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Time-course and accumulation of triclabendazole and its metabolites in bile, liver tissues and flukes collected from treated sheep

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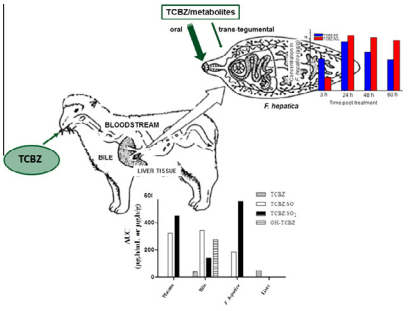
HIGHLIGHTS

- Only the TCBZ sulpho-metabolites were recovered in plasma from TCBZ treated sheep.
- These metabolites were also the main analytes accumulated within adult flukes.
- TCBZ was the main compound accumulated in liver tissue from TCBZ treated sheep.
- The hydroxy-metabolite was recovered in bile from treated sheep.
- Oral ingestion seems to be the main route of drug entry into the flukes.

GRAPHICAL ABSTRACT



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ABSTRACT

The flukicidal compound triclabendazole (TCBZ) has a complex metabolic pattern that includes the systemic presence of its sulphoxide (TCBZ.SO) and sulphone (TCBZ.SO_2) metabolites, usually recovered from the bile of treated animals. The aim of the current work was to evaluate the time-course and pattern of *in vivo* accumulation of TCBZ/metabolites into adult *Fasciola hepatica* specimens recovered from infected sheep. Twelve (12) healthy Corriedale sheep were orally infected with one hundred (100) metacercariae of the TCBZ-susceptible Cullomptom isolate of *F. hepatica*. Sixteen weeks after infection, animals were intraruminally treated with TCBZ (10 mg/kg). At 3, 24, 48 and 60 h post-treatment (pt), animals were sacrificed (n = 3/time period) and samples of blood, bile, liver tissue and adult *F. hepatica* specimens were collected. The concentrations of TCBZ/metabolites were measured by HPLC. TCBZ.SO and TCBZ.SO_2 were the only molecules recovered in the bloodstream, with peak plasma concentrations of 10.8 μg/mL (TCBZ.SO) and 12.6 μg/mL (TCBZ.SO_2). The same metabolites were also the main analytes accumulated within the adult flukes, reaching peak concentrations between 6.35 μg/g (TCBZ.SO) and 13.9 μg/g (TCBZ.SO_2) at 24 h pt, which was coincident with the time when the maximum plasma concentration was attained. Low levels of TCBZ parent drug (0.14 μg/g at 24 h pt) were measured within collected flukes. TCBZ parent drug and its sulpho- and hydroxy-derivatives were recovered in bile collected from treated sheep between 3 and 60 h pt. Although relatively high concentrations of hydroxy-TCBZ (ranging from 0.86 to 10.1 μg/mL) were measured in bile, this metabolite was not recovered within the flukes at any time pt. Finally, TCBZ parent drug was the main compound accumulated in liver tissue over the 60 h pt period. The time-course and drug concentration patterns within the adult liver fluke after TCBZ treatment followed a similar trend to those observed in plasma. Overall, the data reported here confirm that oral ingestion is a main route of drug entry into the trematode *in vivo* exposed to TCBZ/metabolites.

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However, the presence of TCBZ within the adult fluke (despite being absent in the systemic circulation) may be related to some degree of trans-tegumental diffusion from bile or by a direct oral ingestion from portal blood.

1. Introduction

Triclabendazole [6-chloro-5(2-3 dichlorophenoxy)-2-methyl thio-benzimidazole] or TCBZ, an halogenated benzimidazole thiol derivative, shows high efficacy against both the mature and immature stages of *Fasciola hepatica* in sheep and cattle; this is a differential feature compared to other available trematodicidal drugs (Boray et al., 1983). It has been the drug of choice for treating liver fluke infections in livestock for over 20 years and, more recently, has been used successfully to treat human cases of fascioliasis (Fairweather, 2005, 2009). As a consequence of its excellent activity against *F. hepatica*, it has been extensively used and this has led to the selection of TCBZ-resistant populations, which is now a worrying problem in several areas of the world (Fairweather, 2005, 2009, 2011).

After its oral/intraruminal administration to sheep, TCBZ is rapidly metabolized to the sulphoxide (TCBZ.SO) and sulphone ($TCBZ.SO_2$) metabolites (Alvinerie and Galtier, 1986; Mohammed Ali et al., 1986; Hennessy et al., 1987; Ceballos et al., 2010), with plasma peak concentration attained at 18–22 h (TCBZ.SO) and 36–42 h ($TCBZ.SO_2$) post-treatment (pt). Neither TCBZ nor any other metabolites are detected in plasma. Hydroxylation of TCBZ and its two main metabolites also occurs in the liver, but the products are secreted into the bile, mainly in their conjugated form (Hennessy et al., 1987). Maximum levels of the hydroxylated compounds are reached after 8 h (OH-TCBZ), 21 h (OH-TCBZ.SO) and 36 h (OH-$TCBZ.SO_2$) pt (Hennessy et al., 1987). TCBZ.SO and $TCBZ.SO_2$ are the two main (unconjugated) metabolites in both plasma and bile. In plasma, these metabolites bind strongly (>99%) to plasma proteins, mainly albumin (Hennessy et al., 1987), extending the systemic exposure of the drug] in the host. Trans-tegumental diffusion and oral ingestion are the two potential routes available for the entry of drugs into *F. hepatica*. Since adult liver flukes are blood-consuming parasites, plasma protein binding may have an important role in the accumulation of drug into the parasite due to oral ingestion. Therefore, the fluke could be potentially exposed to high drug levels in the bile (surrounding medium). *Ex vivo* studies have demonstrated that TCBZ and all of its metabolic products can diffuse through the tegument of the fluke (Mottier et al., 2004a). Trans-tegumental diffusion appears to be the main route of benzimidazole entry into *F. hepatica*, at least under *ex vivo* conditions (Mottier et al., 2006). Furthermore, the *in vitro* uptake of TCBZ occurs even when the oral route has been closed by ligation (Bennett and Köhler, 1987; Mottier et al., 2006), which suggests that diffusion could play a role in drug uptake *in vivo*. However, it is likely that the drug-parasite interaction could differ *in vivo* because parasites are exposed to a changing drug concentration profile over time in a variable physiological environment. Such a variable environment may be consistent with the different pattern of TCBZ metabolites reported in plasma and bile *in vivo*. Consequently, the contribution of oral ingestion and/or diffusion through the external surface as potential mechanisms of the entry of TCBZ/metabolites into *F. hepatica* needs to be further clarified. The aim of the current work was to evaluate the *in vivo* time-course and patterns of TCBZ/metabolites in adult *F. hepatica* specimens recovered from infected sheep over a 60-h time period.

2. Materials and methods

2.1. Chemicals

Pure reference standards (97–99%) of TCBZ, its sulphoxide (TCBZ.SO) and sulphone ($TCBZ.SO_2$) metabolites, and the hydroxylated compounds (OH-TCBZ, OH-TCBZ.SO, OH-$TCBZ.SO_2$) were kindly provided by Novartis Animal Health (Basel, Switzerland). The different solvents (HPLC grade) and buffer salt used for sample extraction or the chromatographic method were purchased from Baker Ind. (Phillipsburg, USA).

2.2. Animals and experimental design

Twelve (12) healthy male Corriedale sheep (45.8 ± 7.3 kg), aged 14–16 months, were involved in this trial. Animals were housed during the experiment and for 15 days before the start of the study. Animals were fed on a commercial balanced diet. Water was provided *ad libitum*. Animal procedures and management protocols were carried out in accordance with 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) and with internationally accepted animal welfare guidelines (AVMA, 2001).

Animals were each orally infected with one hundred (100) metacercariae of a TCBZ-susceptible *F. hepatica* isolate, named Cullompton. For details of the history of the Cullompton isolate, see Fairweather (2011). Sixteen weeks after infection, sheep were treated with TCBZ (Fasinex® 5%, Novartis) at 10 mg/kg by the intraruminal (i.r.) route. Three animals were killed at each of 4 time-points after treatment (3, 24, 48 and 60 h) and blood, bile, liver and liver fluke samples were collected. Blood was collected by jugular venepuncture into heparinized tubes and immediately centrifuged at 3000*g* for 15 min to obtain the plasma. After dissection of the animal, samples of liver and bile (from the gall-bladder) were taken. To recover *F. hepatica* adult specimens, the liver, common bile ducts and the gall-bladder of each sheep were removed and opened. The fluke specimens were rinsed extensively with saline solution (NaCl 0.9% w/v) to remove bile and/or adhering materials. All obtained samples were placed into plastic tubes and frozen at −20 °C until analysis by high performance liquid chromatography (HPLC).

2.3. Analytical procedures

2.3.1. Extraction of drug from plasma samples

TCBZ and its metabolites were extracted from plasma as previously described (Virkel et al., 2006). Samples (1 mL) were spiked with 10 μL of oxibendazole (OBZ) (100 μg/mL), which was used as an internal standard (IS). After addition of 2 mL of acetonitrile, samples were shaken for 20 min (multivortex) and then centrifuged at 2500*g* for 15 min. The supernatants were recovered and evaporated to dryness in a vacuum concentrator (Speed-Vac®, Savant, Los Angeles, USA). The dry extracts were reconstituted in 300 μL of mobile phase and an aliquot of 50 μL was injected into the HPLC system.