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Time-dependent changes to the tegumental system and gastrodermis of adult *Fasciola hepatica* following treatment *in vivo* with triclabendazole in the sheep host

E. Toner[a], G.P. Brennan[a], R.E.B. Hanna[b], H.W. Edgar[b], I. Fairweather[a,*]

[a] *Parasite Therapeutics Research Group, School of Biological Sciences, The Queen's University of Belfast, Medical Biology Centre, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom*
[b] *Veterinary Sciences Division, Agri-Food and Biosciences Institute (AFBI), Stormont, Belfast BT4 3SD, United Kingdom*

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ABSTRACT

Eight indoor-reared cross-bred 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 (pt). 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 transmission electron microscopy (TEM). Disruption to the ultrastructure of the tegument became increasingly severe over time pt. Flukes recovered at 48 h pt showed widespread blebbing of the apical plasma membrane and swelling of the mucopolysaccharide masses surrounding the basal infolds. There was evidence of reduced secretory activity in the tegumental cells and spacing between the cells. Sloughing of the tegumental syncytium was observed at 72 h pt. The subtegumental musculature, parenchyma and tegumental cells were severely disrupted. At 96 h pt, all of the flukes were totally devoid of tegument. Disruption to the subtegumental tissue and somatic musculature was severe, and was so extreme in some specimens that the tegumental cells were barely discernible. Disruption to the gastrodermis was also progressive, though not as severe as disruption to the tegument. There was a general decline of secretory activity with time pt. Autophagic activity was apparent from 48 h pt and became more widespread with increasing time, culminating in breakdown of the gastrodermal cell cytoplasm. The mitochondria were swollen and electron-lucent and the cisternae of the granular endoplasmic reticulum were dilated and fragmented from 72 h pt.

1. Introduction

Triclabendazole (TCBZ) remains the most effective drug to treat liver fluke infections, because of its wide spectrum of activity that encompasses both early juvenile and adult flukes (Boray et al., 1983). Most of the studies relating to drug action of TCBZ against the liver fluke have been carried out *in vitro*. A number of different tissues have been examined – the tegument, gut, testis and vitellaria – and their response to individual TCBZ metabolites evaluated (Stitt and Fairweather, 1992, 1993, 1994, 1996; Halferty et al., 2009; Toner et al., 2009, 2010a). The concensus from these studies is that TCBZ action is directed against the microtubule component of the cytoskeleton, as is typical of benzimidazole drugs (see reviews by Fairweather, 2005,

* Corresponding author. Tel.: +44 28 90972298 27; fax: +44 28 90975877 28.
E-mail address: i.fairweather@qub.ac.uk (I. Fairweather).

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2009). Little is known about how quickly the drug acts to cause elimination of the flukes *in vivo* and whether the morphological changes seen previously *in vitro* are an accurate representation of what occurs in the ruminant host.

The present study is part of a number of investigations monitoring morphological changes in the fluke over a 4-day period following treatment of *F. hepatica*–treated sheep with TCBZ. A paper describing surface changes has already been published (Toner et al., 2010b), as has a paper dealing with histological changes in the reproductive organs (Hanna et al., 2010). The current article presents data on internal changes to the tegumental system, the somatic musculature and the gastrodermal cells, as visualised by transmission electron microscopy (TEM). It is hoped that interpretation of these changes will help to clarify the mechanisms that produce the alterations visible externally and ultimately lead to the elimination of the flukes from the host.

2. Materials and methods

2.1. Trial protocol

Full details of the trial design have been given by Toner et al. (2010b), so will not be repeated here. Briefly, 8 indoor-reared sheep of the Dorset × Suffolk breed were selected for the trial and weighed between 68 and 76 kg. The sheep were infected with the TCBZ-susceptible Cullompton isolate of *F. hepatica* and 6 sheep were treated at 12 weeks post-infection with TCBZ at a concentration of 10 mg/kg. Flukes were recovered at autopsy 48 h, 72 h and 96 h post-treatment (pt) and prepared for TEM analysis. Six flukes per sheep were processed at 48 h pt; 6 flukes were processed from one of the TCBZ-treated sheep at 72 h pt, and 2 from the second sheep, as only 7 flukes were recovered from this sheep. At 96 h pt, only 4 flukes in total were recovered from the 2 sheep: of the 4, 2 flukes were processed for TEM. Six flukes from each of the 2 untreated control sheep were recovered at the same time as the 96 h-treated sheep. The sheep were housed indoors throughout the trial period, with water and haylage provided *ad libitum*.

2.2. Tissue preparation for TEM

Initially, the flukes were lightly flat-fixed for 30 min at room temperature in 4% (w/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) containing 3% (w/v) sucrose. Flukes were then dissected into oral cone, anterior midbody, posterior midbody and tail regions, and transverse slices (3 mm in thickness) were taken from these sections. Specimens were then free-fixed for 4 h in 4% (w/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) and subsequently washed in 0.1 M sodium cacodylate buffer (pH 7.4) containing 3% (w/v) sucrose at 4 °C overnight. Fluke sections were then post-fixed in 1% osmium tetroxide for 1 h and again washed in 0.1 M sodium cacodylate buffer (pH 7.4) at 4 °C overnight. Flukes were next dehydrated in an ascending series of ethanols, infiltrated and embedded in Agar 100 resin and polymerised for 48 h at 60 °C. Ultrathin sections (60–70 nm thick) were cut using a Reichert Ultracut E ultramicrotome, mounted on uncoated copper grids, double-stained with uranyl acetate and lead citrate, and viewed in a FEI CM 100 transmission electron microscope operating at an accelerating voltage of 100 keV.

3. Results

3.1. Fluke recovery and condition

Flukes recovered from each of the untreated control sheep were active and had full gut contents. Forty-eight hours after treatment with TCBZ, the flukes were of a similar condition. At 72 h pt, 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: they were inactive, had no gut contents, appeared to be very elongate and thin, and were greenish in colour. After 96 h treatment with TCBZ, only 4 flukes were recovered from the two sheep. All of the flukes were dead and had no gut contents. Three of the four flukes were recovered from the gall bladder and they were very elongate and thin and had a greenish colour. Numbers of flukes recovered at each time period pt are given in Toner et al. (2010b: Table 1).

3.2. Changes to the tegumental syncytium, the underlying tegumental cells and the somatic musculature

All of the images were taken from the posterior midbody region as this was consistently seen to be the most affected region of the fluke (the reader is referred to Toner et al. (2010b) for comparable SEM images).

3.2.1. Control flukes

Control fluke morphology appeared normal. For images of normal tegumental syncytium, tegumental cells and muscle ultrastructure the reader is referred to Halferty et al. (2009) and Fairweather et al. (1999).

3.2.2. Forty-eight hours post-treatment in vivo with TCBZ (10 mg/kg); flukes recovered from the liver

The tegumental syncytium was still intact following 48 h treatment *in vivo*. The apical surface had a convoluted appearance, due to the deep and complex organisation of the apical invaginations (Fig. 1A), and surface blebbing was observed (Fig. 1B). T1 and T2 secretory bodies were present throughout the syncytium, both at the apex (Fig. 1B) and at the base (Fig. 1C), although they were relatively few in number. There was minor swelling of the mucopolysaccharide masses surrounding the basal infolds, although the infolds themselves remained tightly closed (Fig. 1C). In the sub-tegumental muscle blocks, the muscle fibres appeared to be loosely-packed, and spaces were present around the muscle bundles (Fig. 1D and E). T1 and T2 secretory bodies were present in low numbers within the respective T1 and T2 tegumental cells, and Golgi complexes were absent. Autophagic vacuoles were present within the cell bodies (Fig. 1F). Mitochondria were present in normal numbers throughout the syncytium and within the tegumental cells, and retained a normal morphology (Fig. 1A, C and F).