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Nematicidal Activity of Flubendazole and Its Reduced Metabolite on a Murine Model of *Trichinella spiralis* Infection

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Key Words
Trichinella spiralis · Flubendazole · Reduced flubendazole · Efficacy

Abstract
Background: Flubendazole (FLBZ) is a broad-spectrum benzimidazole anthelmintic compound. The parent FLBZ is metabolized to its reduced (R-FLBZ) and hydrolyzed (H-FLBZ) metabolites. There are no data on the potential nematicidal activity of R-FLBZ, the main plasma metabolite found in sheep and mice. The goal of the current work was to assess the efficacy of FLBZ and R-FLBZ against *Trichinella spiralis* in a mouse model. ***Methods:*** Both compounds were administered to Balb/c mice infected with *T. spiralis* as either a cyclodextrin aqueous solution or as a carboxymethylcellulose suspension. Treatments were performed orally (5 mg/kg) at 1 day after infection with *T. spiralis*. The efficacy of the treatments was assessed at day 6 after infection. ***Results:*** While the efficacy obtained for FLBZ and R-FLBZ administered as a solution was 94 and 98%, respectively, the efficacies obtained after the treatment with FLBZ suspensions were 38% (FLBZ) and 64% (R-FLBZ). ***Conclusion:*** Under the current experimental conditions, a high nematicidal efficacy of both FLBZ and R-FLBZ administered as solution preparations was observed.

Introduction

The characterization of the pharmacokinetic and/or pharmacodynamic patterns has been relevant to optimize drug use in the treatment of different bacterial and parasitic infections [1–3]. Benzimidazole-methylcarbamates (BZD) are broad-spectrum anthelmintic compounds widely used in human and veterinary medicine to control nematode, cestode and trematode infections [4]. The broadly used molecules on this chemical family include albendazole (ABZ), fenbendazole (FBZ), flubendazole (FLBZ) and mebendazole. The BZD methylcarbamates have only limited water solubility, which allows their preparation as suspensions/tablets for oral administration. For BZD anthelmintics, dissolution is the rate-limiting step in the systemic availability of the active drug/metabolites [5]. It has been previously reported that the use of complexing agents such as cyclodextrins (CD) increases the water solubility of both FLBZ and ABZ [6] and their systemic drug exposure in mice [3, 7]. After the oral administration of FLBZ as a solution or suspension, both formulations demonstrated a preventive chemopro-

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phylactic effect in *Echinococcus granulosus*-infected mice [8]. However, since anthelmintic efficacy of BZD compounds is closely related to the presence of sustained concentrations at the site of parasite location, the improved pharmacokinetic behaviour observed after the administration of FLBZ as a CD solution permits higher drug exposure of the hydatid cysts, which enhances its clinical efficacy in echinococcosis [3].

The methylcarbamate BZD compounds are extensively metabolised in all mammalian species studied [9, 5]. A common pattern among different BZD is the short life of the parent drug and the predominance of their metabolic products in the bloodstream and all tissues and fluids of the host [5], as well as in parasites recovered from BZD-treated animals [10–12]. The primary metabolites are usually products of oxidative and hydrolytic processes and are all more polar and water soluble than the parent drug. For example, ABZ and FBZ are oxidized into their sulphoxide metabolites named ABZ-sulphoxide and oxfendazole, respectively. These metabolites have higher water solubility than the sulphides (parent drugs). Furthermore, while ABZ and FBZ have a greater affinity for β-tubulin (the putative mode of BZD action), the sulphoxide derivatives ABZ-sulphoxide and oxfendazole bind to a lesser extent. As a consequence, the parent compounds have a higher anthelmintic potency compared to the sulphoxidated metabolites, as previously demonstrated in different in vitro [13, 14] and ex vivo [15] studies. The presence of a ketone group in position-5 determines a different biotransformation pattern for FLBZ (compared to other BZD) in the host. The FLBZ parent compound and its reduced (R-FLBZ) and hydrolyzed (H-FLBZ) metabolites have been identified in the bloodstream of treated poultry [16], sheep [17] and mice [3]. The anthelmintic potency of FLBZ appears to be similar to that described for ABZ or FBZ [14, 15]. While H-FLBZ is an inactive metabolite, no data is available on the anthelmintic activity of the R-FLBZ, which is the main FLBZ metabolite systemically available in sheep [17] and mice [3]. Although some in vitro work on the effect on *Fasciola hepatica* egg hatching [18] and *E. granulosus* protoscolices [7] has been done, further information of the anthelmintic activity of the main FLBZ metabolite is needed. Since R-FLBZ may contribute to the final FLBZ anthelmintic effect, it would be relevant to assess the individual nematicidal activity of this metabolite. The *Trichinella spiralis*/mouse model is a suitable method to assess anthelmintic efficacy under in vivo conditions because of its low cost and the use of a low amount of drugs.

The main research goal of this work was to compare the in vivo nematicidal effect of FLBZ and R-FLBZ formulated as a CD-based aqueous solution or suspensions on a murine model of *T. spiralis*.

Materials and Methods

Chemicals

FLBZ and R-FLBZ were kindly provided by Janssen Animal Health (Beerse, Belgium). Carboxymethylcellulose (CMC) of low-viscosity grade was purchased from Anedra (Buenos Aires, Argentina). Roquette, France, kindly supplied us the hydroxypropyl-β-cyclodextrin (HPBCD; Kleptose-B-CD).

FLBZ and R-FLBZ Formulations

The FLBZ and R-FLBZ CD-based solutions were prepared by dissolution of 50 mg of pure FLBZ or R-FLBZ and 10 g of HPBCD in 100 ml of deionized water (pH = 1.2). The pH was adjusted using hydrochloric acid (25 mM). The formulations were shaken during 48 h (40°C) and then were filtrated through 0.45 μm filter (Whatman, Piscataway, N.J., USA). FLBZ and R-FLBZ suspensions (0.5 mg/ml) were prepared by addition of pure FLBZ and R-FLBZ in deionized water with CMC (0.5% p/v, pH = 6.0) under shaking during 6 h. FLBZ and R-FLBZ suspensions were vigorously shaken before its intragastric administration to mice.

Animals

Balb/c mice (6 months of age at the start of the experiments) were used. The animals were housed in a temperature-controlled (21 ± 2°C), light-cycled (12-hour 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).

Experimental Design

Balb/c mice (n = 60) were orally infected with 500 L1 muscle larvae of *T. spiralis* per mouse. The L1 larvae were obtained from infected mice carcasses, after artificial digestion following the technique described by Ruiz et al. [19]. The animals were divided into six experimental groups (n = 10) as follows: Control-sol, unmedicated control group treated with the CD solution; Control-susp, unmedicated control group treated with CMC suspension; FLBZ-sol, treated with FLBZ formulated as a CD-based solution; R-FLBZ-sol, treated with R-FLBZ formulated as a CD-based solution; FLBZ-susp, treated with FLBZ formulated as a suspension; and R-FLBZ-susp, treated with R-FLBZ formulated as a suspension. Treatments were performed orally (5 mg/kg) by intragastric administration (0.3 ml animal) at 1 day after infection with the L1 *T. spiralis* larvae. The anthelmintic efficacy of FLBZ and R-FLBZ against preadult *T. spiralis* stages was assessed on day 6 after infection, according to Lopez-García et al. [20]. After sacrifice, the number of worms remaining in the gut were isolated and counted following the method described by Denham and Martinez [21]. Efficacy of the treatments was calculates as: %E = (mean of worms

in C – mean of worms in T)/mean of worms in C × 100, in which C represents the unmedicated control group and T the treated group. The geometric mean was used as it most accurately represents the distribution of nematode populations within each group.

Nematode counts in each experimental group were compared by a nonparametric (Kruskal-Wallis) test using the Instat 3.0 Software (Graph Pad Software, La Jolla, Calif., USA). A probability of $p < 0.05$ was considered statistically significant.

Results and Discussion

All infected animals included in the unmedicated control groups developed *T. spiralis* worms in their small intestine. The nematode counts (mean ± SD) obtained for all experimental groups are shown in table 1. There were no statistical differences ($p > 0.05$) between the number of worms recovered from the Control-CD group (106 ± 77 nematodes) and the Control-CMC group (100 ± 94 nematodes), indicating that the vehicle from both preparations did not affect the nematode burdens. Significantly ($p < 0.05$) lower nematode counts were observed in animals treated with FLBZ or R-FLBZ as a CD-based solution. Efficacies of 94 and 98%, were observed for FLBZ-sol and R-FLBZ-sol, respectively. Conversely, animals treated with the suspension showed similar ($p > 0.05$) nematode counts compared to those observed in the Control-CMC group, with efficacies as low as 38% (FLBZ) and 64% (R-FLBZ). These results clearly demonstrate the nematicidal effect of R-FLBZ under the current experimental conditions, and the importance of improved drug solubility in the clinical efficacy of BZDs compounds. The relevance of this finding is supported by the fact that R-FLBZ is the main FLBZ metabolite found in the bloodstream of treated sheep, which has demonstrated equivalent ability to accumulate into target parasites (*Moniezia* spp.) to that observed for the parent FLBZ compound [17].

After the oral administration of BZD as a suspension, drug particles must dissolve to facilitate its absorption through the gastrointestinal mucosa and its diffusion through the nematode cuticle [22]. The water solubility of BZD compounds drastically increases at low pH values. Thus, the dissolution of BZD compounds occurs mainly in the stomach. The short gastrointestinal transit time in mice determine a shorter time for dissolution of the administered drug suspension. The drug that cannot dissolve in gastrointestinal contents pass down and is excreted in the faeces without exerting its action [5]. After administration of FLBZ or R-FLBZ as a CD-based solution, the dissolution of the active molecule is not a limiting step for drug efficacy. It would be expected that worms were exposed to a higher amount of drug, explaining the high efficacy observed after the use of FLBZ and R-FLBZ as a CD-based solution. These results are consistent with previous studies where the use of an ABZ-CD [23, 24, 7] and FLBZ-CD solution [3, 7] resulted in improved anthelmintic efficacy.

In conclusion, the R-FLBZ metabolite contributes to the nematicidal activity observed for FLBZ. Furthermore, the use of pharmacotechnically based strategies would be helpful to improve the clinical efficacy of BZD anthelmintics for the treatment of different parasitic diseases. Altogether, the data reported here is a further contribution to the understanding of the relative anthelmintic potency of FLBZ as its active R-FLBZ metabolite, and the effect of drug formulation on BZD anthelmintic activity against *T. spiralis* in mice.

Table 1. Preadult *T. spiralis* counts (mean ± SD) in mice treated with the vehicle without FLBZ of a CD-based solution or CMC suspension, and in mice treated (5 mg/kg) with FLBZ and its reduced metabolite (R-FLBZ) formulated either as a solution or suspension

	Control	FLBZ	R-FLBZ
CD-solution			
Worm counts	106 ± 77^a	17 ± 29^b	6.3 ± 14^b
Efficacy[1]	–	94%	98%
CMC-suspension			
Worm counts	100 ± 93^a	56 ± 64^c	55 ± 148^c
Efficacy[1]	–	38%	64%

The treatment efficacy is also shown. Different superscript letters are statistically different at $p < 0.05$.

[1] Efficacy was calculated using geometric mean.

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