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ORIGINAL PAPER

Albendazole treatment in cystic echinococcosis: pharmacokinetics and clinical efficacy of two different aqueous formulations

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Abstract The pharmacokinetic (PK) behaviour and clinical efficacy of albendazole (ABZ) against hydatid cysts in mice were assessed after treatment with two different ABZ pharmaceutical formulations. BalbC mice received ABZ (0.5 mg/kg) prepared either as solution or suspension (50 μg/ml) for oral administration (PK study). Blood samples were collected up to 16 h post-treatment and processed to measure ABZ/metabolites concentrations in plasma. The clinical efficacy assessment was performed in BalbC mice infected 8 months earlier with *Echinococcus granulosus* protoscoleces. Infected animals were allocated into three experimental treatment groups: (a) untreated control, (b) ABZ-solution treated, (c) ABZ-suspension treated. Both treated groups received ABZ (0.5 mg/kg) administered under two different therapeutic schemes: dosing every 48 h over 30 days (regimen I) or treated every 12 h during 15 days (regimen II). Experimental mice were sacrificed 12 h after treatment, and cysts were recovered, weighed and processed for transmission electron microscopy. Enhanced ABZ sulphoxide (the main ABZ metabolite) concentration profiles were measured in animals treated with the ABZ solution. Any positive clinical response was obtained after treatment every 48 h (30 days therapy). However, consistent with the observed PK results, both ABZ formulations were clinically effective in infected mice treated with a 12-h dosing interval (15 days therapy).

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Introduction

Hydatidosis or cystic echinococcosis (CE) is caused by the larval stage of the tapeworm *Echinococcus granulosus* and produces a long-lasting infection in humans and animals, which is a serious public health problem worldwide (Jenkins et al. 2005; Moro and Schantz 2006). People become infected after ingestion of eggs passed into the environment with faeces from definitive hosts. The resulting oncosphere larvae penetrate the intestinal wall and enter to the bloodstream to develop into a slow-grown metacestode or hydatid cyst. The liver and the lungs are the most commonly affected organs, although other organs can also be affected (Ammann and Eckert 1995). Initially, the cyst is small and patients are asymptomatic. The induction of morbidity depends on the number, size, involved organ and localization of the cyst within the organ, and the pressure of cyst on surrounding tissues (Eckert and Deplazes 2004). Furthermore, the possibility of cysts rupture and protoscoleces dissemination can result in anaphylactic reaction or in a secondary CE caused by the spilled of large number of protoscoleces, which have the potential to differentiate

into new hydatid cysts (El-On 2003). Surgery has been the only treatment available before the introduction of benzimidazole (BZD) anthelmintics (Menezes da Silva 2003) and is still probably the most common treatment for CE (Ammann and Eckert 1995).

BZD anthelmintics are chemically classified as BZD thiols, halogenated BZD, pro-BZD and BZD methylcarbamates (Lanusse and Prichard 1993). The BZD methylcarbamate compounds mebendazole (MBZ), flubendazole (FLBZ) and albendazole (ABZ) have been used to treat CE in humans (Davis et al. 1986). The main disadvantage of MBZ treatment is the high doses (40–50 mg/kg) and prolonged (3–6 months) treatment that are needed to reach effective results (Bartoloni et al. 1992; Rippman et al. 1992; Teggi et al. 1993; WHO 2001). On the other hand, a low in vivo FLBZ efficacy has been reported by different authors (Davis et al. 1986; Recco et al. 1984). Therefore, ABZ is routinely used pre- and post-operatively in humans and as a treatment of choice for inoperative CE. According to WHO recommendations, ABZ is given in daily doses of 10 to 15 mg/kg of body weight in two divided doses postprandially for 3 to 6 month (WHO 2001). ABZ has shown to be significantly more effective than MBZ in the treatment of whole hydatid cysts and liver cysts (Teggi et al. 1993). However, the effectiveness of BZD treatments, including ABZ, is lower than 80%, with only 8–20% of patients with successful treatment indicated by disappearance or significant decrease in the size of *E. granulosus* cysts (Pawlowski et al. 2001; Todorov et al. 1998; Davis et al. 1989). The age of the cysts, the age of the patient, the localization of the cysts and their morphological features have been indicated as factors that may influence the therapeutic results (Teggi et al. 1993). However, poor drug bioavailability is considered as an important cause of erratic therapeutic success in CE treatment.

BZD anthelmintics have only limited water solubility, and small differences in drug solubility may have a major influence on their absorption and resultant pharmacokinetic behaviour (Lanusse and Prichard 1993). These compounds must be dissolved in the gastrointestinal (GI) fluids to allow the absorption through the GI mucosa to reach the bloodstream and, from the bloodstream, to achieve sustained concentrations in the parasite surrounding medium. Poor/erratic GI absorption is a common inconvenient for the systemic availability of enterally administered BZD in most species (Lanusse and Prichard 1993). Due to its improved GI absorption compared to MBZ, and the achievement of relatively high plasma concentrations of the active ABZ sulphoxide (ABZSO) metabolite after oral administration, ABZ has replaced MBZ as the drug of choice against CE (Davis et al. 1989; WHO 1996; Horton 2003).

The anthelmintic activity of BZD compounds not only depend on its binding to parasite ß-tubulin but also on their ability to reach high and sustained concentrations at the site of parasite location that allow the delivery of effective concentrations of the compound at the receptor within the parasite cells, in sufficient time, to cause the therapeutic effect (Thompson and Geary 1995). The critical factors for success appear to be the ability of the drug to penetrate the complex structure of the cyst's wall, which mainly depends on drug lipophilicity, the concentration gradient across the wall of the cyst and the persistence of adequate levels of the parent drug/active metabolite at the site of parasite location. As a consequence, a higher systemic availability of the parent drug/active metabolite obtained by either an enhancement on drug absorption or a shorter dosing interval may correlate with an enhanced antiparasitic effect.

The aim of the current experimental work was to compare the plasma pharmacokinetic behaviour and the clinical efficacy of two different ABZ formulations (aqueous solution and aqueous suspension) against hydatid cyst developed in a murine model. The efficacy of the two therapeutic regimens was evaluated by the comparison of cysts weights and ultrastructural morphological changes induced by each ABZ treatment.

Materials and methods

Chemicals

Reference standards (97–99% pure) of ABZ, ABZSO, albendazole sulphone ($ABZSO_2$) and oxibendazole (OBZ, used as internal standard) were supplied by Schering Plough (Kenilworth, USA). All the solvents (acetonitrile and methanol) used during the extraction and drug analysis were high-performance liquid chromatography (HPLC) grade and purchased from Sintorgan® (Buenos Aires, Argentina). Water was double distilled and deionized using a water purification system (Simplicity®, Millipore, Brazil).

ABZ formulations

ABZ solution (50 μg/ml) was prepared by dissolution of ABZ pure standard in deionized water. The pH of the formulation was adjusted to 1.2 using hydrochloric acid (25 mM). The formulation was shaken (12 h) and then was filtrated through 0.45 μm filter (Whatman, NJ, USA). The final ABZ concentration was determined (n=4) by HPLC using the method described below. The ABZ suspension (50 μg/ml) was prepared by addition of ABZ pure standard in deionized water (pH=7.0) under shaking (12 h). ABZ suspension was vigorously shaken before its intragastric administration to mice. For the clinical efficacy studies, ABZ formulations were freshly prepared every 3 days and maintained under refrigeration (3–5°C).

Animals

BalbC mice (8 week old at the starting of the experiments) were used as experimental subject. Animals were housed in a temperature-controlled (22±1°C), light-cycled (12 h light/dark cycle) room. Food and water were provided ad libitum.

Pharmacokinetic study

Experimental design

Eighty-eight (88) healthy mice were divided into two groups (n=44). ABZ formulated either a solution (group I) or suspension (group II) that was given orally (0.3 ml) using an intragastric tube at the same dose (0.5 mg/kg). This dose rate was lower than that previously reported (García et al. 2003; Stettler et al. 2004), and it was limited by the maximum amount of ABZ able to be delivered from 0.3 ml of a solution prepared at 50 μg/ml. Blood obtained from sacrificed animals (n=4 per collection point) was collected in heparinized plastic tubes at the following times post-treatment: 5, 15 and 30 min, and 1, 2, 4, 6, 8, 10, 12 and 16 h. Blood samples were centrifuged at 2,000×g for 15 min, and the recovered plasma was stored at −20°C until analysed by HPLC.

Assessment of drug concentrations

Plasma samples (100 μl) plus 900 μl of water were spiked with 10 μl of OBZ (stock solution 10 μg/ml) as internal standard. ABZ, ABZSO and $ABZSO_2$ were extracted using disposable C18 cartridges (Strata®, Phenomenex, CA, USA) previously conditioned with 0.5 ml of methanol, followed by 0.5 ml of water. All samples were injected into the cartridges and then sequentially washed with 1 ml of water and eluted with 2 ml of methanol. The elutant was evaporated to dryness in a vacuum concentrator (Speed-Vac®, Savant, CE), then reconstituted with 100 μl of the same mobile phase used on the HPLC system.

Experimental and fortified plasma samples were analyzed for ABZ, ABZSO and $ABZSO_2$, and the internal standard by HPLC. Fifty microlitres of each sample were injected in a Shimadzu 10A HPLC system (Shimadzu Corporation, Kyoto, Japan), using two pumps (LC-10AS), an autosampler (SIL-10A) with a 50-μl loop, an UV detector (SPD-10A) reading at 292 nm, a column oven (Eppendorf TC-45, Eppendorf, Madison, WI, USA) set at 30°C and a controller (Shimadzu Class LC10, Kyoto, Japan). The C18 reversed-phase column was Phenosphere® (5 μm, 250×4.6 mm, Phenomenex, Torrance, CA, USA). Elution from the stationary phase was carried out at a flow rate of 1.2 ml/min using acetonitrile and ammonium acetate buffer (0.025 M, pH 6.6) as a mobile phase. The elution gradient linearly changed from 27:73 (acetonitrile:ammonium acetate buffer) to 50:50 in 5 min, then maintained for 5 min and modified to 27:73 in 3 min, which was then maintained over 3 min.

Identification of ABZ, ABZSO, $ABZSO_2$ and OBZ was undertaken by comparison with the retention times of 97–99% pure reference standards. A complete validation of the analytical procedures for extraction and quantification of ABZ/metabolites in each plasma sample was performed before starting the analysis of experimental samples. Retention times for ABZSO, $ABZSO_2$, OBZ and ABZ were 5.76, 7.09, 9.82 and 11.71 min, respectively. Plasma calibration curves for each analyte were constructed by least squares linear regression analysis, obtaining correlation coefficients (r) between 0.9945 and 0.9959. Mean absolute recovery percentages for concentrations ranging between 0.1 and 2 μg/ml (n=5) were 81.1% (ABZSO), 76.4% ($ABZSO_2$) and 92.4% (ABZ) with percentages of residual standard deviation (%RSD) of 5.2%, 4.3% and 6.4%, respectively. The limits of detection (LOD) obtained were 0.015 (ABZSO), 0.033 ($ABZSO_2$) and 0.02 μg/ml (ABZ). The limit of quantification (LOQ) was defined as the lowest measured concentration with a %RSD <20%, an accuracy of ±20% (measured as percentage of relative error) and an absolute recovery ≥70%. The LOQ obtained for the molecules assayed was 0.1 μg/ml. Values below LOQ were not included in the pharmacokinetic analysis.

Pharmacokinetic analysis of the data

The concentration vs. time curves for ABZSO in plasma for each experimental group was fitted with the PKSolutions™ computer software (Summit Research Services, Ashland, OH, USA). The peak concentration (C_{max}) and time to peak concentration (T_{max}) of ABZSO were read from the plotted concentration–time curve. The area under the concentration–time curve (AUC) for ABZSO was calculated by the trapezoidal rule (Gibaldi and Perrier 1982) and further extrapolated to infinity by dividing the last experimental concentration by the terminal slope (per hour). The AUC value was considered as an indicator of the relative bioavailability of the two ABZ formulations assayed.

Clinical efficacy study

Protoscoleces collection

Protoscoleces of *E. granulosus* were collected aseptically from liver hydatid cysts of natural infected cattle, slaughtered in two abattoirs located in the southeast of the Buenos Aires province, Argentina. The obtained protoscoleces were washed several times with phosphate-buffered saline at

pH 7.2 to yield only viable protoscoleces. Vitality was assessed by muscular movements (evaluated under light microscope), motility of flame cells and the methylene blue exclusion test (Elissondo et al. 2004).

Experimental design

To prove the efficacy of both ABZ formulations (solution or suspension), different therapeutic regimens were evaluated on previously infected BalbC mice. The infection was performed by the intraperitoneal injection of 1,500 protoescoleces per animal, dissolved in 0.5 ml of medium 199. After 8 months of infection, the animals were allocated into three experimental groups: (a) untreated control group (animals received with 0.3 ml of water as placebo), (b) ABZ-solution-treated group [animals treated with 0.3 ml of the ABZ solution (50 μg/ml) by intragastric application at the dose rate of 0.5 mg/kg] and (c) ABZ-suspension-treated group [animals treated with 0.3 ml of the ABZ suspension (50 μg/ml) by the same route and dose than described for the ABZ-solution-treated group]. The infected animals were involved in the assessment of two different therapeutic schemes: regimen I, ABZ was administered every 48 h during 30 days; regimen II, ABZ was administered at 12-h intervals over 15 days. Due to limitations on availability of hydatid cyst material obtained from local abattoirs, the two different therapeutic regimens were evaluated in separate experimental phases with seven (regimen I) and ten (regimen II) infected animals for the treated group. Mice were euthanised at the end of the treatment period, and necropsy was carried out immediately thereafter. At necropsy, the peritoneal cavity was opened, and the hydatid cysts were carefully removed. The cyst weight was recorded using an analytical balance (Mettler AJ150L, Mettler-Toledo AG, Greinfensee, Switzerland).

Electron microscopy

Samples of hyadatid cysts (germinal and laminated layers) were obtained from mice of each experimental group and processed as described by Elissondo et al. (2007). Briefly, samples were fixed with 3% glutaraldehyde in sodium cacodylate buffer for 72 h at 4°C. Then, several washes in cacodylate buffer were made and post-fixed in 2% OsO_4 in cacodylate buffer. They were dehydrated by sequential incubations in increasing concentrations of acetone 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 diamond knife, stained with uranyl-acetate-saturated solution and lead citrate, and examined with a JEOL 100 CXII transmission electron microscope (JEOL, USA) at 80 kV.

Statistical analysis of the data

Pharmacokinetic parameters are presented as mean ± SD and were compared by Student's *t* test. For the clinical efficacy study, the cyst weights (reported as mean ± SD) were compared by analysis of variance (ANOVA). The Tuckey's range test was used to indicate the order of significance when a significant *F* value was obtained. A value of $P<0.05$ was considered statistically significant. The statistical analysis was performed using the Instat 3.0 Software (Graph Pad Software, San Diego, CA, USA).

Results

ABZSO and $ABZSO_2$ were the recovered metabolites in mice plasma after the oral administration of both ABZ formulations. $ABZSO_2$ plasma concentrations were under the LOQ in both ABZ-treated groups, which precluded any pharmacokinetic analysis. Figure 1 shows the mean plasma concentrations of ABZSO obtained after the oral administration of two different aqueous formulations of ABZ (solution and suspension) to mice. The main disposition kinetic data for ABZSO in both the ABZ solution and the suspension-treated mice are summarized in Table 1. After treatment with both formulations, ABZSO plasma concentrations rapidly increased to reach similar C_{max} values of 0.37 μg/ml (solution) and 0.34 μg/ml (suspension), obtained at 26 and 45 min (T_{max}) post-treatment, respectively. A higher ($P<0.05$) AUC value (>263%) was obtained after treatment with the ABZ solution, compared to that obtained for the suspension formulation. Moreover,

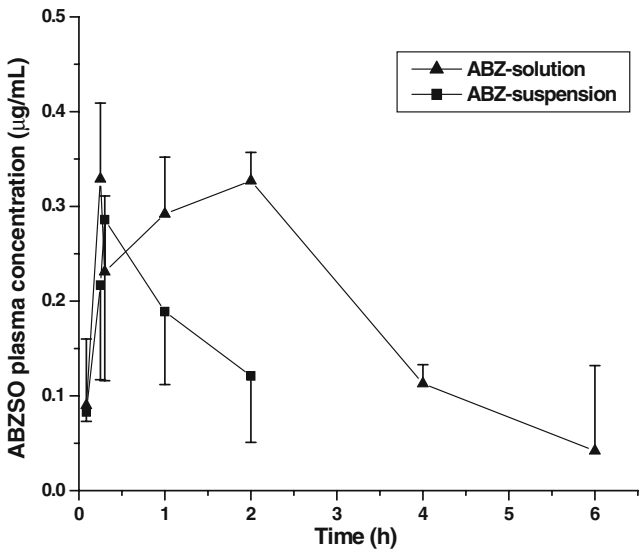

Fig. 1 Plasma concentration profiles (mean ± SD) of albendazole sulphoxide (ABZSO) after intragastric administration of ABZ (0.5 mg/kg) formulated either as an aqueous solution or an aqueous suspension (50 μg/ml)

Table 1 Pharmacokinetic parameters (mean ± SD) for albendazole sulphoxide (ABZSO) obtained after the intragastric administration of albendazole (ABZ) to mice (0.5 mg/kg), formulated as either aqueous solution or aqueous suspension

	ABZ solution	ABZ suspension
Cmax (μg/ml)	0.37±0.03	0.34±0.11
Tmax (min)	26.2±19.4	45.0±15.0
AUC (μg h/ml)	1.20±0.17	0.33±0.04*
PDP (min)	5–360	5–120

C_{max} peak plasma concentration, T_{max} time to peak plasma concentration, *AUC* area under the concentration–time curve, *PDP* plasma detection period

*$P<0.05$, statistically significant differences

a longer plasma detection period of the sulphoxide metabolite was observed after the ABZ solution administration (6 h) compared to the suspension (2 h).

Hydatid cysts developed in all the infected animals involved in the clinical efficacy study. The therapeutic regime I (ABZ treatment every 48 h over 30 days) did not cause any alteration on the weight of the cysts recovered from infected mice. Any significant difference was observed between treatment groups (both formulation) with less than 2% reduction on cyst weight compared to those recovered from untreated animals. However, a significant decrease on cyst weights upon both treatments, ABZ solution (0.80±0.47 g) and ABZ suspension (0.49± 0.31 g), compared to the untreated control group (5.23± 1.91 g) were obtained with the shorter (12 h) dosing interval over 15 days treatment (regimen II). No statistical differences were observed in cyst weights between treatment with ABZ formulated as solution or suspension.

The ultrastructural appearance of the germinal and laminated layers after transmission electron microscopy (TEM) analysis of cysts recovered from untreated and ABZ-treated infected mice after regimens I and II are shown in Figs. 2, 3, 4, and 5 and 6, 7, 8, and 9, respectively. 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. In contrast, cysts recovered from mice treated with ABZ formulated either

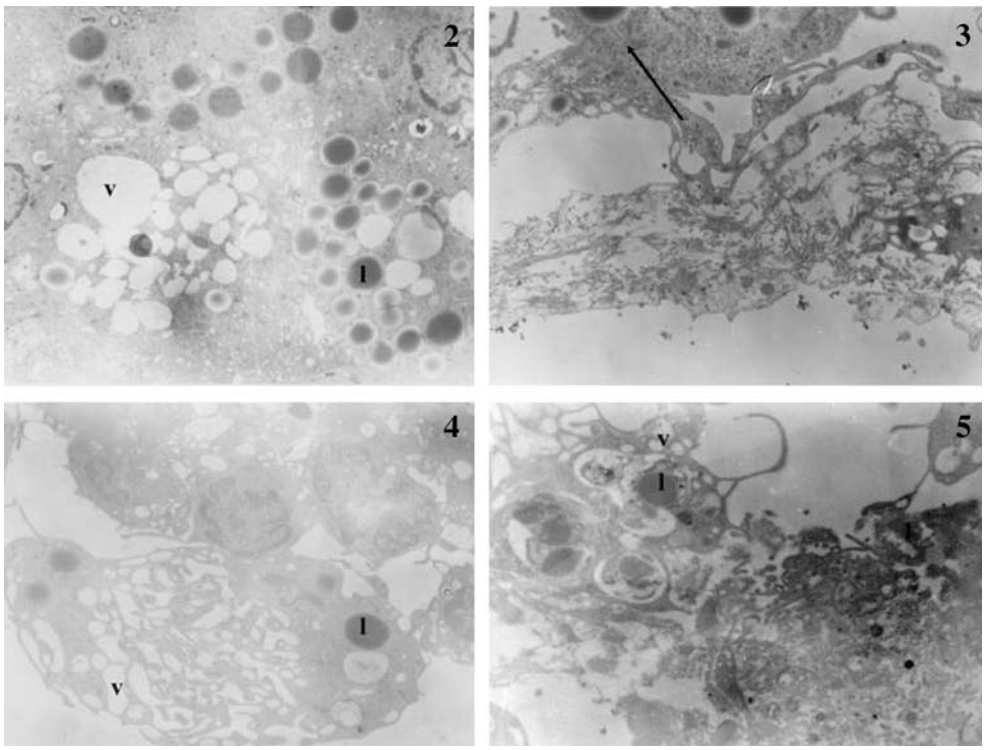

Fig. 2 Transmission electron microscopy (TEM) of hydatid cysts recovered from artificially infected mice treated with albendazole (ABZ; regimen I). ABZ-suspension-treated group. Note the vacuolation of the distal cytoplasm (*v* vacuoles) and presence of lipid droplets (*l;* ×4,000)

Fig. 3 Transmission electron microscopy (TEM) of hydatid cysts recovered from artificially infected mice treated with albendazole (ABZ; regimen I). Cyst recovered from the same group as the previous one. Note the tissue severely affected and lipid droplets (*arrow;* ×6,000)

Fig. 4 Transmission electron microscopy (TEM) of hydatid cysts recovered from artificially infected mice treated with albendazole (ABZ; regimen I). ABZ-solution-treated group. It shows the presence of numerous vacuoles (*v*) and lipid droplets (*l;* ×4,000)

Fig. 5 Transmission electron microscopy (TEM) of hydatid cysts recovered from artificially infected mice treated with albendazole (ABZ; regimen I). The same to the previous one, note the damage tissue with vacuoles (*v*) and lipid droplets (*l;* ×5,000)

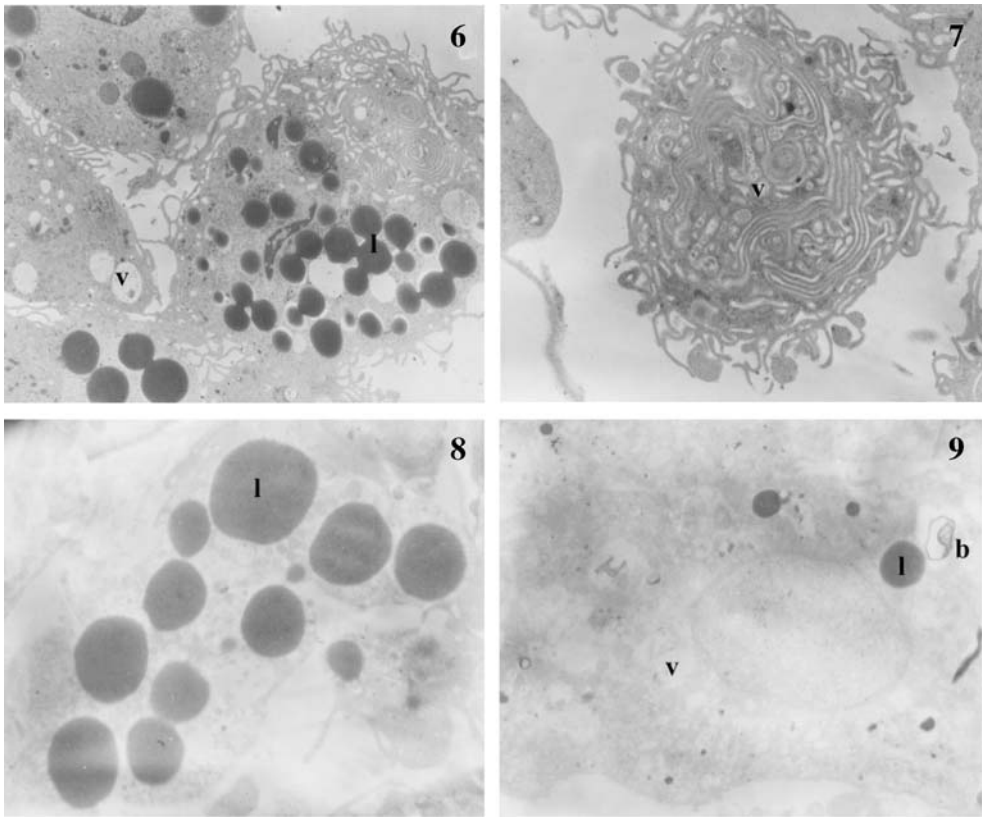

Fig. 6 Transmission electron microscopy (TEM) of hydatid cysts recovered from artificially infected mice and treated with albendazole (ABZ; regimen II). ABZ-suspension-treated group. Note the completely altered germinated layer with presence of vacuoles (*v*) and numerous lipid droplets (*l*; ×4,000)

Fig. 7 Transmission electron microscopy (TEM) of hydatid cysts recovered from artificially infected mice and treated with albendazole (ABZ; regimen II). The same to the previous one. Note the damaged tissue and vacuoles (*v*) (6000x)

Fig. 8 Transmission electron microscopy (TEM) of hydatid cysts recovered from artificially infected mice and treated with albendazole (ABZ) (Regimen II). ABZ-solution treated group. Note lipid droplets (*l;* ×8,000)

Fig. 9 Transmission electron microscopy (TEM) of hydatid cysts recovered from artificially infected mice and treated with albendazole (ABZ; regimen II). Just as the previous one, note the altered tissue with vacuoles (*v*), residual lamellar body (*b*) and lipid droplets (*l;* ×8,000)

as a solution or a suspension showed a completely damaged germinal layer with vacuolated areas, numerous lipid droplets and residual lamellar bodies.

Discussion

The lack of water solubility is an important limitation for the formulation of BZD compounds, which only allows their preparation as suspensions, pastes or granule for oral administration. The mucous surface in the GI tract behaves as a lipid barrier for the absorption of active substances, so that absorption depends on lipid solubility and degree of ionization at GI pH levels. However, drug particles must dissolve in the enteric fluids to facilitate absorption of the BZD molecule through the GI mucosa. The dissolution rate of an enterally delivered compound influences the rate and extent of its absorption (systemic bioavailability), its maximal plasma concentration, its subsequent distribution to target tissues/parasites and its overall disposition kinetics.

ABZ parent drug was not detected in plasma at any time after its intragastric administration, as a solution or suspension, to mice. ABZSO (active metabolite) was the main analyte identified in plasma between 5 min and either 2 (ABZ suspension) or 6 h (ABZ solution) pos-treatment. The oral administration of ABZ formulated as a solution, correlated with an increased plasma availability of ABZSO compared to that observed after the administration of the aqueous suspension. In fact, the relative bioavailability of the sulphoxide metabolite increased 263% after the treatment with the solution compared to the suspension. These results agree with previous studies, where the use of different formulations was assessed to increase ABZ bioavailability. Indeed, the use of ABZ–cyclodextrin complexes (García et al. 2003; Castillo et al. 1999; Garcıa-Rodriguez et al. 2001) ABZ solid dispersions (Kohri et al.

1999), ABZ complexation with povidone (Evrard et al. 2002) or an ABZ soybean oil emulsion (Shuhua et al. 2002) have been shown to markedly enhance the ABZSO plasma levels in comparison with the conventional suspension. As similar C_{max} values for ABZSO were observed after the administration of both ABZ formulations, the higher AUC value obtained after the ABZ solution was related to the longer ABZSO residence in the bloodstream (longer detection in plasma) observed in this experimental group. Interestingly, the ABZSO C_{max} value observed in mice in the present experiment was similar to that reported in humans dosed with a ten times higher dose (6–8 mg/kg; Dayan 2003). This is likely related to the lower absorption of ABZ in man compared to mouse (Dayan 2003). Considering that, even after the ABZ solution administration, ABZSO is detected in plasma up to only 6 h post-treatment, ABZ administration for time intervals no longer than 12 h would be necessary to assure a sustained drug exposure of the *E. granulosus* cyst.

Available scientific evidence indicates that higher drug bioavailability correlates with improved efficacy of BZD anthelmintics against CE developed in mice (García et al. 2003; Stettler et al. 2004; Xiao et al. 1988; Ceballos et al. 2006). The administration of ABZ over 30 days with a 48-h-apart dosing interval (regimen I) did not reach efficacious anthelmintic activity against CE in mice. In fact, equivalent cyst weighs were observed in ABZ-treated and untreated control animals. In contrast, when ABZ was given twice a day for 15 days (regimen II), a significantly lower cyst weight was observed in both ABZ-treated groups (solution and suspension), where the mean cyst weight was reduced between 85% and 91%. It is, therefore, clear that the sustained drug–parasite contact occurring after a shorter therapeutic interval induced a higher drug effect. Interesting, in assessing regimen II (both ABZ formulations administered every 12 h), the enhanced drug availability obtained after the ABZ solution administration did not increase the drug effect on cyst weight. It is likely that the enhanced ABZSO concentrations obtained after the ABZ solution administration are not sufficient to induce a higher anthelmintic effect on cyst development compared to the concentration profiles achieved after the ABZ suspension administration. In conclusion, a marked anthelmintic effect of ABZ was observed only upon 15 days of treatment twice a day. This effective treatment period was shorter than that reported by other authors in analogous murine models (Stettler et al. 2004; Shuhua et al. 2002; Rodrigues et al. 1995).

No ultrastructural changes (TEM analysis) were observed in the germinal membrane of cysts recovered from mice of the untreated control groups. In contrast, the germinal layer of cysts recovered from ABZ-treated mice was markedly altered with vacuolated areas and numerous lipid droplets (Figs. 2, 3, 4, 5, 6, 7, 8, 9). The same ultrastructural changes were observed after treatments with both formulations (solution and suspension). However, the damage extension appears to be broader after the ABZ solution compared to the suspension, which may correlate with the enhanced drug availability measured on the bloodstream. On the other hand, although in dosing regimen I a clear effect in terms of cyst weight reduction was not observed, TEM analysis showed similar qualitative ultrastructural changes to those observed when the animals were treated every 12 h over 15 days (regimen II), where metacestodes had undergone considerable degenerative alterations. The internal tissue was extensively distorted, showing increase on the number of lipid droplets, vacuoles and residual lamellar bodies.

In conclusion, the exposure of the hydatid cysts to enhanced drug concentrations obtained after the administration of ABZ at 12 h interval during 15 consecutive days would correlate with increased efficacy in mice. The increased ABZ absorption observed after the administration of the solution was not sufficient to determine an enhanced anthelmintic effect under our experimental conditions. However, we consider that the complementary results on ABZ kinetic behaviour and hydatid cyst damage summarized in this paper may provide useful information to optimize hydatid disease chemical treatment.

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