COMPARATIVE PERFORMANCE OF FLUBENDAZOLE AND ALBENDAZOLE IN CYSTIC ECHINOCOCCOSIS:

EX VIVO ACTIVITY, PLASMA/CYSTS DISPOSITION AND EFFICACY IN INFECTED MICE

Running title: FLBZ AND ABZ IN CYSTIC ECHINOCOCCOSIS

Ceballos Laura*, Elissondo Celina, Sánchez Bruni Sergio, Denegri Guillermo, Lanusse Carlos, Alvarez Luis.

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.

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.

Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina.

*Corresponding author: Dr. Laura Ceballos, 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. Tel/fax: +54-2293-439850.

e-mail address: ceballos@vet.unicen.edu.ar
Abstract
The need to identify improved therapy against cystic echinococcosis (CE) has motivated pharmacology-based research. The comparative pharmacological performance of the benzimidazole compounds flubendazole (FLBZ) and albendazole (ABZ) was addressed here. The goals of the work were: a) to evaluate the ex vivo activity of FLBZ, ABZ and their respective metabolites against *Echinococcus granulosus* protoescoleces; b) to compare the plasma and cysts disposition kinetics for both drugs in infected mice; and c) to compare the clinical efficacy of FLBZ and ABZ against CE in mice. Ex vivo study: *E. granulosus* protoescoleces were incubated with FLBZ, reduced-FLBZ (R-FLBZ), ABZ and ABZ-sulphoxide (ABZSO) (10 nmol/mL). Protoescoleces viability was monitored by the methylene blue exclusion test and scanning electron microscopy (SEM). Pharmacokinetic study: Balb/c mice with CE, were allocated in two different groups and orally treated with either FLBZ or ABZ (5 mg/kg) both formulated as a cyclodextrin-based solution. Blood and cysts samples were taken up to 12 h post-treatment and analyzed by HPLC. Efficacy study: CE infected Balb/c mice were divided into three groups, unmedicated control, FLBZ and ABZ treated groups. Oral treatments were performed twice a day during 25 days. After treatment, all animals were killed and the weight of the cysts was recorded. Loss of protoescoleces viability was observed after drug incubation. FLBZ was detected in plasma (AUC= 1.8 μg.h/mL) and cysts (AUC= 0.3 μg.h/g) collected from treated-infected animals. Conversely, ABZSO was the only active molecule measured in plasma (AUC= 4.4 μg.h/ml) and cysts (AUC= 1.5 μg.h/g) after ABZ treatment. FLBZ induced a 90% reduction on cyst weight in comparison to those collected from untreated control mice (P<0.05). However, no differences on cysts weight were observed between the ABZ treated (8.2 g) and unmedicated control group (10.5 g). Due these results we consider that flubendazole offers a great potential to become a drug of choice in the treatment of cystic echinococcosis.

Keywords: flubendazole, albendazole, cystic echinococcosis.
Introduction

The cystic Echinococcosis (CE) disease in humans occurs as a result of infection by the larval stages of tae
cestodes of the genus Echinococcus, named *Echinococcus granulosus*. The life cycle of *E. granulosus* involves
dogs and other canids as definitive hosts for the intestinal tapeworm, as well as domestic and wild ungulates as
intermediate hosts for the tissue-invading metacestode (larval) stage. The metacestode (hydatid cyst) is a fluid-
filled, spherical, unilocular cyst that consists of an inner germinal layer of cells supported by a laminated
membrane. Each cyst is surrounded by a host-produced layer of granulomatous adventitial reaction. Small
vesicles called brood capsules bud internally from the germinal layer and produce multiple protoscoleces (PSC)
by asexual division. In humans, the slowly growing hydatid cysts can attain a volume of several liters and
contain many thousands of PSC. The signs and symptoms depend on the location and size of hydatid cysts

Currently, the treatment of the disease involves surgical removal of either the entire cyst or its contents by
puncture and aspiration (PAIR) (14), as well as, chemotherapy based on the use of benzimidazole (BZ)
methylcarbamate compounds (30). As a chemical class, the BZ methylcarbamates have only limited water
solubility which only allows their preparation as tablets for oral administration in humans. Small differences in
drug solubility may have a major influence on their absorption and resultant pharmacokinetic behaviour (21). It has
been previously reported that the use of complexing agents such as hydroxypropyl-β-cyclodextrin (CDs) increases
both the water solubility of the BZ methylcarbamates flubendazole (FLBZ) and albendazole (ABZ) (6), and their
systemic drug exposure in different species (8, 13). ABZ formulated as either a suspension or capsules is the drug
most frequently used to treat CE in human. Although beneficial results (positive therapeutic response in ~75%
of cases) have been reported in different studies (10, 32), more often the success rate is somewhat lower than
expected. Furthermore, the prolonged treatment period required for CE therapy, is frequently associated with
side effects (17). Flubendazole (FLBZ), an alternative BZ methylcarbamate compound, failed to affect *E.*
granulosus cysts in man after its oral administration as a suspension or tablets (9, 29). However, the improved kinetic behaviour of FLBZ administered as a CDs-based solution resulted in enhanced FLBZ clinical efficacy on CE developed in mice (8), showing that the increased FLBZ bioavailability induced by CDs would impact on the amount of drug reaching the hydatid cysts and its overall antiparasitic effect.

The anthelmintic efficacy after a drug treatment may depend not only on the activity of the parent drug, but also on the activity of active metabolite/s. In the case of ABZ, two metabolites are found in the systemic circulation after its administration to different animal species, ABZ-sulphoxide (ABZSO) and ABZ-sulphone (ABZSO₂) (4, 23, 28, 31). While ABZSO is an active metabolite, the sulphone derivative do not exerts anthelmintic action. The main metabolic pathways for FLBZ include reduction to form reduced-FLBZ (R-FLBZ), and hydrolysis of the methylcarbamate group to form the hydrolyzed-FLBZ metabolite (H-FLBZ). R-FLBZ was the main analyte recovered from the bloodstream of sheep (25) and mice (8) treated with FLBZ, in which only trace amounts of H-FLBZ were detected. Likewise, while H-FLBZ is an inactive metabolite, some biological activity has been described for R-FLBZ (1). In order to gain further insight on the activity of drugs used against CE, the potential contribution of the parent compound and its active metabolites on the final anthelmintic effect need to be considered.

The ability of drugs to reach high and sustained concentrations at the site of parasite location in the body depends on their pharmacokinetic and metabolic pattern in the host. Thus, the characterization of the pharmacokinetic and/or pharmacodynamic behaviour of drug/metabolites has been relevant to achieve optimized drug treatments for different parasitic infections (24). In the search for improved therapy for CE, the goals of the current work were: a) to compare the ex vivo activity of FLBZ, ABZ and their respective metabolites against E. granulosus PSC; b) to characterize the plasma drug exposure and cyst concentration.
profiles for FLBZ and ABZ in mice infected with *E. granulosus*; and c) to compare the clinical efficacy of FLBZ and ABZ orally administered as a CDs-solution to mice infected with *E. granulosus*.

**Materials and Methods**

**Chemicals**

Janssen Animal Health (Beerse, Belgium) kindly provided pure analytical standards of FLBZ, and its metabolites R-FLBZ and H-FLBZ. Reference standards of ABZ, ABZSO and oxibendazole (OBZ, used as internal standard) were purchased from Sigma-Aldrich (Dorset, UK). The solvents used for the chemical extraction and chromatographic analysis were HPLC grade (Baker, Inc., Phillipsburg, NJ, USA). The CDs was kindly supplied by Cargill Inc. (Hammond, IN, USA).

**FLBZ/ABZ solutions and formulations**

The FLBZ, R-FLBZ, ABZ and ABZSO solutions used for *ex vivo* studies were prepared by dissolution of 10 mg of pure standard of each drug in 100 mL of methanol. The FLBZ and ABZ formulations used in the efficacy and pharmacokinetic studies were prepared by dissolution of 50 mg of pure FLBZ or ABZ and 10 g of CDs in 100 mL of dionized water. The pH was adjusted to 1.2 using hydrochloric acid (25 mM). The formulations were shaken during 48 h (40 °C) and then were filtrated through 0.2 µm filter (Whatman, Clifton, NJ, USA). The final FLBZ/ABZ concentration was confirmed by HPLC.

**Protoscoleces collection**

PSC of *E. granulosus* were collected aseptically from hydatid cysts obtained from liver of infected cattle slaughtered in an abattoir 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 (5).
Experimental design

Ex vivo study

The culture protocols were carried out as previously described (11) using 199 medium (Gibco, Invitrogen, Buenos Aires, Argentina) supplemented with 100 IU penicillin, 100 µg/mL streptomycin, and 4 mg/mL glucose. Cultures were performed (by triplicate) in 10 mL of incubation medium at 37°C without changes of medium (12). Viable and free PSC (1500 per Leighton tube) were incubated with 10 nmol/mL of FLBZ, R-FLBZ, ABZ or ABZSO. PSC incubated with culture medium alone or culture medium supplemented with methanol was used as controls. Culture tubes were followed microscopically everyday. Samples of PSC (approximately 70–90 PSC in 180 µL of incubation medium) from both control and treated groups were taken every 6 days for up to 54 days. Vitality of the PSC was monitored using the methylene blue exclusion technique. Additionally, ultrastructure studies with scanning electron microscopy (SEM) were performed. Samples were fixed with 2.5% glutaraldehyde in sodium cacodylate buffer for 48 h at 4°C. Then, several washes in cacodylate buffer were made. The specimens were dehydrated by sequential incubations in increasing concentrations of ethanol and were finally immersed in hexamethyl-disilazane for 5 min, 1 h and then overnight. They were then sputter-coated with gold (100 Å thick) and inspected on a JEOL JSM-6460 LV (Peabody, MA, USA) scanning electron microscope operating at 15 kV.
In vivo studies

Animals

Balb/c mice (4 months old at the starting of the experiments) were used. The animals were housed in temperature controlled (21 ± 2 ºC), light-cycled (12h light/dark cycle) room. Food and water were provided ad libitum. Animal procedures and management protocols were approved by the Ethics Committee according to the Animal Welfare Policy (act 087/02) of the Faculty of Veterinary Medicine, Universidad Nacional del Centro de la Provincia de Buenos Aires (UNCPBA), Tandil, Argentina (http://www.vet.unicen.edu.ar ). The animals were used for the following studies:

Pharmacokinetic study

Balb/c mice (n= 88) were infected by intraperitoneal (i.p.) inoculation of 1500 PSC/animal, suspended in 0.5 mL of medium 199. Six months post-infection, the animals were allocated into two experimental groups (44 animals/group): the FLBZ and ABZ groups received a single dose of either FLBZ- or ABZ-CDs solution, respectively. Both formulations were given orally at the same dose rate (5 mg/kg), using an intragastric tube. Blood and cysts samples were obtained from sacrificed animals (n= 4 per collection point) at the following times post-treatment: 5, 15 and 30 min, 1, 2, 3, 4, 6, 8, 10 and 12 h. Blood samples were centrifuged at 2000 x g for 15 minutes and the recovered plasma was stored at -20 ºC until analysis by HPLC. Cysts sample were washed several times with physiologic solution and stored at -20 ºC until analysis by HPLC.

HPLC dosajes and pharmacokinetic analysis of the concentrations data

Plasma (100 µL) and cysts (0.5 g) samples were spiked with OBZ as internal standard (1 µg/mL). After 5 min, plasma and cyst samples were supplemented with 1.5 mL of acetonitrile HPLC grade. Later samples were shaked 15 min in multi-tube-vortexer (VWR, Scientifics Products, West Chester, PA, USA) and centrifuged at 3800 x g for 10 min. The supernatant was concentrated to dryness in a vacuum concentrator (Speed-Vac®,
Savant, CE, USA) and then reconstituted with 150 µL of mobile phase. Finally, 50 µL of this solution was injected into the chromatographic system.

Chromatography was performed on a Shimadzu HPLC equipment (Shimadzu Corporation, Kyoto, Japan), with an ultraviolet visible spectrophotometric detector (UV) (SPD-10A) reading at 292 nm, a C18 reversed-phase column (5 µm, 250 mm x 4.6 mm, Kromasil®, Sweden) were used for analysis of FLBZ, ABZ and its metabolites. The calibration curves for each analyte constructed by least squares linear regression analysis, showed good linearity with correlation coefficients \( \geq \) than 0.994. Mean absolute recoveries percentages for concentrations ranging between 0.01 and 4 µg/mL (n= 5) were 90.2 (H-FLBZ), 92.1 (R-FLBZ) and 90.2% (FLBZ) with CV of 10.7; 7.4 and 10.6 %, respectively and 81.1 (ABZSO), 76.4 (ABZSO2) and 92.4 % (ABZ) with CV of 5.2, 4.3 and 6.4%, respectively. The limit of quantification (LOQ) was defined as the lowest measured concentration with a CV \( \leq \) 20% an accuracy of ± 20 % and an absolute recovery \( \geq \) 70 %. The LOQ for all assayed analytes were 0.01 µg/mL.

The peak concentration (Cmax) and time to peak concentration (Tmax) were read from the plotted concentration-time curves of each analyte. The plasma and cyst area under the concentration time-curve (AUC) were calculated by the trapezoidal rule (15), using the PKSolutionTM computer program (Summit Research Services, Ashland, OR, USA).

**Efficacy study**

Balb/c mice (n= 30, body weight 25 ± 5 g) were infected with PSC of *E. granulosus* as above described (Pharmacokinetic study). At nine months post-infection, the animals were allocated into the following experimental groups (10 animals/group): unmedicated control group, animals received CDs (10%) as a placebo; FLBZ group, animals were treated with the FLBZ-CDs solution; and ABZ group, animals were treated with the
ABZ-CDs solution. All treatments were performed by intragastric inoculation (0.3 mL/animal) every 12 h for 25 consecutive days. The FLBZ and ABZ dose rate was 5 mg/kg. At the end of the treatment period, the animals were sacrificed, and necropsy was carried out immediately thereafter. The cysts present were removed from the peritoneal cavity. The weight of the cysts collected from each animal was recorded.

Statistical analysis

The results obtained in the ex vivo studies are presented as vitality (%), arithmetic means ± SD. The observed differences 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. The pharmacokinetic parameters are presented as arithmetic means ± SD. Non-parametric test (Mann-Whitney) was used for the statistical comparison of the pharmacokinetic data obtained from the two experimental groups. For the clinical efficacy study, cyst weights (arithmetic mean ± SD) were compared by ANOVA. The Tuckey’s range test was used to indicate the order of significance when a significant F value was obtained. In all cases, 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

Figure 1 shows the survival of *E. granulosus* PSC exposed to FLBZ, R-FLBZ, ABZ or ABZSO (10 nmol/mL). Control PSC incubated in the absence of drug were not altered and remained viable (94.1 ± 1.8%) after 36 days of incubation. In contrast, loss of PSC viability in FLBZ-treated cultures was observed after 6 days, with a 35.4 ± 0.7% reduction in the number of viable parasites. The number of dead PSC increased with the drug exposure time, and the viability decreased to 54.8% after 18 days of culture. The maximal protoscolicidal effect of FLBZ was observed after 25 days of incubation when the percentage of vital PSC was only 17.3%. From days 30 onwards, viable parasites could no longer be observed in cultures treated with 10 nmol/mL of FLBZ. The treatment with either ABZ or ABZSO also showed a protoscolicidal effect, which was reached later compared to that observed for FLBZ, with 61% (ABZ) and 67.4% (ABZSO) of parasites remaining viable in culture after 18 days, and the percentage of vital PSC decreased to 29.1% (ABZ) and 52.2% (ABZSO) at 30 days of culture.

Likewise, the PSC incubation with R-FLBZ was associated with a loss of viability similar to that observed for ABZSO. In this case, the viability diminished from 68.4% at 18 days of incubation to 65.9% (24 days) and 58.5% (30 days) (Figure 1).

The primary site of drug damage was the parasite tegument, where the presence of numerous blebs could be observed by inverted microscope (data not shown). SEM analysis demonstrated the drug-induced morphological and structural damage in FLBZ, R-FLBZ, ABZ and ABZSO-treated PSC (Figure 2). Hence, the results of vitality tests coincide with the tissue damage observed at the ultrastructural level by SEM. However, the damage was faster and appeared to be broader after FLBZ treatment, where a complete loss of morphology was observed at 25 days p.i. At this time, all PSC showed rostellar disorganization and complete shedding of microtriches. The internal tissue was severely affected, resulting in the loss of its integrity and an increase in the number of lipid droplets (Figure 2).
FLBZ and R-FLBZ were the main analytes detected in plasma and cyst obtained after the oral administration of FLBZ to infected mice. The concentrations profiles (mean ± SD) of FLBZ and R-FLBZ either in plasma or within recovered cysts are shown in Figure 3. FLBZ achieved the peak plasma concentration at 30 min after its oral administration, being detected up to 6 h post-treatment (Figure 3 shown only up to 4 h). Cmax values of 1.56 ± 0.3 and 1.02 ± 0.1 µg/mL were observed for FLBZ and R-FLBZ, respectively. Table 1 summarizes the main pharmacokinetic parameters obtained for FLBZ and R-FLBZ after the oral administration of FLBZ to mice. There were no significant differences (P > 0.05) between the concentration of FLBZ and R-FLBZ measured in cysts samples (Table 1). Both, the cyst concentrations of FLBZ and R-FLBZ results 10-fold lower than those observed in plasma. The main pharmacokinetic parameters obtained for ABZ metabolites in plasma and cyst of infected mice are shown in Figure 4. The parent drug was not detected at any time post-treatment, neither in plasma nor in cysts. ABZSO and ABZSO2 were the main metabolites recovered in plasma. Similarly, both metabolites were measured in cysts recovered from ABZ-treated mice. Higher peak plasma concentrations (Cmax) were observed for ABZSO (2.60 ± 0.5 µg/mL) compared to ABZSO2 (0.40 ± 0.1 µg/mL). A similar pattern was observed in cysts, where ABZSO concentrations were 5-fold higher than those observed for ABZSO2. These results were reflected in a higher AUC value for ABZSO both in plasma (4.40 ± 0.4 µg.h/mL) and cysts (1.50 ± 0.1 µg.h/g) compared to those obtained for ABZSO2 (0.70 ± 0.2 and 0.30 ± 0.1 µg.h/mL-g in plasma and cysts, respectively) (Table 2).

All infected animals involved in the efficacy study developed cysts in their abdominal cavity. Figure 5 shows the weight (mean ± SD) of the cysts recovered from unmedicated control and treated mice. There were no statistical differences (P > 0.05) between cysts weight recovered from the unmedicated group (10.5 ± 8.7 g) and ABZ treated group (8.23 ± 6.6 g). Conversely, after oral administration of FLBZ to mice the cysts weight (0.98 ± 0.8 g) were significantly lower (P < 0.05) than those obtained in both the unmedicated and ABZ-treated group.
Discussion

The pharmacological activity mediating BZ action is based on the binding to parasite β-tubulin, which produces subsequent disruption of the tubulin-microtubule dynamic equilibrium (18). While the sulphides (ABZ, fenbendazole) have a greater affinity for β-tubulin, their oxidized metabolites (ABZSO and oxfendazole, respectively) bind to a lesser extent. As a consequence, the parent compounds have a higher anthelmintic potency compared to the sulphoxide metabolites, as it was previously demonstrated in different in vitro (19, 22) and ex vivo (27) studies. The anthelmintic potency of FLBZ appears to be similar to that described for ABZ or fenbendazole (20, 27). No data are available on the anthelmintic activity of the R-FLBZ, which is the main FLBZ metabolite found in plasma of sheep (25) and mice (8), with the exception of an observed effect on Fasciola hepatica egg hatching for that metabolite (1). In the current experiment, all compounds involved in the ex vivo study (FLBZ, R-FLBZ, ABZ and ABZSO) decreased the PSC vitality. Nevertheless, the PSC cultured in the presence of FLBZ were killed more effectively and faster compared to those incubated with ABZ. While, after 30 days of incubation, PSC vitality was reduced 70 and 50% by ABZ or ABZSO respectively, FLBZ killed 99% of PSC in the same time period. The faster activity of FLBZ has been previously demonstrated by Elissondo et al (12), who obtained similar outcome after culture of PSC with different concentrations of FLBZ. Furthermore, the current study describes for the first time the protoscolicidal effect of R-FLBZ, which resulted similar to that observed for ABZSO.

In agreement with kinetic data previously obtained in mice (8), a high FLBZ plasma concentration was observed after its administration as a CDs-solution. Furthermore, the current observations shows a similar behaviour after ABZ administration as a CDs-based solution in mice, compared to that obtained after the administration of ABZ as a conventional suspension (7). CDs increased FLBZ and ABZ water solubility (6), which accounted for enhanced absorption and bioavailability in mice, compared with the conventional suspension formulation (8). After the oral administration of FLBZ to mice, FLBZ and R-FLBZ were the main
analytes recovered in plasma. The ratio plasma AUC FLBZ/R-FLBZ was 1.33, as a result of the predominance of FLBZ over R-FLBZ in mice plasma after the administration of FLBZ. This plasma profile is different to that described in sheep, where R-FLBZ was the main metabolite found in the bloodstream (25). In the current work, as previously reported in other species (23, 28, 4, 31) following ABZ administration, ABZSO and ABZSO$_2$ were the only analytes recovered in plasma, being ABZSO the main metabolite measured up to 4 h (Figure 4) and accounting for 86% of the total analytes measured in plasma.

The higher the concentration profiles achieved at the tissue/fluid of parasite location, the greater the amount of drug reaching the target parasite (2). Consequently, the enhancement in plasma drug exposure induced by formulation in CDs impacts on the amount of drug reaching the hydatid cysts and its overall antiparasitic effect. The FLBZ/R-FLBZ pattern found in cysts was similar to that observed in plasma, resulting in a cyst AUC ratio of 1.25. However, cyst concentrations represented only 19% (FLBZ) and 21% (R-FLBZ) to those measured in plasma. ABZ metabolites cyst accumulation appears to be higher, representing 34% (ABZSO) and 43% (ABZSO$_2$) of the plasma concentrations.

Even when animals were treated at the same dose (5 mg/kg) of both FLBZ and ABZ, a higher cyst concentration of ABZ metabolites was observed, compared to that measured for FLBZ/R-FLBZ. The greater ABZSO cyst concentrations could be explained by its higher concentrations in the bloodstream. Furthermore, since in the murine model hydatid cysts develop “free” in the whole abdominal cavity, the drug can reach the cyst only from the peritoneal fluid. The greater water solubility of ABZSO compared to that reported for FLBZ or R-FLBZ (26) could determine its high concentration in the peritoneal fluid, and consequently the greater amount of drug reaching the target parasite. In fact, ABZSO have been detected in high concentrations in organics aqueous fluids as intestinal fluids (3) and urine (16).
After 25 days treatment period, a clear reduction on cyst weight was observed following the administration of the FLBZ-solution, compared to the unmedicated control mice (Figure 5). The mean cysts weight was reduced by 90% in the FLBZ treated mice, which agree with previously reported results (8). Contrarily, under our experimental conditions, ABZ treatment did not result in significantly changes in cyst weight compared to the unmedicated mice (14% reduction, P > 0.05). Since ABZ has previously demonstrated an excellent activity against hydatid cyst developed in the murine model (7, 35, 36), it is likely that the lack of clinical effect observed in the current work may be related to differences in the infectious material (age of cysts, intermediated host) as well as in its management at the time of collection. Furthermore, the relatively short treatment period may limit ABZ action. The shorter period of time required by FLBZ to exert its action is consistent with the *in vitro* results, where FLBZ required 24 days of incubation to kill 90% of protoescoleces, while ABZSO killed 40% in the same time period. The only active molecule that reaches the hydatid cyst after ABZ treatment is the ABZSO active metabolite and cyst accumulation (AUC) resulted 4-fold higher to that observed for FLBZ. However, this difference decreases when the sum of FLBZ + R-FLBZ cysts exposure is considered.

In conclusion, while ABZ efficacy is based on the capacity of its ABZSO metabolite to reach the cyst, after FLBZ administration the ability of both, the parent drug and its reduced metabolite to accumulate into the hydatid cyst, may account for its improved *in vivo* activity. The low FLBZ/R-FLBZ cyst concentration may be compensated by the higher anthelmintic potency of the parent drug. The current results clearly show that if FLBZ systemic availability is improved by different drug-formulation strategies, FLBZ may be a highly useful alternative for CE treatment in humans. The outcome of the drug reported here greatly contributes with comparative data on the pharmacological performance of two BZ compounds for use in the CE treatment.
Acknowledgements

This work was partially supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Agencia Nacional de Promoción Científica y Técnica (ANPCyT both from Argentina). The authors acknowledge Dr. Kathleen Vlaminck, Dr. Leo Van Leemput (Janssen Animal Health, Beerse, Belgium) and Dr. Gustavo Viana (Janssen, Buenos Aires, Argentina) for providing the FLBZ used in the present experimental work. The CDs was kindly supply by Cargill Inc. (Hammond, IN, USA).
References


Figure 1: Survival (mean ± SD) of *E. granulosus* protoscoleces after exposure to either culture medium (alone or supplemented with methanol, control group), flubendazole (FLBZ), reduced flubendazole (R-FLBZ), albendazole (ABZ), or albendazole sulphoxide (ABZSO) at the same concentrations (10 nmol/mL) over 54 days. ▲ - Control medium, △ - Control medium + MEOH, ■ - FLBZ, □ - R-FLBZ, ● - ABZ, ○ - ABZSO.

Figure 2: Representative images of the scanning electron microscopy (SEM) of protoscoleces cultured *in vitro* for 6 days in presence of medium containing methanol (10 µL) (Control group) or flubendazole (FLBZ), reduced flubendazole (R-FLBZ), albendazole (ABZ), and albendazole sulphoxide (ABZSO). a) evaginated control protoscolex. b) invaginated control protoscolex. c) evaginated protoscolex cultured with FLBZ. Note the extensive drug-induced damage with contraction in soma region as well as the tegument markedly altered and also shedding of microtriches and disorder on the rostellum. d), e) and f) evaginated protoscolex cultured with R-FLBZ. The altered tegument of soma region and loss of microtriches on the rostellum can be observed. Detail of the rostellum from protoscolex are shown in Figure d. and invaginated protoscolex with an extensive damage affecting the tegument. g) evaginated altered protoscolex in presence of ABZ. The shedding of microtriches can be observed in the scolex region either on suckers or on rostellum, h) invaginated protoscolex clearly altered after cultured in presence of ABZSO.

Figure 3: Plasma and cystic concentration profiles (mean ± SD) for flubendazole (FLBZ) and reduced-FLBZ (R-FLBZ) after FLBZ oral administration (5 mg/kg), as a hydroxypropyl-β-cyclodextrin-solution, to infected mice.
Figure 4: Plasma and cystic concentration profiles (mean ± SD) for albendazole sulphoxide (ABZSO) and albendazole sulphone (ABZSO₂) after oral administration of ABZ (5 mg/kg) as a hydroxypropyl-β-cyclodextrin-solution, to infected mice.

Figure 5: Box plot representation showing the comparative distribution of the weight (g) of hydatid cysts recovered from either unmedicated, flubendazole (FLBZ) and albendazole (ABZ) treated mice. Both treatments were given at 5 mg/kg, every 12 h during 25 days. A significant weight cysts reduction (P<0.05) was achieved in FLBZ treated animals compared to the control group.
Pharmacokinetic parameters (mean ± SD) for flubendazole (FLBZ) and its reduced metabolite (R-FLBZ) obtained in plasma and cyst after the oral administration of FLBZ to mice (5 mg/kg).

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Plasma</th>
<th>Cysts</th>
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<tbody>
<tr>
<td></td>
<td>FLBZ</td>
<td>R-FLBZ</td>
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<tr>
<td>AUC₀₋ₜ (µg.h/mL-g)*</td>
<td>1.80 ± 0.3ᵃ</td>
<td>1.35 ± 0.3ᵃ</td>
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<tr>
<td>Cmax (µg/mL-g)**</td>
<td>1.56 ± 0.3ᵃ</td>
<td>1.02 ± 0.1ᵇ</td>
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<tr>
<td>Tmax (h)</td>
<td>0.30 ± 0.0ᵃ</td>
<td>0.40 ± 0.1ᵃ</td>
</tr>
<tr>
<td>Dp</td>
<td>5 min-6 h</td>
<td>5 min - 6 h</td>
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</tbody>
</table>

AUC₀₋ₜ: Area under the concentration vs. time curve from time 0-t; Cmax: Peak plasma concentration; Tmax: Time to the Cmax; Dp: Detection period. Different letters indicate statistical differences in the pharmacokinetic parameter (P< 0.05). *Plasma AUC is expressed as µg.h/mL and cyst AUC as µg/h/g. ** Plasma Cmax is expressed as µg/mL and cyst Cmax as µg/g.
Table 2

Pharmacokinetic parameters (mean ± SD) for albendazole sulphoxide (ABZSO) and albendazole sulphone (ABZSO\(_2\)) obtained in plasma and cyst after the oral administration of albendazole (ABZ) to mice (5 mg/kg).

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Plasma ABZSO</th>
<th>Plasma ABZSO(_2)</th>
<th>Cysts ABZSO</th>
<th>Cysts ABZSO(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(_{0-t}) (µg.h/mL-g)</td>
<td>4.40 ± 0.4(^a)</td>
<td>0.70 ± 0.2(^a)</td>
<td>1.50 ± 0.1(^b)</td>
<td>0.30 ± 0.1(^c)</td>
</tr>
<tr>
<td>Cmax (µg/mL-g)</td>
<td>2.60 ± 0.5(^a)</td>
<td>0.40 ± 0.1(^b)</td>
<td>0.50 ± 0.1(^b)</td>
<td>0.10 ± 0.0(^c)</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.50 ± 0.1(^a)</td>
<td>0.50 ± 0.1(^a)</td>
<td>1.00 ± 0.0(^a)</td>
<td>1.00 ± 0.0(^a)</td>
</tr>
<tr>
<td>Dp</td>
<td>5 min – 6 h</td>
<td>5 min ± 4 h</td>
<td>5 min ± 6 h</td>
<td>5 min ± 4 h</td>
</tr>
</tbody>
</table>

**AUC\(_{0-t}\):** Area under the concentration vs. time curve from time 0-t; **Cmax:** Peak plasma concentration; **Tmax:** Time to the Cmax; **Dp:** Detection period. Different letters indicate statistical differences in the pharmacokinetic parameter (P< 0.05).

*Plasma AUC is expressed as µg.h/mL and cyst AUC as µg.h/g. ** Plasma Cmax is expressed as µg/mL and cyst Cmax as µg/g.
Figure 1
Figure 3

PLASMA

Time (h)

0 1 2 3 4

0.0 0.5 1.0 1.5 2.0

Plasma concentration (µg/mL)

FLBZ
R-FLBZ

Cysts concentration (µg/g)

Time

0.0 0.08 0.12

FLBZ
R-FLBZ

5 min 15 min 30 min 1 h 2 h 4 h

Time
Figure 4

**PLASMA**

- ABZSO
- ABZSO₂

**Cysts**

- ABZSO
- ABZSO₂

<table>
<thead>
<tr>
<th>Time</th>
<th>Cysts concentration (µg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 min</td>
<td>0.2</td>
</tr>
<tr>
<td>15 min</td>
<td>0.4</td>
</tr>
<tr>
<td>30 min</td>
<td>0.6</td>
</tr>
<tr>
<td>1 h</td>
<td>0.8</td>
</tr>
<tr>
<td>2 h</td>
<td>0.4</td>
</tr>
<tr>
<td>4 h</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Figure 5

A box plot showing the distribution of cyst weight (g) for different groups: CONTROL, ABZ, and FLBZ. The plot indicates significant differences among the groups, with letters (a and b) denoting statistical significance.