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Tegumental surface changes in adult *Fasciola hepatica* in response to treatment *in vivo* with triclabendazole in the sheep host

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ABSTRACT

Eight indoor-reared crossbred sheep with no pre-exposure to *Fasciola hepatica* were infected by oral gavage with 200 metacercarial cysts of the triclabendazole (TCBZ)-susceptible Cullompton isolate of *F. hepatica*. At 12 weeks post-infection, sheep were dosed with 10 mg/kg triclabendazole. Two sheep per time period were euthanized at 48 h, 72 h and 96 h post-treatment. Two control sheep were euthanized alongside the 96 h triclabendazole-treated sheep. Flukes were recovered from each of the sheeps' liver and, if present, from the gall bladder, and they were processed for scanning electron microscopy (SEM).

Flukes recovered 48 h post-treatment were active. Disruption to the tegument took the form of swelling, widespread blebbing and some loss of the tegument covering the spines. By 72 h post-treatment, all flukes recovered were dead and a number were recovered from the gall bladder. Typically, the posterior end of the flukes was elongated and in this region the tegumental syncytium had sloughed away. Tegumental loss was more widespread on flukes recovered from the gall bladder. At 96 h post-treatment, only one fluke was recovered from the liver and three from the gall bladder. All the flukes were dead and they were totally devoid of tegumental syncytium.

1. Introduction

The liver fluke, *Fasciola hepatica*, is a parasite of considerable importance in the veterinary and agri-food sectors, in temperate regions of the world. As a disease, fascioliasis is also emerging as a significant zoonotic infection of humans, with upward of 180 million people at risk of infection (WHO, 2007). Recent trends suggest that climatic changes may favour transmission of the intermediate stages of the parasite, so prevalence may increase further as a result (Kenyon et al., 2009; Mas-Coma et al., 2009). Triclabendazole (TCBZ) continues to be the mainstay of chemotherapy against liver fluke infection (Fairweather, 2009). It is the only drug that can target the migratory juvenile stages from just 3 days post-infection (p.i.), as well as adult flukes (Boray et al., 1983). In the ruminant host, TCBZ is metabolised to a number of different forms: TCBZ sulphoxide (TCBZ.SO), TCBZ sulphone (TCBZ.SO_2) and the equivalent hydroxy metabolites (Hennessy et al., 1987). Much is known about the absorption, plasma kinetics, tissue distribution, metabolism and elimination of TCBZ in the host (Hennessy et al., 1987; Virkel et al., 2006, 2009; Mestorino et al., 2008; Lifschitz et al., 2009; Ceballos et al., 2010). In contrast, little is known about the time-course of drug action in the host.

A number of morphological studies have been carried out to examine changes to the tegumental surface of the fluke and its internal tissues following treatment with TCBZ, with a view to gain information on its mechanism of action. The studies were conducted either *in vitro*

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or *in vivo*, using the rat as experimental host (Stitt and Fairweather, 1993, 1994; Robinson et al., 2002; McConville et al., 2006; Meaney et al., 2006, 2007; Devine et al., 2009, 2010; Halferty et al., 2009; Toner et al., 2009, 2010). *In vitro* experiments have involved individual metabolites of TCBZ and made use of very rich culture media, which may lessen the impact of drug action to a certain extent. By way of contrast, in the *in vivo* situation, the fluke is exposed to a number (and mixture) of metabolites, each of which has a greater or lesser impact on the fluke (e.g. Halferty et al., 2009). Moreover, the adult fluke has to contend with other potentially damaging factors, such as bile, that could exacerbate drug action (Fairweather et al., 1999). Consequently, it is important to know if the interpretation of data on drug action derived from the *in vitro* and rat experiments accurately represent what happens to the fluke in its natural ruminant host. Also, the speed at which TCBZ acts against the fluke. In efficacy studies, the animal is left for a certain length of time post-treatment (p.t.) (anything between 2 weeks and 17 weeks p.t.: e.g. Boray et al., 1983; Dorchies et al., 1983; Richards et al., 1990; Martínez-Moreno et al., 1997; Gaasenbeek et al., 2001; Walker et al., 2004) before it is sacrificed and flukes recovered, yet drug action may be faster than this.

The present study was carried out to fill this gap in our knowledge. Flukes were recovered at different time-points p.t. (48–96 h). They were prepared for morphological analysis by scanning and transmission electron microscopy (SEM and TEM, respectively), histology and whole-mount staining. The data presented here is of SEM observations of surface changes to the tegument of the fluke. The tegument is an important tissue, as it represents the interface between the parasite and its host and it is the main route by which TCBZ metabolites enter the fluke (Mottier et al., 2006; Toner et al., 2009, 2010). The tegument also plays a number of important roles in fluke biology, including protection against the host immune response (Fairweather et al., 1999) and maintenance of its integrity is essential for the survival of the fluke in the face of a stress situation, such as that provided by drug action.

2. Materials and methods

2.1. Sheep trial

This trial was conducted in 2008 under a project licence, held by Dr. R.E.B. Hanna, and was carried out at the Agri-Food and Biosciences Institute (AFBI) in Stormont, Belfast.

2.1.1. Animals and parasite material

Eight indoor-reared sheep of the Dorset × Suffolk breed, with no pre-exposure to *F. hepatica,* were selected for the trial. To verify that animals were free from infection with *F. hepatica,* faecal samples were collected from all animals 7 days prior to artificial infection and examined for the presence of fluke eggs. Infection was also ruled out via blood sampling and a coproantigen *F. hepatica* ELISA (BioK201; Bio-X Diagnostics, Jemelle, Belgium). Animals were each infected with 200 metacercarial cysts of the TCBZ-susceptible Cullompton isolate of *F. hepatica*. This isolate was obtained in 1998 as a field isolate from an abattoir in Cullompton, Devon, UK and has been shown to be susceptible to TCBZ *in vivo* (McCoy et al., 2005; Halferty et al., 2008; McConville et al., 2009a; Hanna et al., 2010) and to TCBZ.SO *in vitro* (Robinson et al., 2002; McConville et al., 2006; Devine et al., 2009; Halferty et al., 2009; Toner et al., 2009, 2010).

2.1.2. Treatment and necropsy

The eight sheep were divided randomly into two groups: six TCBZ-treated and two control. At 12 weeks post-infection, sheep were orally dosed with 10 mg/kg TCBZ ("Fasinex"; 5%, w/v). This is the recommended dose rate and has been used in a number of studies (e.g. Hennessy et al., 1987; Virkel et al., 2006; Mestorino et al., 2008). The control group received no treatment. Two animals were killed at each of three time-points following anthelmintic dosing: at 48 h, 72 h and 96 h. Two sheep from the control group were taken at the same time as the 96 h TCBZ-treated sheep. Sheep were euthanized by captive bolt stunning followed by exsanguination and their livers, gall bladders and kidneys removed. Flukes were recovered from the bile ducts and, if present, from the gall bladder of the sheep and placed in warm (37 °C) RPMI-1640 medium (Sigma–Aldrich Co. Ltd., Poole, Dorset, UK). Livers were dissected into approximately 5 sections and any remaining flukes were retrieved by crumbling the liver in warm (37 °C) RPMI-1640 medium. Total fluke counts were obtained for each group. Where only partial flukes were retrieved, the count was taken as the higher number of either region collected (either anterior or posterior). Flukes were recovered for SEM analysis.

2.1.3. Tissue preparation for SEM

Initially, the flukes were lightly flat fixed for 1 h at room temperature in 4% (w/v) aqueous glutaraldehyde and subsequently free fixed at 4 °C in a 3:1 mixture of 4% (w/v) aqueous glutaraldehyde and 1% osmium tetroxide overnight. Flukes were then rinsed in 70% (v/v) ethanol and dehydrated in an ascending series of ethanols. Following this, the flukes were dried in hexamethyldisilazane, mounted (with the ventral or dorsal surface facing upwards) on aluminium stubs and sputter-coated with gold–palladium. The flukes were viewed using a FEI Quanta 200 scanning electron microscope, operating at an accelerating voltage of 10 keV.

3. Results

3.1. Visual observations

Flukes recovered from each of the untreated control sheep were active and had full gut contents. The surface architecture of the flukes appeared normal. For images of normal morphology, the reader is referred to the paper by McConville et al. (2009b: Figs. 1–6).

Forty-eight hours after treatment with TCBZ, all of the flukes recovered were active and had full gut contents. By 72 h p.t. with TCBZ, all flukes recovered were inactive and contained limited gut contents. Some flukes appeared to be very elongate and were translucent. Three flukes were recovered from the gall bladder. These flukes were inac-