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Comparative assessment of albendazole and triclabendazole ovicidal activity on *Fasciola hepatica* eggs

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ABSTRACT

The benzimidazole compounds albendazole (ABZ) and triclabendazole (TCBZ) are both effective against *Fasciola hepatica*, although ABZ is only effective against adult flukes. Additionally, ABZ is a broad-spectrum nematodicidal compound with well-known ovicidal activity. However, no data on the ovicidal effect of TCBZ against *F. hepatica* eggs are available. The work reported here evaluated the comparative ovicidal effect of ABZ, TCBZ and their sulphoxide metabolites on *F. hepatica* eggs recovered from bile of sheep artificially infected with either a TCBZ-susceptible (Cullompton) or a TCBZ-resistant (Sligo) isolate of *F. hepatica*. Additionally, the effects of different non-flukicidal methylcarbamate benzimidazole compounds on the hatching of *F. hepatica* eggs were evaluated. Eggs (500 eggs/mL, $n = 4$) were incubated for 12 h either with TCBZ, TCBZ sulphoxide (TCBZ.SO), ABZ (5, 10 and 20 nmol/mL) or without drug (untreated control) (Experiment 1). Additionally, the effect of TCBZ and TCBZ.SO (5 nmol/mL) on egg hatchability was examined after a long (15 days) drug exposure (Experiment 2). Furthermore, the ovicidal effect of ABZ and ABZ.SO at different concentrations (5, 1, 0.5, 0.1and 0.05 nmol/mL) (Experiment 3), and the effect of fenbendazole (FBZ), oxfendazole (OFZ), mebendazole (MBZ), flubendazole (FLBZ) (5 nmol/mL) and reduced-FLBZ (R-FLBZ) (2 μg/mL) on fluke eggs, were evaluated after a 12-h exposure (Experiment 4). Egg hatch was assessed by direct microscopic observation after incubation at 25 °C for 15 days. TCBZ and TCBZ.SO did not affect egg hatch after a 12-h incubation. A similar result was obtained after a much longer drug exposure (15 days) (Experiment 1 and 2). However, a significant ($P < 0.05$) inhibition of egg hatch was observed in ABZ- and ABZ.SO-incubated eggs (Experiments 1 and 3). Additionally, the non-flukicidal compounds (Experiment 4) affected egg hatchability, particularly FLBZ and R-FLBZ. In conclusion, ABZ and ABZ.SO had a clear inhibitory effect on egg development of *F. hepatica*. However, the most extensively used flukicidal compound, TCBZ, and its main sulphoxide metabolite, did not affect egg hatch, even in TCBZ-susceptible flukes.

1. Introduction

Fascioliasis, caused by the trematode liver fluke *Fasciola hepatica*, is the cause of considerable loss in sheep and cattle production systems all over the world (Roberson and Courtney, 1995). Benzimidazoles (BZD) are broad-spectrum anthelmintic compounds widely used in human and

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veterinary medicine to control nematode, cestode and trematode infections (McKellar and Scott, 1990). The BZD compounds currently marketed as anthelmintics can be grouped as BZD thiazolyls, BZD methylcarbamates, pro-BZD and halogenated BZD thiols (Lanusse and Prichard, 1993). Only a few molecules within the BZD chemical family demonstrate activity against *F. hepatica* (Fairweather and Boray, 2005). The halogenated derivative triclabendazole (TCBZ) is the most effective because of its excellent activity against adult and juvenile flukes (Boray et al., 1983). Consequently, it is the most widely used and this has led to the selection and emergence of TCBZ-resistant fluke populations in several areas of the world (data reviewed by Fairweather, 2005, 2009). TCBZ is structurally quite different from other BZD, having neither a carbamate nor a thiazolyl ring in the carbon 2 position (Campbell, 1990), but having three chloride atoms in its molecule. Albendazole (ABZ) is the only BZD methylcarbamate recommended for the control of fascioliasis in domestic animals, despite its activity being restricted to flukes older than 12 weeks (McKellar and Scott, 1990). Fenbendazole (FBZ), a similar BZD methylcarbamate widely used in veterinary medicine as a nematodicidal drug, is not as effective as ABZ against *F. hepatica*, but a single treatment of 5 mg/kg reduced *F. gigantica* infection in sheep by up to 95% (Roberson and Courtney, 1995).

The BZD anthelmintics are extensively metabolised in all mammalian species studied (Gottschall et al., 1990; Lanusse and Prichard, 1993). TCBZ parent drug is not detected in plasma after its oral administration to sheep, indicating that it is completely removed from portal blood by the liver following absorption (Hennessy et al., 1987). TCBZ is oxidised to form the metabolites triclabendazole sulphoxide (TCBZ.SO) and triclabendazole sulphone (TCBZ.SO_2). Since TCBZ.SO is the major metabolite found in the plasma of TCBZ-treated sheep, and only low concentrations of the parent compound are detected in bile (unpublished observations), TCBZ.SO has been postulated to be responsible for the activity against liver flukes (Fairweather, 2005, 2009). Similar to that observed for TCBZ, ABZ is not found in the bloodstream after enteral administration to sheep (Marriner and Bogan, 1980) and cattle (Prichard et al., 1985). ABZ oxidations lead to more polar and less anthelmintically active metabolites. In terms of binding to parasite tubulin, the ABZ parent drug is more potent than its sulphoxide metabolite (ABZ.SO), while the sulphone (ABZ.SO_2) is an inactive derivative (Lacey, 1990; Lubega and Prichard, 1991).

In addition to their excellent nematodicidal efficacy, BZD anthelmintics demonstrate ovicidal activity, as eggs of many nematode species fail to hatch after BZD exposure in the gut contents (Lacey, 1988). Additionally, some BZD methylcarbamate compounds have activity against *F. hepatica* eggs (Coles and Briscoe, 1978). However, as far as we know, no data on the ovicidal effect of TCBZ against *F. hepatica* eggs is available. The main goals of the current trial were: (a) to evaluate the comparative ovicidal effect of TCBZ, ABZ and their respective sulphoxide metabolites on *Fasciola hepatica* eggs obtained from TCBZ-susceptible and -resistant isolates; and (b) to assess the effects of different non-flukicidal methylcarbamate benzimidazole compounds on the hatching of *F. hepatica* eggs.

2. Material and methods

2.1. Chemicals

Pure reference standards of TCBZ and TCBZ.SO (Novartis Animal Health, Basel, Switzerland), ABZ FBZ, OFZ, MBZ (Schering Plough, Kenilworth, USA), FLBZ and its reduced metabolite (R-FLBZ) (Janssen Animal Health, Beerse, Belgium) were used for the experimental assays. The solvents (methanol or DMSO) used for drug dissolution were of analytical grade (Anedra, Buenos Aires, Argentina).

2.2. Collection of F. hepatica eggs

Six (6) Corriedale sheep were orally infected with 200 metacercariae of *F. hepatica* contained in a gelatine capsule. Four (4) animals were infected with a TCBZ-susceptible isolate (named Cullompton) and the other two (2) sheep with a TCBZ-resistant isolate (named Sligo). For details of the history of the two isolates, see Robinson et al. (2004) and McConville et al. (2009). Sixteen (16) weeks after infection, the animals were stunned and exsanguinated immediately. Animal procedures and management protocols were approved by the Ethics Committee according to 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), and to internationally accepted animal welfare guidelines (AVMA, 2001). *F. hepatica* eggs (both isolates) were directly recovered from the bile of each infected sheep. After several washes with tap water, eggs were suspended in water (500 eggs/mL).

2.3. Experimental design

Four separate studies (Experiments 1–4) were performed, as follows:

Experiment 1: It was performed to evaluate the comparative ovicidal effect of the fukicidal compounds TCBZ, TCBZ.SO and ABZ on *Fasciola hepatica* eggs obtained from a TCBZ-susceptible or a TCBZ-resistant *F. hepatica* isolate. Fluke eggs (500/mL, $n = 4$) from both isolates (TCBZ-susceptible or -resistant) were incubated (25 °C) for a 12-h period with either TCBZ, TCBZ.SO or ABZ at a final concentration of either 5, 10 or 20 nmol/mL. They are pharmacologically relevant concentrations obtained from previous studies where the bile concentrations of these BZD compounds were measured after conventional treatments in sheep (Hennessy et al., 1987; Alvarez et al., 2000). A 12-h period of drug–egg contact represents the approximate time of egg exposure to the drug after an *in vivo* treatment. Untreated eggs were incubated as control assays. Untreated and treated eggs were gently washed (3×) to facilitate drug removal, and kept in darkness at 25 °C for 15 days. After this period, the trematode eggs were exposed to daylight for 1 h. When this time had elapsed, 1 mL of 10% (v/v) buffered formalin was added to each tube in order to stop egg hatching. Hatched and unhatched eggs were evaluated using an optical microscope (40× magnification). Approximately 80 eggs were counted to estimate the proportion of hatched eggs in each tube.