado N, Rodríguez-Caabeiro F, Jiménez A, Criado A, De Armas C (1989) In vitro effects of levamisol and ivermectin against *Echinococcus granulosus* protoscoleces. Int J Parasitol 19:945–947

Casado N, Criado A, Jimenez A, De Armas C, Brasa C, Pérez-Serrano J, Rodríguez-Caabeiro F (1992) Viability of *Echinococcus granulosus* cysts in mice following cultivation in vitro. Int J Parasitol 22:335–339

Casado N, Pérez Serrano J, Denegri G, Rodríguez-Caabeiro F (1996) Development of a chemotherapeutic model for in vitro drug screening in *Echinococcus granulosus* cysts: assessment of the effects of albendazole and albendazole sulphoxide combination-therapy. Int J Parasitol 26:59–65

Chinnery JB, Morris DL (1986) Effect of albendazole sulphoxide on viability of hydatid protoscoleces in vitro. Trans R Soc Trop Med Hyg 80:815–817

Da Silva N, Guyatt H, Bundy D (1997) Anthelmintics—a comparative review of their clinical pharmacology. Drugs 53:769–788

Davis A, Pawlowski ZS, Dixon H (1986) Multicenter trials of benzimidazole-carbamates in human echinococcosis. Bull World Health Organ 64:333–388

Elissondo MC, Dopchiz MC, Brasesco M, Denegri G (2004) *Echinococcus granulosus*: first report of microcysts formation from protoscoleces of cattle origin using the in vitro vesicular culture technique. Parasite 11(4):415–418

Elissondo MC, Dopchiz MC, Ceballos L, Alvarez L, Sánchez Bruni S, Lanusse C, Denegri G (2006) In vitro effects of flubendazole on *Echinococcus granulosus* protoscoleces. Parasitol Res 98:317–323

El-On J (2003) Benzimidazole treatment of cystic echinococcosis. Acta Trop 85:243–252

Friedman PA, Platzer EG (1978) Interaction of anthelmintic benzimidazoles and benzimidazole derivatives with bovine brain tubulin. Biochim Biophys Acta 544:605–614

Gemmell MA, Parmenter SN (1983) Effects of prazicuantel against eggs of *Taenia hydatigena* and protoscoleces and metacestodes of *Echinococcus granulosus*. Vet Med Rev 1:3–9

Heath DD, Chevis RAF (1974) Mebendazole and hydatid cysts. Lancet 2:218–219

Heath DD, Christie MJ, Chevis RA (1975) The lethal effect of mebendazole on secondary *Echinococcus granulosus* cysticerci

of *Taenia pisciformis* and etrathyridia of *Mesocestoides corti*. Parasitology 70:273–285

Lacey E (1988) The role of the cytoskeletal protein, tubulin, in the mode of action and mechanism of drug resistance to benzimidazoles. Int J Parasitol 18:885–936

Lubega G, Prichard R (1991) Interaction of benzimidazole anthelmintics with *Haemonchus contortus* tubulin: binding affinity and anthelmintic efficacy. Exp Parasitol 73:203–209

Luider PJ, Robotti G, Meister FP, Bircher J (1985) High oral doses of Mebendazole interfere with growth of larval *Echinococcus multilocularis* lesions. J Hepatol 1:369–377

Morris DL, Chinnery JB, Ubhi C (1987) A comparison of the effects al albendazole, its sulphone metabolite, and mebendazole on the viability of protoscoleces of *Echinococcus granulosus* in an in vitro culture system. Trans R Soc Trop Med Hyg 81:804–806

Pérez-Serrano J, Casado N, Denegri G, Rodríguez-Caabeiro F (1994) The effects of albendazole and albendazole sulphoxide combination-therapy on *Echinococcus granulosus* in vitro. Int J Parasitol 24:219–224

Pérez-Serrano J, Denegri G, Casado N, Rodríguez-Caabeiro F (1997) Treatment of experimental echinococcosis with albendazole and albendazole sulphoxide. Int J Parasitol 27:1341–1345

Pérez-Serrano J, Martínez J, Denegri G, Casado N, Bodega G, Rodríguez-Caabeiro F (2001) In vitro effect of albendazole and albendazole sulphoxide on 70 and 60 kDa heat shock proteins in *Echinococcus granulosus* protoscolices. Res Rev Parasitol 61:67–71

Recco P, Hornus E, Frejevus J, Micheau P, Bessieres MH, Roques C, Linas, MD (1984) Hydatidose pleurale disseminee et hydatidoses hepatiques. Traitment post-operatoire par flubendazole a propos de 3 cas. Bull Soc Fr Parasitol 3:115–118

Richards KS, Morris DL, Daniels D, Riley EM (1988) *Echinococcus granulosus*: the effects of praziquantel, in vivo and in vitro, on the ultrastructure of equine strain murine cysts. Parasitology 96:323–336

Urrea-Paris MA, Moreno MJ, Casado N, Rodríguez-Caabeiro F (1999) *Echinococcus granulosus*: praziquantel treatment against the metacestode stage. Parasitol Res 85:999–1006

Urrea-Paris MA, Moreno MJ, Casado N, Rodríguez-Caabeiro F (2000) In vitro effect of praziquantel and albendazole combination therapy of the larval stage of *Echinococcus granulosus*. Parasitol Res 86:957–964

Urrea-Paris MA, Moreno MJ, Casado N, Rodríguez-Caabeiro F (2002) Relationship between the efficacy of praziquantel treatment and the cystic differentiation in vivo of *Echinococcus granulosus* metacestode. Parasitol Res 88:26–31

Verheyen A (1982) *Echinococcus granulosus*: the influence of mebendazole therapy on the ultrastructural morphology of the germinal layer of hydatid cysts in man and in mice. Z Parasitenkd 67:55–65

Walker M, Rossignol JF, Torgerson P, Hemphill A (2004) In vitro effects of nitazoxanide on *Echinococcus granulosus* protoscoleces and metacestodes. J Antimicrob Chemother 54(3):609–616

Xiao SH, You JQ, Yang YQ, Guo HF, Zhang CW, Chai JJ, Zhang WL (1988) Histological alterations and drug concentrations in endocysts and cysts fluid of hydatid cysts harboring in mice treated with praziquantel. Acta Pharmacol Sin 9:461–464