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Enhanced chemoprophylactic and clinical efficacy of albendazole formulated as solid dispersions in experimental cystic echinococcosis

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ABSTRACT

Cystic echinococcosis is a chronic, complex, and still neglected disease. Although albendazole has demonstrated efficacy, only about one-third of patients experience complete remission or cure and 30–50% of treated patients develop some evidence of a therapeutic response. Different strategies have been developed in order to improve the albendazole water solubility and dissolution rate. The aim of the current work was to investigate the chemoprophylactic and clinical efficacy of an albendazole:poloxamer 188 solid dispersion formulation on mice infected with *Echinococcus granulosus* metacestodes. Albendazole formulated as solid dispersion had greater chemoprophylactic and clinical efficacy than albendazole alone. The improved in therapeutic efficacy could be a consequence of the increase in the systemic availability of albendazole sulfoxide. The work reported here demonstrates that in vivo treatment with albendazole:poloxamer 188 impairs the development of the hydatid cysts. This new pharmacotechnically based strategy could be a suitable alternative for treating cystic echinococcosis in humans.

1. Introduction

Cystic echinococcosis (CE), a zoonosis caused by the larval stage of *Echinococcus granulosus*, is characterized by long term growth of hydatid cysts in humans and mammalian intermediate hosts (McManus et al., 2012). This parasitic infection is a chronic, complex, and still neglected disease (Brunetti et al., 2011).

Currently four treatment approaches are in use: surgery, PAIR (puncture, aspiration, injection of protoscolicidal agent, reaspiration), chemotherapy with benzimidazoles (BZ), and watch and wait for inactive, clinically silent cysts (Stojkovic et al., 2009). The appropriate treatment depends on cyst characteristics (for hepatic cysts, size and stage are the most important criteria), the therapeutic resources available, and the physician's preference. The level of evidence supporting one therapeutic modality over the other is low because only few prospective, randomized studies comparing different treatments are available (Brunetti and White, 2012).

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Surgery is usually complemented by antiparasitic drugs, and in inoperable cases, chemotherapy is the only option. The drugs commonly used for anti-hydatid cysts treatment are BZ, such albendazole (ABZ) and mebendazole (McManus et al., 2012). ABZ belongs to type II biopharmaceutical classification system, with low aqueous solubility (0.01 mg/ml in water at 25 °C) and high permeability (Jung et al., 1998). Consequently, this compound has to be administrated at higher doses or as multiple doses in order to provide therapeutic concentrations and acceptable anthelmintic efficacy (Cook, 1990). According to WHO recommendations, ABZ is given in daily doses of 10–15 mg/kg of body weight in two divided doses postprandially for 3–6 month (WHO, 2001). Although albendazole has demonstrated efficacy, only about one-third of patients experience complete remission or cure and 30–50% of treated patients develop some evidence of a therapeutic response. Furthermore, between 20 and 40% of patients with hydatid cysts do not respond to medical management with ABZ (Moro and Schantz, 2009).

Therapeutic failures following oral administration with ABZ have been primarily linked to the poor drug absorption rate resulting in low drug level in plasma and hydatid cysts (Daniel-Mwambete et al., 2004). It is well known that the slow dissolution rate of ABZ leads generally to a poor and erratic absorption from the gastro-intestinal tract (Castro et al., 2012). Moreover, the low solubility drugs offer only few formulation alternatives, limiting the administration routes available (Alanazi et al., 2007; Vogt et al., 2008; Castro et al., 2012). Therefore, the increasing of aqueous solubility and dissolution rate of ABZ is a relevant goal to optimize the chemotherapeutic treatment of CE.

Different strategies have been developed in order to improve to ABZ water solubility and dissolution rate such as preparation of oil in water emulsion (Shuhua et al., 2002), incorporation into liposomes (Wen et al., 1996) and complexation with cyclodextrins (Kata and Schauer, 1991). Increased systemic bioavailability of albendazole was also reported when drug co-administered with a fatty meal (Lange et al., 1988), fruit juice (Nagy et al., 2002), cosolvent (Torrado et al., 1997), or with surfactants (del Estal et al., 1994). In addition, several clinical studies have demonstrated that enhanced systemic availability of the parent drug/active metabolite obtained by increased drug absorption correlates with an improved antiparasitic effect (Torrado et al., 1997; Wen et al., 1996; Mingjie et al., 2002; Shuhua et al., 2002; García et al., 2003; Ceballos et al., 2006, 2008, 2009; Liu et al., 2012).

Solid dispersions (SDs) are one of the most successful strategies to improve drug dissolution rate of poorly soluble drugs. SDs are molecular mixtures of poorly water soluble drugs in hydrophilic carriers, which present a drug release profile that is driven by the polymer properties (Vasconcelos et al., 2007). Different materials have been evaluated as carriers. The first SDs generation involved the use of crystalline carries (Levy, 1963; Sekiguchi et al., 1964) and sugars (Kanig, 1964), while for the second generation several types of hydrophilic polymers such as polyethylene glycols (Wang et al., 2004; Janssens et al., 2008), polyvinylpyrrolidone (Marín et al., 2002; Konno et al., 2008) among others, have been evaluated. Recently, some studies evidenced that the dissolution rate may be improved using carriers, which possess surface activity or self-emulsifying properties. This third generation of SDs were more efficient at enhancing bioavailability of poorly soluble drugs and SDs thus obtained were more stable owing mainly to a reduction of drug recrystallization (Vasconcelos et al., 2007).

Poloxamers are polyoxyethylene–polyoxypropylene block copolymer nonionic surfactants that have been widely used as wetting and solubilizing agents. Furthermore, these compounds are used in a variety of oral, parenteral, and topical pharmaceutical formulations and are generally regarded as nontoxic and nonirritant materials (Castro et al., 2012). Poloxamers are not metabolized in the body (Collett and Popli, 2000). Previous works have demonstrated a significant increase on dissolution rate of ABZ using Poloxamer 188 (P 188) as carrier in SDs or physical mixtures (Castro et al., 2010, 2012). Moreover, the in vitro dissolution rate of ABZ formulated as SDs showed an acceptable correlation with the in vivo pharmacokinetic studies. Increased systemic availability was obtained when ABZ was administered as ABZ:P 188 SDs, with a 50% enhancement in systemic exposure (AUC values) compared to treatment with a simple ABZ suspension. Consistently, the Cmax increased 130% following treatment with P 188 based SDs ABZ formulation. The enhanced bioavailability of ABZ in SDs containing P 188 as carrier could be attributed to the improved dissolution rate and the surfactant effects of this carrier (Castro et al., 2012).

The aim of the current work was to investigate the chemoprophylactic and clinical efficacy of an ABZ:P 188 solid dispersion formulation on mice infected with *E. granulosus* metacestodes.

2. Materials and methods

2.1. Chemicals

For the preparation of SDs the following materials were used: ABZ (Pharmaceutical grade, Parafarm, Buenos Aires, Argentina), and POLOXAMER 188 (BASF, Germany). All other reagents were of analytical grade.

2.2. ABZ formulations

SDs were prepared by melting of ABZ and poloxamer (1:1) in a water bath at 63 °C. The mixtures were homogenized by stirring. The resulting homogenous preparations were rapidly cooled and pulverized. The 212-micron particle size fraction was obtained by sieving and kept in a screw-capped glass vial until use.

ABZ suspension (2.1 mg/ml) was prepared by dissolution of ABZ pure standard in deionized water (pH = 7.0) under shaking (12 h). The ABZ:P188 (4.2 μg/ml) was prepared by dissolution of ABZ:P 188 (1:1) in deionized water (pH = 7.0) under shaking (24 h). ABZ suspension and ABZ:P 188 were vigorously shaken before its intragastric administration to mice.

2.3. Protoscoleces collection

Protoscoleces of *E. granulosus* were collected aseptically from liver and lung hydatid cysts of infected cattle slaughtered in an abattoir located in the southeast of Buenos Aires province, Argentina. Viability was assessed by the methylene blue exclusion test (Elissondo et al., 2006).

2.4. Experimental animals and infection

Animal procedures and management protocols were carried out in accordance with the 2011 revised form of *The Guide for the Care and Use of Laboratory Animals* published by the U.S. National Institutes of Health. Unnecessary animal suffering was avoided throughout the study. Female CF-1 mice ($n = 80$; body weight $25\,g \pm 5$) were infected by intraperitoneal inoculation with 1500 *E. granulosus* protoscoleces/animal, suspended in 0.5 ml of medium 199 (Gibco). The animals were housed in a temperature-controlled ($22 \pm 1\,^{\circ}C$), light-cycled (12-h light/dark cycle) room. Food and water were given ad libitum.

2.5. Experimental design

Two different experimental designs were conducted: a chemoprophylactic efficacy study (which simulates a cyst rupture during surgical practice and the concomitant drug treatment) and, a clinical efficacy study (simulating an experimental secondary hydatidosis).

2.5.1. Chemoprophylactic efficacy study

The treatment started 24 h after the infection. Forty CF-1 mice were allocated into 4 experimental groups (10 animals/group) and treated as follows: (a) unmedicated control group, animals receiving distilled water as a placebo; (b) control group P 188 in distilled water, (c) ABZ suspension treated group; (d) ABZ:P 188-treated group. Treatments were performed daily during 30 days by intragastric administration (0.3 ml/animal) at the dose rate of 25 mg/kg. Five months after infection, mice were euthanized, and necropsy was carried out immediately thereafter.

2.5.2. Clinical efficacy study

At 4 months post-infection, mice were allocated into the following experimental groups (10 animals/group) and treated as follows: (a) unmedicated control group, animals receiving distilled water as a placebo; (b) control group P 188 in distilled water, (c) ABZ group, animals treated with the ABZ suspension; (d) ABZ:P 188 group, treated with the solid dispersion formulation. Dose rate and regimen administration were the same as those used for chemoprophylactic efficacy study. At the end of the 30 day treatment period, animals were euthanized, and necropsy was carried out immediately thereafter.

2.6. Determination of parasite weight and efficacy rate of treatments

At necropsy in both the prophylactic and efficacy studies, the peritoneal cavity was opened, and the hydatid cysts were carefully removed. The weight of the cysts collected from each individual animal was recorded using an analytical balance. The efficacy of treatments (based on the weight of cysts from infected mice), was calculated by use of the following formula: the mean weight of untreated control group minus the mean cysts weight of treated group divided by the mean cysts weight of untreated group.

Table 1
Chemoprophylactic efficacy study. Mean (±SD) weights (g) of hydatid cysts recovered at five months post-infection from artificially infected mice from the unmedicated control and from the ABZ:P 188 and ABZ suspension treated groups. Treatments were given at 25 mg/kg, every 24 h over 30 days following infection.

	Chemoprophylactic efficacy study	
	Wet weight (g) of cysts Mean ± SD	% of efficacy
Unmedicated control group	13.29 ± 5.06	
P188	10.13 ± 2.96	
ABZ-P188	0.30 ± 0.34[a,b]	97.74
ABZ suspension	1.52 ± 0.76[a]	88.56

[a] $P < 0.01$, statistically significant differences between treated group vs control group.
[b] $P < 0.01$, statistically significant differences between ABZ:P 188 group vs ABZ group.

2.7. Morphologic study

Samples of cysts recovered from each mouse were processed for scanning and transmission electron microscopy (SEM and TEM) as described by Elissondo et al. (2007).

2.8. Statistical analysis

Cysts weights (reported as mean ± SD) were compared statistically by nonparametric method (Kruskal Wallis test). Differences in mean cyst weight between treated groups were analyzed with Wilcoxon matched-pairs test. A value of $P < 0.05$ was considered statistically significant. The statistical analysis was performed using R Software (R development core team, 2007).

3. Results

3.1. Chemoprophylactic efficacy study

Table 1 summarizes the cyst weights (mean ± SD) recorded after treatments on the different experimental groups (Unmedicated control, P 188, ABZ:P 188 and ABZ suspension treated groups) involved in chemoprophylactic efficacy studies. There was no statistically significant difference ($P > 0.05$) between the mean cyst weight of control groups (i.e. control distilled water and P 188 groups). ABZ:P 188 had a greater chemoprophylactic efficacy than ABZ suspension (Table 1). All the infected mice (10/10) from the untreated control group and ABZ-treated group developed hyaline hydatid cysts in the abdominal cavity, whereas in 1 out of the 10 ABZ:P 188 treated mice the infection did not progress. Significant differences ($P < 0.01$) were observed in the weight of the cyst recovered from unmedicated mice (13.29 ± 5.06 g) compared to that obtained of

Table 2
Clinical efficacy study. Mean (±SD) weights (g) of the hydatid cysts recovered at five months post-infection from artificially infected mice from the Unmedicated control and from the ABZ:P 188 and ABZ suspension treated groups. Treatments were performed after 4 months of inoculation during 30 days at the doses rate of 25 mg/kg, every 24 h.

	Clinical efficacy study	
	Wet weight (g) of cysts Mean ± SD	% of efficacy
Unmedicated control group	12.45 ± 3.38	
P188	11.96 ± 3.79	
ABZ-P188	1.15 ± 0.63[a]	90.76
ABZ suspension	3.48 ± 3.08[a]	72.05

[a] $P < 0.01$, statistically significant differences between treated group vs control group.

treated groups. Moreover, mice that received ABZ:P 188 exhibited a significantly greater reduction ($P < 0.01$) in the weight of cysts (0.30 ± 0.34 g) compared to ABZ suspension treated mice (1.52 ± 0.76 g).

3.2. Clinical efficacy study

Hydatid cysts developed in all the infected animals involved in the efficacy studies (Table 2). There was no statistically significant difference ($P > 0.05$) between the mean cyst weight of control distilled water (12.45 ± 3.38) and P 188 (11.96 ± 3.79) groups. Both treatments resulted in a statistically significant reduction ($P < 0.01$) on the cysts weight compared to those obtained for unmedicated mice. Although the mean cyst weight reduction ABZ:P 188 group was higher (1.15 ± 0.63 g) than that of ABZ suspension group (3.48 ± 3.08), no difference was found in mean cyst weight between both groups ($P > 0.05$) (Table 2). However, differences in the ultrastructural changes observed in the germinal layer of cysts recovered from both ABZ-treated groups were detected.

3.3. Morphologic study

Fig. 1 shows the ultrastructural appearance of the germinal and laminated layers after SEM and TEM analysis of cysts recovered from unmedicated mice. All cysts in the samples removed from control mice appeared turgid, showing no observable collapse of the germinal layer and no change in ultrastructure were detected (Fig. 1a). TEM analysis of cysts recovered from the untreated control group revealed typical features of *E. granulosus* metacestodes, with a distinct acellular outer laminated layer and a germinal layer without alterations (Fig. 1b).

In contrast, the ultrastructural study of cysts developed in mice treated with the ABZ:P 188 or ABZ suspension from chemoprophylactic and clinical studies revealed changes in the germinal layer (Figs. 2 and 3). However, the damage extension appears to be greater after ABZ:P 188 compared to the ABZ suspension treatment. Regarding with the ultrastructural study at SEM, debris of cells could be observed (ABZ:P 188 treatment) (Figs. 2a and 3a) or only few cells with an intact morphology (ABZ suspension treatment) (Figs. 2c and 3c). TEM analysis of cysts from treated mice revealed the internal tissue distorted with the presence vacuolated areas. Likewise, ultrastructural damage induces by ABZ:P 188 (Figs. 2b and 3b) were greater than those induces by ABZ suspension (Figs. 2d and 3d).

4. Discussion

Although ABZ is considered the chemotherapeutic treatment of choice for human echinococcosis, it was developed primarily to target parasites in the gastrointestinal tract. For that reason, low bioavailability outside the intestine was considered important for the optimal performance of the drug. However, in order to effectively treat humans for hydatid cysts this feature is considered undesirable (Shuhua et al., 2002).

Available scientific evidence indicates that higher drug bioavailability correlates with improved efficacy of BZD anthelmintics against CE developed in mice (Mingjie et al., 2002; Shuhua et al., 2002; Ceballos et al., 2006, 2008, 2009; Liu et al., 2012). ABZ:P 188 SDs increased ABZ water solubility, which accounted for its enhanced absorption and bioavailability in mice (Castro et al., 2012). In the present study, ABZ:P 188 solid dispersion at the same dose used by Castro et al. (2012) was tested on mice experimentally infected with protoscoleces of *E. granulosus*. Two different experimental designs were conducted: a chemoprophylactic efficacy study (which simulates a cyst rupture during surgical practice and the concomitant drug treatment) and, a clinical efficacy study (simulating an experimental secondary hydatidosis).

After oral administration of ABZ, both formulations (ABZ:P 188 and ABZ suspension) demonstrated a preventive chemoprophylactic effect. Nevertheless, ABZ formulated as solid dispersion had greater chemoprophylactic efficacy than ABZ alone. Firstly, 1 out of the 10 of the ABZ:P 188 treated mice did not develop any cyst while in all mice of ABZ suspension group the infection progressed. A deleterious drug effect on *E. granulosus* protoscoleces at the time of infection may help to explain the lack of cyst development. Additionally, statistically significant differences in cyst weight were detected between both treated groups (Table 1). Therefore, ABZ:P 188 may not only reduce the number of cysts that develop but may also inhibit the development of secondary hydatidosis in mice.

Hydatid cysts developed in all the infected animals involved in the clinical efficacy studies. Significantly ($P < 0.01$) lower cyst weights were observed in mice of both treated groups compared to those recovered from untreated mice. Although a clear reduction on cyst weight was observed after the administration of ABZ:P 188, no significant differences between the two formulations were detected ($P > 0.05$, Table 2). On the other hand, even when animals were treated at the same dose of both ABZ:P 188 and ABZ suspension, the presence of ABZ metabolite (ABZSO) after 18 h of the last dose, was detected only in cysts recovered from ABZ:P 188 treated group (data not shown). This result could be explained by the improved drug concentration profiles in the bloodstream described by Castro et al. (2012).

No ultrastructural changes (SEM and TEM analysis) were observed in the germinal layer of cyst recovered from unmedicated mice. In contrast, the germinal layer

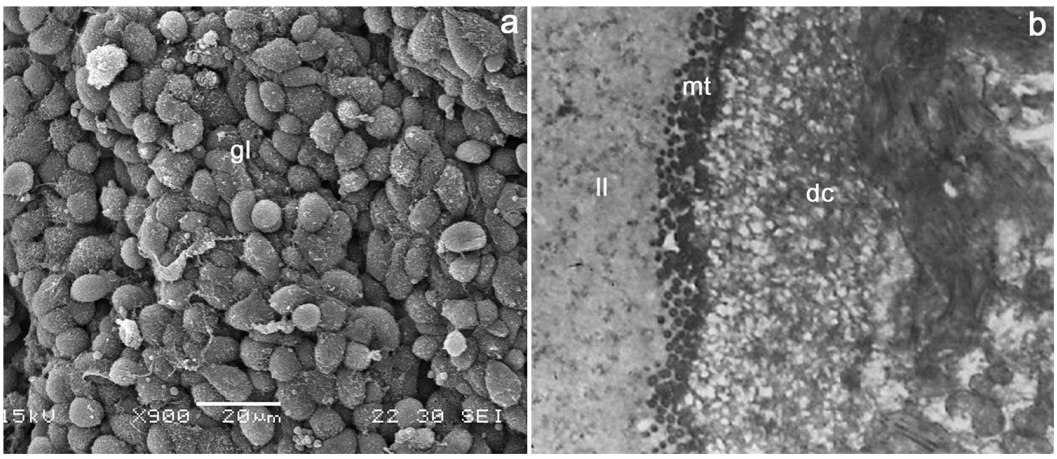

Fig. 1. Scanning and transmission electron microscopy (SEM and TEM, respectively) of hydatid cysts recovered from untreated control mice. (a) SEM (900×) image of hydatid cysts (gl = germinal layer). (b) TEM (12,000×) image of hydatid cysts (ll = laminar layer; mt = microtriches; dc = distal citoplasm).

of cysts recovered from ABZ:P 188 and ABZ-suspension treated mice in both efficacy studies was markedly altered. Nevertheless, the damage extension appears to be greater after the treatment with ABZ:P 188, which may correlate with the enhanced drug availability at the site of cyst location produced by the higher plasma concentration/time (Castro et al., 2012). The ultrastructural changes observed by SEM included the loss of the characteristic multicellular appearance of the germinal layer. The alterations observed by TEM were distorted increased vacuolation of the distal cytoplasm and severe damage of the internal tissue. The ultrastructural changes induced by both ABZ formulations were similar to those described for other BZ compounds such as flubendazole and mebendazole (Ceballos et al.,

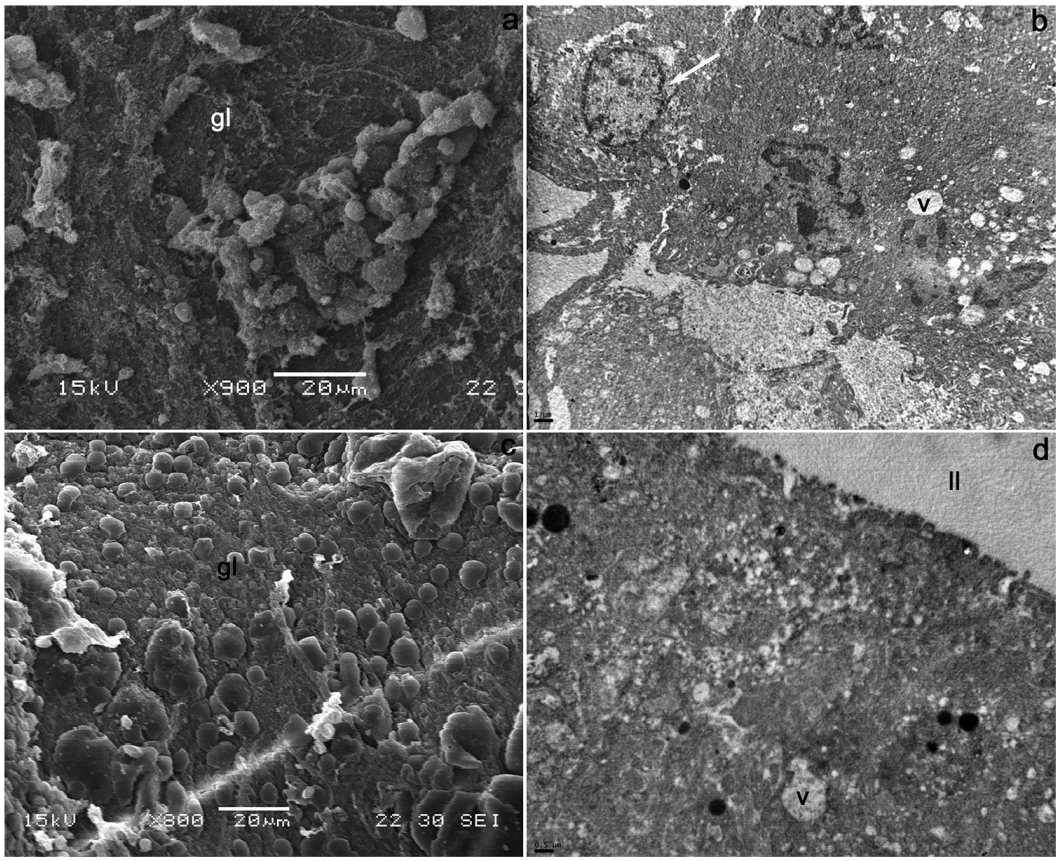

Fig. 2. Representative images of SEM and TEM of hydatid cysts recovered from infected mice treated with ABZ-P188 and ABZ suspension during chemoprophylactic efficacy study. (a)–(b) Cysts recovered from mice treated with the ABZ:P 188 (25 mg/kg). (a) SEM image of hydatid cysts. Alterations in the germinal layer (gl) and only debris of cells can be observed (900×). (b) TEM Scanning image of hydatid cysts. Note the damage tissue and the presence of numerous vacuoles (v vacuoles; 500×). Arrow indicates an apoptotic nucleus (12,000×). (c)–(d) Cysts recovered from mice treated with the ABZ suspension (25 mg/kg). (c) SEM image of hydatid cysts. The germinal layer is altered (800×). (d) TEM Scanning image of hydatid cysts. Note the presence of vacuoles (v vacuoles; ll laminar layer; 10,000×).

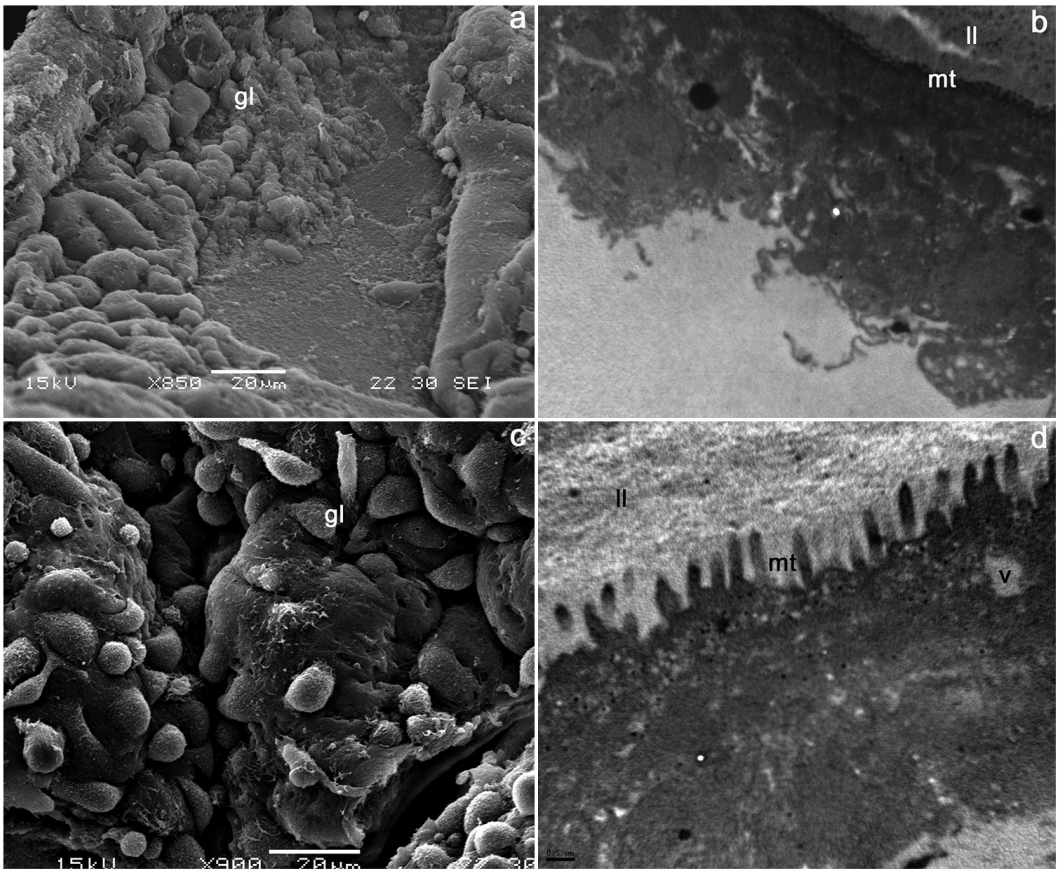

Fig. 3. Representative images of SEM and TEM of hydatid cysts recovered from infected mice treated with ABZ:P 188 and ABZ suspension during clinical efficacy study. (a) SEM image of hydatid cysts. Note the extensive damage of the germinal layer (gl). Only cellular debris could be observed (850×). (b) TEM Scanning image of hydatid cysts. Note the altered germinated layer (mt microtriches; ll laminar layer; 10,000×). (c)–(d) Cysts recovered from mice treated with the ABZ suspension (25 mg/kg). (c) SEM image of hydatid cysts. The germinal layer is altered (900×). (d) TEM Scanning image of hydatid cysts. Note the presence of vacuoles (18,000×).

2009, 2010). The benzimidazole anthelmintics inhibit cytoskeletal tubulin polymerization affecting cell division, secretory transport systems and absorption.

It should be noted that ABZ:P 188 induced greater ultrastructural damage when drug was administered at the time point of infection (chemoprophylactic efficacy study) than when the treatment started 4 months post-infection (clinical efficacy study). This is in accordance with other studies performed with different drugs (Urrea-Paris et al., 1999). The explanation for this could be in the different developmental stage of the parasite material before the treatment, namely protoscolex or hydatid cyst, respectively (Urrea-París et al., 2002). The laminar layer is an effective barrier against the drugs (Urrea-Paris et al., 1999) and physiological and inmunological reaction of the host (Gottstein and Hemphill, 1997). The efficacy of ABZ:P 188 in chemoprophylactic studies was superior (97.74%) to that obtained when the treatment started 4 months p.i. (90.76%).

In conclusion, the therapeutic efficacy of ABZ:P 188 was superior to ABZ suspension for the treatment of *E. granulosus* infection in mice. The improved in therapeutic efficacy could be a consequence of the increase in the systemic availability of ABZSO. The work reported here demonstrates that in vivo treatment with ABZ:P 188 impairs the development of the hydatid cysts. This new pharmacotechnically-based strategy could be a suitable alternative for treating CE in humans.

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