

Flubendazole and ivermectin in vitro combination therapy produces a marked effect on *Echinococcus granulosus* protoscoleces and metacestodes

M. Celina Elissondo · L. Ceballos · L. Alvarez ·
S. Sánchez Bruni · C. Lanusse · G. Denegri

Received: 26 April 2009 / Accepted: 30 April 2009 / Published online: 15 May 2009
© Springer-Verlag 2009

Abstract The aim of the present work was to evaluate the in vitro efficacy of the flubendazole (FLBZ) and ivermectin (IVM) combination against *Echinococcus granulosus* protoscoleces and metacestodes. Protoscoleces and groups of ten peritoneal cysts obtained from BALB/c mice were incubated with the two drugs, either separately or in combination, at the following final concentrations: 10 µg/mL FLBZ, 1 µg/mL FLBZ, 1 µg/mL IVM, 10 µg/mL FLBZ + 1 µg/mL IVM, and 1 µg/mL FLBZ + 1 µg/mL IVM. The maximum protoscolicidal effect was found with the combination 10 µg/mL FLBZ + 1 µg/mL IVM. After 1 day of incubation, the presence of numerous blebs in the tegument of protoscoleces was observed. Ultrastructural studies revealed that the primary site of damage was the tegument of the parasite. The effect of the two drugs on hydatid cysts obtained from mice was more

rapidly detected in cysts treated with the combination of FLBZ + IVM than when drugs were used separately. Ultrastructural studies revealed that the germinal layer of treated cysts lost the multicellular structure feature and underwent considerable degenerative changes after in vitro treatment. The outcomes obtained demonstrated the favorable effect of the combination of FLBZ and IVM against *E. granulosus*.

Introduction

Cystic echinococcosis (CE) caused by the larval stage of the cestode *Echinococcus granulosus* is a life-threatening disease of serious public health and economic concern of global proportion (Eckert and Deplazes 2004). Traditionally, the treatment of echinococcosis relies primarily on surgery and/or chemotherapy, and the treatment strategy depends largely on different factors, such as metacestode size and location, viability status, the interaction between the expanding parasite and the adjacent host tissue, complicating bacterial or fungal infection, and potential complications related to cyst rupture and spillage of protoscoleces (Kern 2003, 2006).

Mebendazole (MBZ) and albendazole (ABZ) were initially tested in animal models and were first used in humans in the late 1970s (Bekhti et al. 1977; Schantz et al. 1982; Morris et al. 1983). Approximately 20–40% of cases do not respond favorably to such chemotherapy. Praziquantel (PZQ), a heterocyclic pyrazinoisoquinoline derivative, has been proposed for use together with benzimidazoles in CE patients (Cobo et al. 1998).

MBZ, ABZ, its metabolite albendazole sulfoxide (ABZSO), and flubendazole (FLBZ) were shown to be

M. C. Elissondo (✉) · G. Denegri
Laboratorio de Zoonosis Parasitarias, Departamento de Biología,
Facultad de Ciencias Exactas y Naturales,
Universidad Nacional de Mar del Plata (UNMDP),
Funes 3250,
7600 Mar del Plata, Argentina
e-mail: mceliss@mdp.edu.ar

L. Ceballos · L. Alvarez · S. Sánchez Bruni · C. Lanusse
Laboratorio de Farmacología, Departamento de Fisiopatología,
Facultad de Ciencias Veterinarias, Universidad Nacional del
Centro de la Provincia de Buenos Aires (UNCPBA),
Campus Universitario,
7000 Tandil, Argentina

M. C. Elissondo · L. Ceballos · L. Alvarez · S. Sánchez Bruni ·
C. Lanusse · G. Denegri
Consejo Nacional de Investigaciones Científicas y Técnicas
(CONICET),
Buenos Aires, Argentina

active against protoscolecemes and metacestodes of *E. granulosus* under in vitro and in vivo conditions (Chinnery and Morris 1986; Morris et al. 1987; Pérez Serrano et al. 1994, 1997; Casado et al. 1996; Elissondo et al. 2006, 2007; Ceballos et al. 2006). On the other hand, PZQ exhibits a high efficacy against protoscolecemes and metacestodes in animal experiments (Urrea-Paris et al. 1999, 2000, 2002) and it was suggested that PZQ exerts a substantial effect against metacestodes and protoscolecemes when applied in combination with ABZ (Urrea-Paris et al. 1999).

Ivermectin (IVM), a macrocyclic lactone endectocide, is used to control nematode and arthropod parasites that cause diseases such as lymphatic filariasis, strongyloidosis, and scabies in humans and parasitic gastroenteritis in farm animals and different ectoparasites as well (McKellar and Benchaoui 1996). Campbell et al. (1983) suggested the total lack of efficacy of IVM on cestodes. However, Casado et al. (1989) found a marked protoscolicidal effect in in vitro incubations. These contradictory results may be explained by the different methodologies used. Thus, Martínez et al. (1999) observed an alteration in heat shock proteins after the in vitro incubation of *E. granulosus* protoscolecemes with IVM, and Pérez-Serrano et al. (2001) later found a tegumental depolarization on the protoscolecemes incubated in vitro with IVM that could be fundamental for the survival of the parasite.

Casado et al. (2002) studied the combination of IVM with ABZ and they demonstrated an improvement of IVM

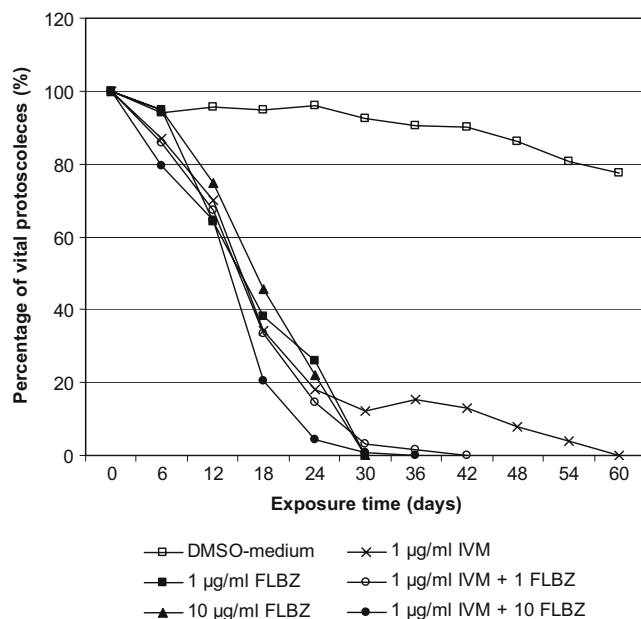


Fig. 1 Survival of *E. granulosus* protoscolecemes after exposure to FLBZ, IVM, and the combination of both drugs. Each point represents the mean percentage of vital protoscolecemes from three different experiments

efficacy when it is combined with ABZ. This treatment also killed protoscolecemes and metacestodes in less time than ABZ alone.

In previous works, we reported the in vitro effect of FLBZ on *E. granulosus* protoscolecemes and metacestodes (Elissondo et al. 2006, 2007). The aim of the present work was to evaluate the in vitro efficacy of the FLBZ and IVM combination against *E. granulosus* protoscolecemes and metacestodes.

Materials and methods

Protoscolecemes collection and culture

Hydatid cysts from the liver and lungs of naturally infected cattle were obtained from an abattoir located in the southeast of Buenos Aires Province, Argentina. Protoscolecemes were collected aseptically and washed several times

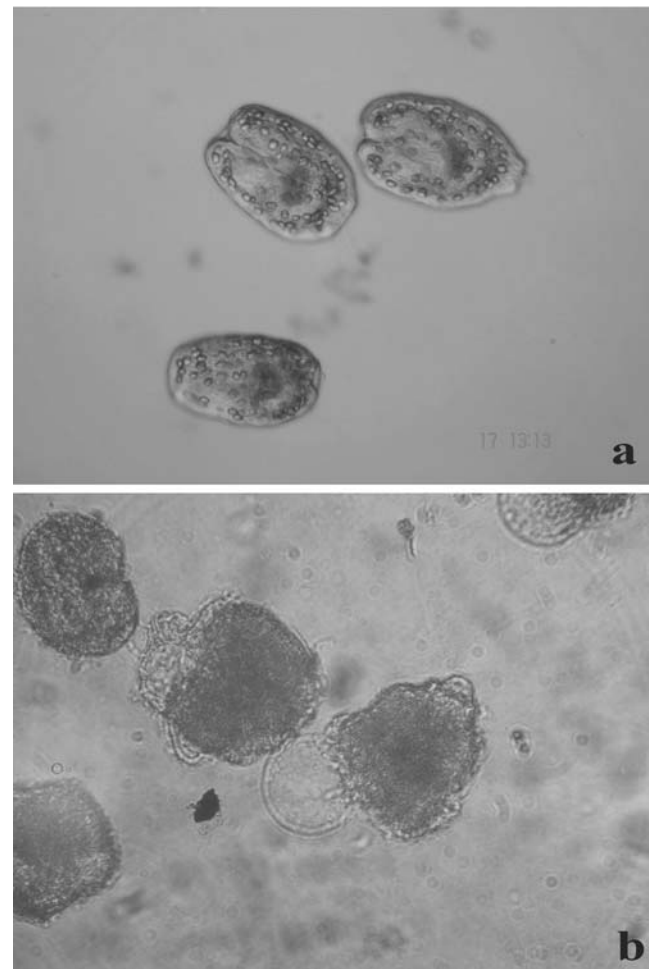
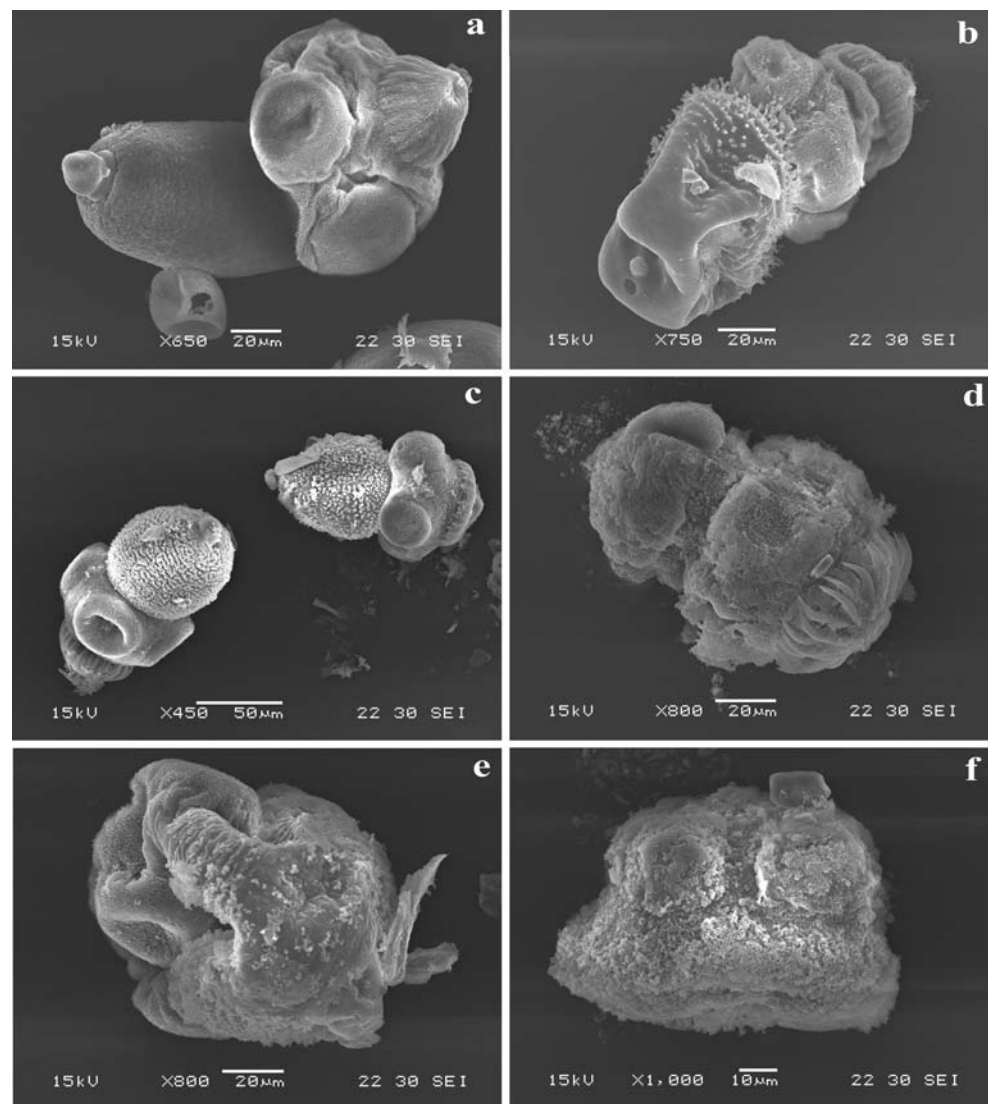


Fig. 2 Light microscopy of *E. granulosus* protoscolecemes incubated in vitro with FLBZ + IVM. **a** Control protoscolecemes ($\times 100$). **b** Altered protoscolecemes after 1 day p.i. with 10 µg/mL FLBZ + 1 µg/mL IVM ($\times 100$)

Fig. 3 SEM of *E. granulosus* protoscoleces incubated in vitro with FLBZ and IVM. **a** Evaginated control protoscolec. **b** Protoscolec incubated with IVM alone (1 $\mu\text{g}/\text{mL}$) during 12 days. Note tegumental alterations of the soma region. **c** Altered protoscoleces after 12 days p.i. with the combination 1 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM. **d–f** Protoscoleces incubated with 10 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM (12 days p.i.). Note the loss of morphology, rostellar disorganization, loss of hooks, shedding of microtriches, and formation of tegumental vesicles



with phosphate-buffered saline (pH 7.2). Viability was assessed by the methylene blue exclusion test (Elissondo et al. 2004). Moreover, other criteria, such as muscular movements, morphological perfectness of the whole body, and motility of flame cells, were taken into account to confirm the viability.

Mouse infection and collection of cysts

BALB/c mice (8 weeks old at the start of the experiments) were infected by intraperitoneal infection of 1,500 protoscoleces in 0.5 mL of medium 199 to produce experimental secondary hydatid disease. The animals were housed in a temperature-controlled ($22\pm 1^\circ\text{C}$), light-cycled (12-h light/dark cycle) room.

At 8 months post-infection, mice with experimental secondary CE were euthanized, and necropsy was carried out immediately thereafter. At necropsy, the peritoneal

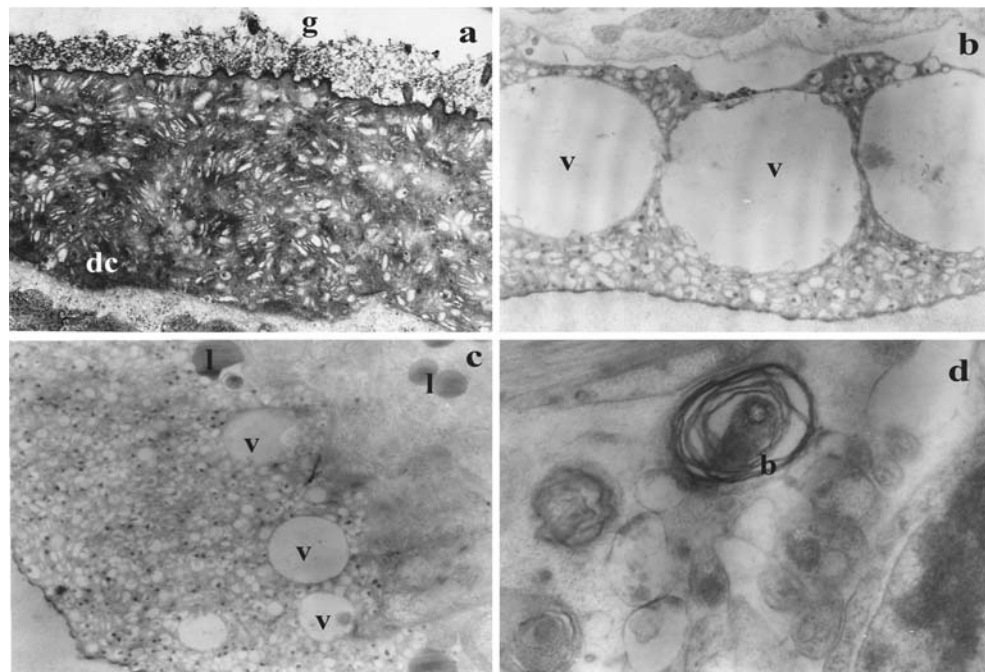
cavity was opened and the hydatid cysts were carefully removed.

Drugs and in vitro incubation procedures

FLBZ (kindly provided by Janssen Animal Health, Beerse, Belgium) and IVM (Sigma, USA) were dissolved in dimethyl sulfoxide (DMSO) at a drug concentration of 1 and 0.1 mg/mL. The two drugs were added to the medium either separately or in combination at the following final concentrations: 10 $\mu\text{g}/\text{mL}$ FLBZ (31.9 μM), 1 $\mu\text{g}/\text{mL}$ FLBZ (3.19 μM), 1 $\mu\text{g}/\text{mL}$ IVM (1.14 μM), 10 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM, and 1 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM.

Protoscoleces (1,500 per Leighton tube) were cultured in medium 199 (Gibco), supplemented with 100 IU penicillin, 100 $\mu\text{g}/\text{mL}$ streptomycin, and 4 mg/mL glucose. Cultures were performed in 10 mL of incubation medium at 37°C

Fig. 4 TEM of *E. granulosus* protoscoleces incubated in vitro with FLBZ + IVM. **a** Soma region of a control protoscolec (12 days p.i.; *g* glycocalix, *dc* distal cytoplasm; $\times 6,700$). **b** Treated protoscolec (12 days p.i., 1 $\mu\text{g}/\text{mL}$ IVM). Note the presence of large vacuoles (*v*) in the distal cytoplasm. **c–d** Protoscolec incubated with 10 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM (12 days p.i.). Internal tissue was severely affected. **c** Note the presence of vacuoles (*v*) and lipid droplets (*l*; $\times 6,700$). **d** Residual lamellar bodies (*b*; $\times 10,000$)



without changes of medium (Elissondo et al. 2006). Protoscoleces incubated with culture medium containing 20 μl DMSO served as controls. Each experiment was repeated three times.

Groups of ten peritoneal cysts obtained from BALB/c mice were also placed in Leighton tubes containing 10 mL of culture medium. The tubes were maintained at 37°C without changes of medium during the entire drug incubation period (Elissondo et al. 2007). The cysts incubated with the culture medium containing DMSO were used as controls. Each experiment was repeated three times.

Evaluation by light and electron microscopy

Culture tubes with protoscoleces were followed up microscopically everyday to determine the appearance of morpho-

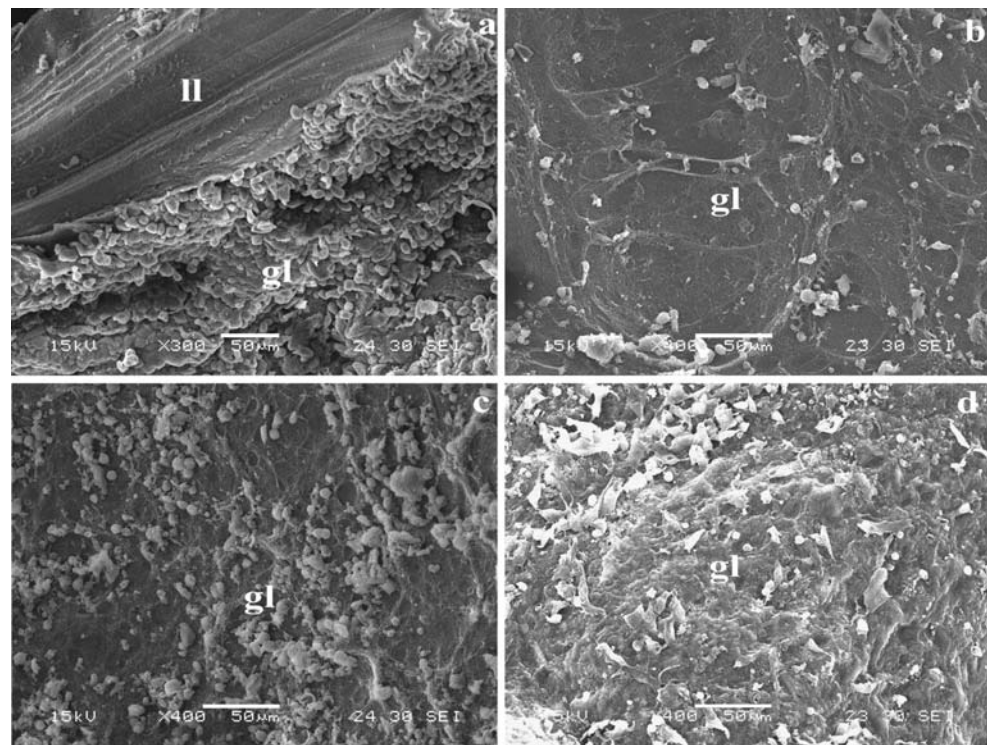
logical alterations. Samples of protoscoleces (approximately 70–90 protoscoleces in 0.5 mL of incubation medium) from each of the dosing groups and the controls were taken every 5 to 6 days for viability assessment using the methylene blue exclusion test. Additionally, ultrastructure studies with scanning and transmission electron microscope (SEM and TEM, respectively) were performed. For ultrastructure studies, protoscoleces cultures were processed as described by Elissondo et al. (2006).

Culture tubes with cysts were followed macroscopically and microscopically everyday. Samples of cysts from each of the dosing groups and the control were taken every 6 days for up to 12 days and then fixed for electron microscopy. The criteria for cysts vitality assessment included loss of turgidity, collapse of cysts, and ultrastructural observation of the germinal layer as described by Elissondo et al. (2007).

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

Parameters of the study	Days p.i.					
	Drug concentrations ($\mu\text{g}/\text{mL}$)					
	Control	1 FLBZ	10 FLBZ	1 IVM	1 FLBZ + 1 IVM	10 FLBZ + 1 IVM
Loss of cyst turgidity	–	9	2	6	2	1
Appearance of collapsed cysts	–	10	6	9	6	2

Fig. 5 SEM of *E. granulosus* metacestodes incubated in vitro with FLBZ + IVM. **a** Control murine cyst with an intact germinal layer (12 days p.i.; *gl* germinal layer, *ll* laminar layer). **b** Murine cyst incubated in vitro with 10 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM (12 days p.i.). Note the extensive damage of the germinal layer. Only cellular debris could be observed. **c** Murine cyst (1 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM, 12 days p.i.). The germinal layer is altered. **d** Murine cyst (1 $\mu\text{g}/\text{mL}$ IVM, 12 days p.i.). Germinal layer showing disintegrated areas



Results

Effects of FLBZ + IVM on *E. granulosus* protoscoleces

The survival of *E. granulosus* protoscoleces after exposure to FLBZ, IVM, and FLBZ + IVM combination is shown in Fig. 1. Control protoscoleces viability was $96.1 \pm 0.8\%$ after 24 days of incubation (Fig. 1). Viability decreased slowly, reaching $77.7 \pm 7.5\%$ at day 60. The maximum protoscolicidal effect was found with the combination 10 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM (Fig. 1); viability was reduced to $4.4 \pm 0.3\%$ after 24 days of incubation compared with $14.5 \pm 4.7\%$ with 1 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM, $18.2 \pm 4.8\%$ with 1 $\mu\text{g}/\text{mL}$ IVM, $22.2 \pm 0.8\%$ with 10 $\mu\text{g}/\text{mL}$ FLBZ, and $26 \pm 1.1\%$ with 1 $\mu\text{g}/\text{mL}$ FLBZ.

The results of the viability tests coincide with the tissue damage observed at the structural level. Protoscoleces incubated with DMSO (control group) revealed no changes in structure throughout the experimental period (Fig. 2a). After 1 day of incubation, the presence of numerous blebs in the tegument of protoscoleces treated with 10 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM was observed by inverted microscope (Fig. 2b). Other alterations such as rostellar disorganization, loss of hooks, and contraction of posterior region could be seen after 2 days of incubation. With the other concentrations and combinations of drugs, the same alterations could be observed later, between 3 and 6 days post-incubation (p.i.).

These results were confirmed on the ultrastructural level by SEM and TEM. Control cultures exhibited no ultrastructural alterations in parasite tissue during the whole incubation period (Figs. 3a and 4a). In contrast, morphological and ultrastructural damages were detected in treated protoscoleces. The primary site of damage was the tegument of the parasite.

At 12 days p.i., observations by SEM and TEM of protoscoleces incubated with IVM alone revealed the presence of tegumental alterations, contraction of the soma region (Fig. 3b), and the presence of large vacuoles in the distal cytoplasm (Fig. 4b). On the other hand, microtriches remained unaltered. The ultrastructural effects observed after FLBZ + IVM incubation were greater than those caused by the drugs incubated separately, although vitality percentages at that time were rather similar (Fig. 1). Moreover, the combination 1 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM produced less marked alterations than 10 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM (Fig. 3c–f). The ultrastructural changes included tegumental alterations (Fig. 3c), rostellar disorganization, loss of hooks, and shedding of microtriches of the scolex region (Fig. 3d). Formation of tegumental vesicles was observed in several protoscoleces (Fig. 3e). In some protoscoleces, loss of morphology was evident (Fig. 3f). Ultrastructural studies by TEM revealed the severely affected internal tissue, resulting in an increase of vacuoles and lamellar residual bodies (Fig. 4c, d).

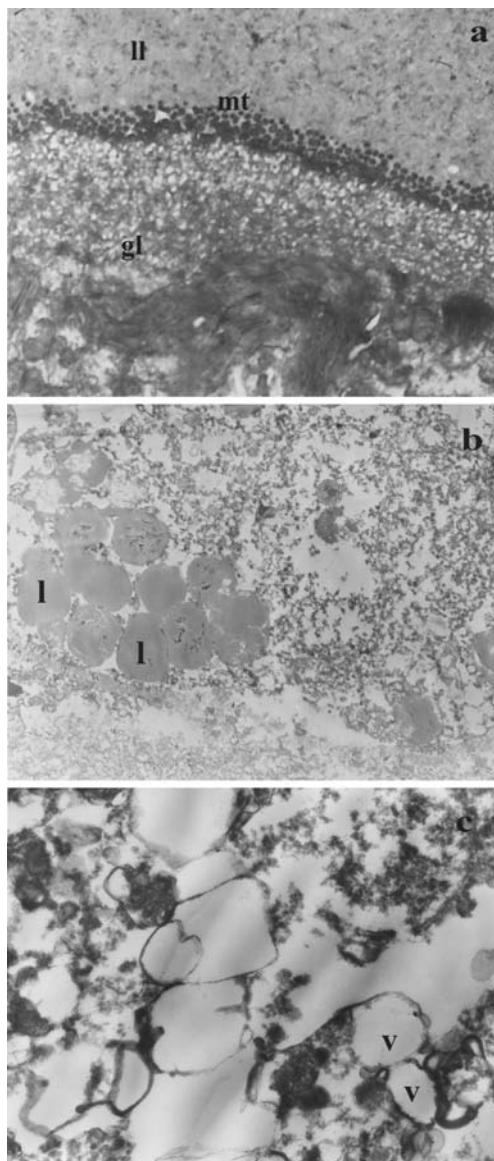


Fig. 6 TEM of *E. granulosus* metacestodes incubated in vitro with FLBZ + IVM. **a** Control cyst (*ll* laminar layer, *mt* microtriches, *gl* germinal layer; $\times 7,500$). **b** Treated murine cyst (12 days p.i., 1 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM). The internal tissue is altered with the presence of lipid droplets (*l*; $\times 8,000$). **c** Murine cyst incubated during 12 days with 1 $\mu\text{g}/\text{mL}$ IVM. Note the presence of numerous vacuoles (*v*)

Effects of FLBZ + IVM on *E. granulosus* cysts

The in vitro results of the incubation of hydatid cysts obtained from mice with the different drugs are shown in Table 1. The effect was more rapidly detected in cysts treated with the combination of FLBZ + IVM than when drugs were used separately.

Control cultures exhibit no ultrastructural alterations in parasite tissue during the whole incubation period (Figs. 5a and 6a). Studies by SEM revealed that the germinal layer of treated cysts lost the multicellular

structure feature (Fig. 5b–d). There was a correlation between the intensity of damage and the drug and/or combination assayed. This fact coincides with the macroscopic changes presented in Table 1. As was mentioned for protoscoleces, the combination 10 $\mu\text{g}/\text{mL}$ FLBZ + 1 $\mu\text{g}/\text{mL}$ IVM produced greater ultrastructural effects than when the drugs were incubated separately (Fig. 5b).

These results were confirmed on the ultrastructural level by TEM (Fig. 6). After 12 days p.i., treated metacestodes underwent considerable degenerative changes after in vitro treatment. The presence of numerous vacuoles and lipid droplets was observed (Fig. 6b, c).

Discussion

In previous studies (Elissondo et al. 2006, 2007), the in vitro and in vivo effect of FLBZ on *E. granulosus* protoscoleces and metacestodes has been established. The present work is the first report of the effect of FLBZ and IVM combination against *E. granulosus* protoscoleces and metacestodes.

There are very few studies on the effects of IVM on cestodes. Casado et al. (1989) demonstrated a rapid protoscolicidal activity of IVM at 100 and 10 $\mu\text{g}/\text{mL}$. Moreover, Pérez-Serrano et al. (2001) recorded a depolarization of the protoscolex tegument which precedes the cestodicidal effect. Later on, Casado et al. (2002) reported that the effects of the in vitro IVM and IVM + ABZ combined treatment against protoscoleces and metacestodes were different. They studied the combination of ABZ + IVM in order to check for possible synergic effects. A higher susceptibility of the parasite was observed when it was incubated with the drug combination.

FLBZ was shown to be more effective and killed protoscoleces and metacestodes in less time than ABZ (Elissondo et al. 2006, 2007). In this work, the combination FLBZ + IVM not only exerted a marked effect on the larval stages of the parasite, but this effect was also observed early compared with the ABZ + IVM combination.

Protoscoleces cultured with FLBZ + IVM were killed considerably faster than protoscoleces cultured with ABZ + IVM. After 18 days of exposure to FLBZ + IVM, viability was approximately $20 \pm 1.4\%$ and it was reduced to $4.4 \pm 0.3\%$ after 24 days of incubation. Casado et al. (2002) reported a vitality of 35% after 18 days of culture with ABZ + IVM, but they did not describe what happened after 18 days of incubation.

Metacestodes incubated with FLBZ + IVM underwent considerable degenerative changes in less time than when the drugs were used separately. Moreover, the observed changes were identical to those reported by Casado et al. (2002). In contrast, they observed loss of turgidity after

3 days in cysts incubated with ABZ + IVM. In this work, these changes were observed during the first day of the experiment.

As evidenced in our experiments, the incubation of drugs separately, as well as in combination, produced ultrastructural alterations with the tegument of the parasite as the primary site of damage. However, treatment efficacy was considerably improved by applying the FLBZ + IVM combination. Ultrastructural changes observed in protoscolexes included tegumental alterations, rostellar disorganization, loss of hooks, shedding of microtriches of the scolex region, and the internal tissue being severely affected.

On the other hand, the ultrastructural effects on cysts were observed on the germinal layer. An increased vacuolization of the distal cytoplasm and the presence of lipid droplets were observed. The intensity of the damage depends on the drug and/or combination assayed. The combination 10 µg/mL FLBZ + 1 µg/mL IVM produced profound morphological and degenerative changes, killing almost all cells of the germinal layer.

Our results at the ultrastructural level are consistent with those reported by Casado et al. (2002). In this work, they suggested that the treatment with IVM produced a depolarization on the surface of the tegument in the initial stage of incubation (3 h), before the ultrastructural changes occur. This depolarization, together with the ultrastructural effects, is, however, not enough to produce the death of the parasite. But, since the tegument is the contact surface of the parasite with the drug, its depolarization, together with the effect of benzimidazole carbamate on the protoscolexes tubulin, could cause more damage to the parasite tissue leading to death.

The outcomes obtained demonstrated the favorable effect of the combination of FLBZ and IVM against *E. granulosus*. However, the IVM concentration (1 µg/mL) used in the present experimental work was much higher than that observed in the plasma of IVM-treated humans in which, after a dose of 15 mg/kg, an IVM peak plasma concentration of 0.045 µg/mL was reported (Fink and Porras 1989). Further thorough studies of FLBZ + IVM chemotherapeutic effect in vitro at a concentration lower than 1 µg/mL should be undertaken.

Additionally, after the in vivo coadministration of both FLBZ and IVM, potential pharmacokinetic interactions between components may occur and need to be addressed. In fact, pharmacokinetic interaction between ABZ and IVM has been recently reported in sheep (Alvarez et al. 2008). Although it was unclear at which level ABZ/metabolites and IVM interact, two possible explanations were as follows: (1) IVM-induced inhibition of ABZ metabolism or (2) drug-to-drug interaction via drug efflux transporters-mediated mechanisms. Moreover, in vivo studies will be

required to evaluate the potential of the combination as a useful option for the treatment of human CE.

Acknowledgements The authors acknowledge Dr. Leo Van Leemput and Dr. Kathleen Vlamincx (Janssen Animal Health, Beerse, Belgium) and Dr. Gustavo Viana (Janssen, Buenos Aires, Argentina) for providing the FLBZ used in the present experimental work and for their critical review of this manuscript. The help of Dr. González and Sr. Chasma is gratefully appreciated. This work was supported by the PICT 02 No. 01-12535, BID 1201/OC-AR.

References

- Alvarez L, Lifschitz A, Entrocasso C, Manazza J, Mottier L, Borda B, Virkel G, Lanusse C (2008) Understanding the pharmacokinetic interaction between ivermectin and albendazole following their combined use in lambs. *J Vet Pharmacol Ther* 31:230–239
- Bekhti A, Schaaps JP, Capron M, Dessaint JP, Santoro F, Capron A (1977) Treatment of hepatic hydatid disease with mebendazole: preliminary results in four cases. *Br Med J* 2:1047–1051
- Campbell WC, Fisher MH, Stapley EO, Albers-Schonberg G, Jacob TA (1983) Ivermectin: a potent new antiparasitic agent. *Science* 221:823–828
- Casado N, Rodríguez-Caabeiro F, Jiménez A, Criado A, De Armas C (1989) In vitro effects of levamisole and ivermectin against *Echinococcus granulosus* protoscolexes. *Int J Parasitol* 19:945–947
- 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
- Casado N, Moreno MJ, Urrea-París MA, Rodríguez-Caabeiro F (2002) Could ivermectin have a synergic effect with albendazole in hydatidosis therapy? 1. In vitro studies. *Parasitol Res* 88:153–159
- Ceballos L, Álvarez L, Sánchez Bruni S, Elissondo C, Dopchiz M, Denegri G, Torrado J, Lanusse C (2006) Development of a cyclodextrin-based flubendazole formulation to control secondary echinococcosis: pharmacokinetics, hydatid cyst morphology and efficacy in mice. *J Vet Pharmacol Ther* 29:85–86
- Chinnery JB, Morris DL (1986) Effect of albendazole sulphoxide on viability of hydatid protoscolexes in vitro. *Trans R Soc Trop Med Hyg* 80:815–817
- Cobo F, Yarnoz C, Sesma B, Fraile P, Aizcorbe M, Trujillo R (1998) Albendazole plus praziquantel versus albendazole alone as a pre-operative treatment in intra-abdominal hydatidosis caused by *Echinococcus granulosus*. *Trop Med Int Health* 3:462–466
- Eckert J, Deplazes P (2004) Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev* 17:107–135
- Elissondo MC, Dopchiz MC, Brasesco M, Denegri G (2004) *Echinococcus granulosus*: first report of microcysts formation from protoscolexes of cattle origin using the in vitro vesicular culture technique. *Parasite* 11:415–418
- Elissondo MC, Dopchiz MC, Ceballos L, Alvarez L, Sánchez Bruni S, Lanusse C, Denegri G (2006) In vitro effects of flubendazole on *Echinococcus granulosus* protoscolexes. *Parasitol Res* 98:317–323
- Elissondo MC, Ceballos L, Dopchiz MC, Andresiuk MV, Alvarez L, Sánchez Bruni S, Lanusse C, Denegri G (2007) In vitro and in vivo effects of flubendazole on *Echinococcus granulosus* metacestodes. *Parasitol Res* 100:1003–1009

- Fink E, Porras A (1989) Pharmacokinetics of ivermectin in animal and humans. In: Campbell W (ed) Ivermectin and abamectin. Springer, New York, pp 113–130
- Kern P (2003) *Echinococcus granulosus* infection: clinical presentation, medical treatment and outcome. *Langenbecks Arch Surg* 388:413–420
- Kern P (2006) Medical treatment of echinococcosis under the guidance of Good Clinical Practice (GCP/ICH). *Parasitol Int* 55:273–282
- Martínez J, Pérez-Serrano J, Bernardina WE, Rodríguez-Caabeiro F (1999) *Echinococcus granulosus*: in vitro effects of ivermectin and praziquantel on HSP60 and HSP70 levels. *Exp Parasitol* 93:171–180
- McKellar Q, Benchaoui H (1996) Avermectins and milbemycins. *J Vet Pharmacol Ther* 19:331–351
- Morris DL, Dykes PW, Dickson B, Marriner SE, Bogan JA, Burrows FG (1983) Albendazole in hydatid disease. *Br Med J* 286:103–104
- Morris DL, Chinnery JB, Ubhi C (1987) A comparison of the effects of 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, Grossman C, Urrea-París MA, Denegri G, Casado N, Rodríguez-Caabeiro F (2001) Depolarization of the tegument precedes morphological alterations in *Echinococcus granulosus* protoscoleces incubated with ivermectin. *Parasitol Res* 87:804–807
- Schantz PM, van den Bossche H, Eckert J (1982) Chemotherapy for larval 235 echinococcosis in animals and humans: report of a workshop. *Z Parasitenkd* 67:5–26
- Urrea-París MA, Moreno MJ, Casado N, Rodríguez-Caabeiro F (1999) *Echinococcus granulosus*: praziquantel treatment against the metacestode stage. *Parasitol Res* 85:999–1006
- Urrea-París 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-París 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