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Time-dependent tegumental surface changes in juvenile *Fasciola gigantica* in response to triclabendazole treatment in goat

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ABSTRACT

Triclabendazole (TCBZ), the anthelmintic drug active against both mature and immature liver flukes, was used to investigate the effect of *in vivo* treatment on the tegumental surface of juvenile *Fasciola gigantica*. Five goats were infected with 150 *F. gigantica* metacercariae each by oral gavage. Four of them were treated with single dose of TCBZ at 10 mg/kg at four weeks post-infection. They were euthanized at 0 (untreated), 24, 48, 72 and 96 h post treatment. Juvenile flukes were manually retrieved from the goat livers and processed for scanning electron microscopy. In control flukes, the anterior region was adorned with sharply pointed spines projecting away from the surface, while in the posterior region, spines become shorter and narrower, loosing serration and with the appearance of distinct furrows and papillae. The dorsal surface retained the same pattern of surface architecture similar to that of ventral surface. Flukes obtained from 24 h post-treatment did not show any apparent change and were still very active. However, there were limited movements and some blebbing, swelling, deposition of tegumental secretions and some flattening displayed by the flukes of 48 h post-treatment. All the worms were found dead 72 h post-treatment and showed advanced level of tegumental disruptions, consisting of severe distortion of spines, sloughing off the tegument to expose the basal lamina, formation of pores and isolated patches of lesions. By 96 h post-treatment, the disruption was extremely severe and the tegument was completely sheared off causing deeper lesions that exposed the underlying musculature. The disruption was more severe at posterior than anterior region and on ventral than dorsal surface. The present study further establishes the time-course of TCBZ action *in vivo* with 100% efficacy against the juvenile tropical liver fluke.

1. Introduction

Fasciolosis, caused by *Fasciola hepatica* and *Fasciola gigantica*, is a serious disease of ruminants worldwide. In some tropical countries, *Fasciola* infection is considered to be the single most important helminth infection of cattle and the prevalence is as high as 30–90% (Spithill et al., 1999). *Fasciola spp*. are also responsible for zoonotic infections of humans as well. About 2.4 million people are infected with this parasite world-wide with possibly 18 million infections undiagnosed and another 180 million people living at risk of infection (Anon, 1995; Mas-Coma et al., 1999). It has been estimated that fasciolosis causes a substantial economic loss of at least $3.2 billion per annum to the live stock industry (Spithill et al., 1999). The economic losses associated with fasciolosis are mainly due to decreased weight gain, milk production and fertility as well as increased costs associated with the need for chemotherapy and condemnation of affected livers at abattoirs (Gajewska et al., 2005). In India, fasciolosis is a serious health problem for live-stock, with the prevalences estimated at 43.28% (Yadav et al., 2009); therefore, the economic losses associated with fasciolosis can be predicted to be huge.

To date, no successful vaccine is commercially available to control fasciolosis, even though many experimental vaccine trials showed varying protection to *Fasciola* infection (Spithill and Dalton, 1998; McManus and Dalton, 2006; Jayaraj et al., 2009).

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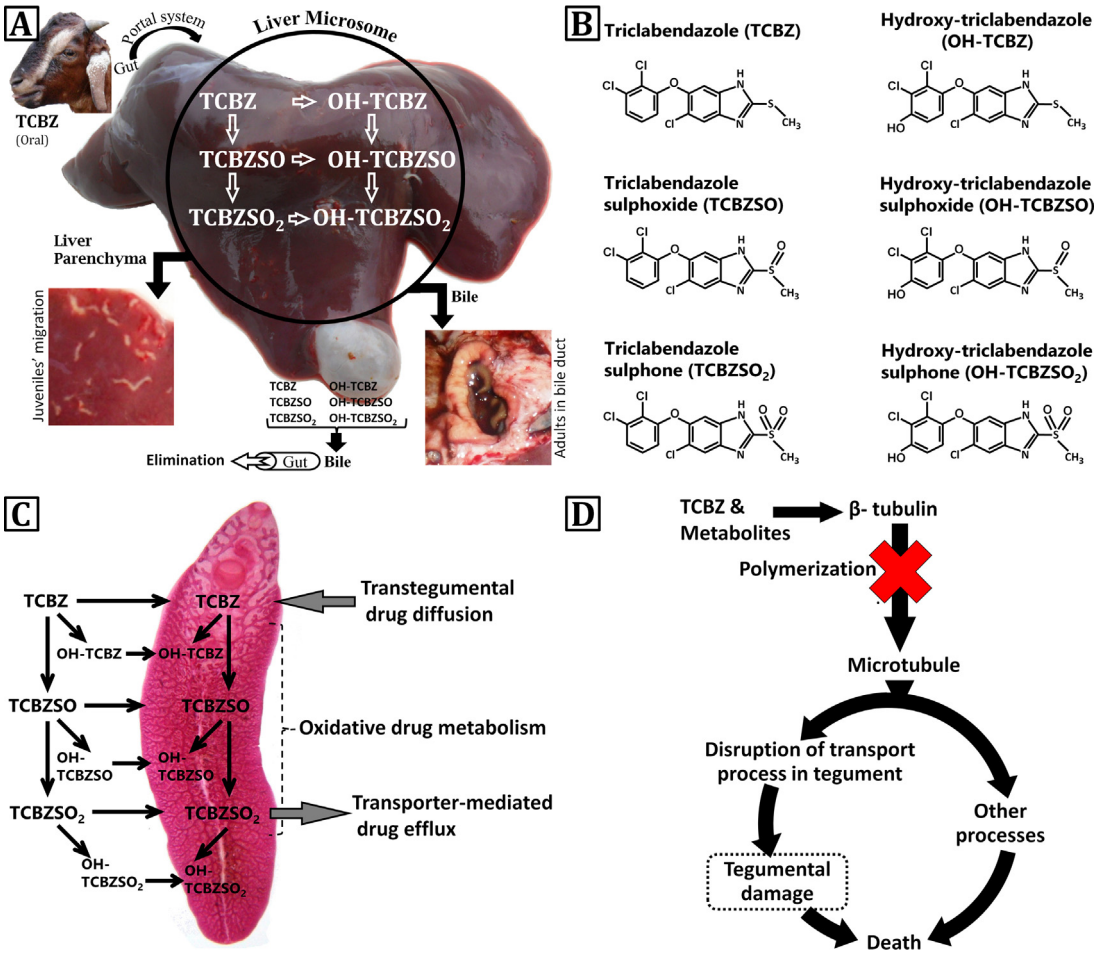

Fig. 1. Mechanisms and pathways involved in triclabendazole (TCBZ) metabolism and pharmacokinetic disposition in goat and TCBZ action on liver fluke. (A) Goats were previously infected with 150 metacercariae of *F. gigantica* and anthelmintic treatment occurred at 4 week post infection with a single oral dose of TCBZ at 10 mg/kg. TCBZ metabolism includes ruminal (including microflora) and hepatic biotransformations into TCBZ-sulphoxide (TCBZSO), TCBZ-sulphone ($TCBZSO_2$), hydroxy-TCBZ (OH—TCBZ), hydroxy-TCBZSO (OH—TCBZSO) and hydroxy-$TCBZSO_2$ (OH—$CBZSO_2$) have been proposed (Virkel et al., 2006). Parent TCBZ rapidly cleared from the blood by the liver (Hennessy et al., 1987) and liver microsomes metabolize into sulpho and hydroxy metabolites (Virkel et al., 2006). Migratory juvenile flukes are exposed to the drug in liver parenchyma (the present study) or these metabolites are excreted into bile duct where adult flukes are bathed in bile and exposed to drug. Eventually, these metabolites are mainly eliminated through faeces (Hennessy et al., 1987). (B) Chemical structures of TCBZ and its metabolites. (C) Molecular interactions of the liver fluke with TCBZ and its sulpho and hydroxy metabolites. The entry of TCBZ, TCBZSO, $TCBZSO_2$ and low amounts of OH—TCBZ, OH—TCBZSO and OH—$CBZSO_2$ in to the fluke is accomplished mainly by diffusion across the tegumental syncytium rather than oral route (Mottier et al., 2006). Within the fluke, the microsomes transform each of these entered drug components into other metabolites, the oxidative drug metabolism (Mottier et al., 2004). *P*-glycoprotein (PGP) is a member of the ATP-binding cassette (ABC) transporters which participate in ATP-dependent efflux mechanism that enable the drug to expel out from the cells (Alvarez et al., 2007). (D) All the TCBZ, TCBZSO and $TCBZSO_2$ contribute to anthelmintic activity and there are variations in the regional specificity in the levels of disruption to the tegument of the fluke (Halferty et al., 2009). These TCBZ and metabolites bind to β-tubulin which blocks the polymerization to form microtubules and consequently disrupt microtubule based processes in the fluke (Stitt and Fairweather, 1993). This would prevent the movement of secretory bodies from the cell body to the tegument that is vital for the maintanace of integrity of the surface membrane which leads to severe progressive damage to the tegument culminating in the death of the fluke (Brennan et al., 2007).

Therefore, the control of fasciolosis is almost exclusively dependent upon chemotherapy. Triclabendazole is the drug of choice because of its potent flukicidal activity, covering the spectrum from early juveniles to reproductively-mature adults (Boray et al., 1983; Rapic et al., 1988). However, the injudicious use can lead to TCBZ-resistance, which is a major problem with the control of *F. hepatica* (Brennan et al., 2007). TCBZ is widely used for treating human *Fasciola* infections as well. Possible mechanisms and pathways involved in TCBZ metabolism and pharmacokinetic disposition in the present study have been depicted in Fig. 1. These mechanisms are well studied in *F. hepatica*, but little is known in *F. gigantica*. Some studies have reported on the action of TCBZ against both adult and juvenile flukes *in vitro* (Meaney et al., 2002; Tansatit et al., 2012). In order to rule out variabilities associated with drug or *ex vivo* culture, such studies must be validated in appropriate ruminant hosts. A huge gap exists between our understanding of anthelmintic action in *F. gigantica* and *F. hepatica*. No information is available on the effects of *in vivo* chemotherapy on intra-mammalian tropical liver fluke. Therefore, in the present study, we aimed to investigate the effect of TCBZ on 4 week old juvenile *F. gigantica* in a goat host, using scanning electron microscopy (SEM) at 24, 48, 72 and 96 h post treatment. In contrast to tests performed *in vitro*, where the flukes are exposed to a single metabolite, the pharmacokinetics of TCBZ *in vivo* is a multistep metabolic pathway where the parasites are exposed to several TCBZ metabolites as a result of hepatic metabolism of administered drug (Hennessy et al., 1987; Virkel et al., 2006; Halferty et al., 2008; Mestorino et al., 2008). The surface tegument represents the front-line of the host-parasite interface, and is the principal route of entry for TCBZ metabolites into the parasite tissues (Toner et al., 2009).

2. Materials and methods

2.1. Animals and parasites

Five male goats, aged approximately 4 months old and weighing between 12 and 16 kg, were selected for the experiment, and