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Praziquantel and albendazole damaging action on in vitro developing *Mesocestoides corti* (Platyhelminthes: Cestoda)

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Abstract

Parasitic flatworms present several steps of body architecture rearrangement during their fast transition from one developmental stage to another, which are, at least in part, responsible for their evasion from host immune response. Besides, different developmental stages present different degrees of susceptibility to drug action, and the identification of more susceptible stages is of importance for the definition of therapeutical approaches. *Mesocestoides corti* (syn. *Mesocestoides vogae*) is considered a good model to study cestode biology because it can be easily manipulated both in vivo and in vitro and due to its relatively close relationship to cestodes of medical relevance, such as those from genera *Echinococcus* or *Taenia*. We have analyzed the damaging action of two broad spectrum anthelmintic drugs (praziquantel and albendazole) throughout the in vitro strobilization process of *M. corti* in order to identify developmental stages or body structures more susceptible to these drugs. Tetrathyridia (larval stage) and segmented-induced worms were cultivated and treated with praziquantel and albendazole. Whole mounted samples, taken from different developmental stages, were fixed and stained with fluorophore-labeled WGA lectin and phalloidin for the analysis of tegument and muscles, respectively. Confocal laser scanning microscopy was used to identify anatomical changes and lesions caused by each anthelmintic drug in a 3D view. We demonstrated that both praziquantel and albendazole cause extensive tissue damage, especially on tegument, and that adult forms were the most susceptible to drug exposure.

Keywords: *Mesocestoides corti*; Tetrathyridia; Anthelmintic drugs; Confocal microscopy; In vitro development

1. Introduction

The knowledge of helminth parasite muscle and tegument anatomy is important for the understanding of host–parasite interactions, the design of anthelmintic drugs, and the comprehension of drug delivery [1,2]. The mechanisms of drug action are better understood from the genetic and biochemical points of view. However, drug effects on parasite body architecture are not very clear [3].

The body surface topography was well examined by scanning microscopy in the platyhelminth parasites [4–6]. However, the examination of surface structures is not sufficient to analyze the inner complexity of tegument or muscle tissues. A confocal laser scanning microscopy (CLSM) allows the study of gross anatomy in a three-dimensional view, and the use of different fluorophore-stained ligands allows the simultaneous analysis of different tissues, structures, and organs [7–9]. Fluorescent-labeled phalloidin, often used for in vitro studies of actin polymerization, can be used as muscular marker to show muscle fiber organization, and, in tapeworms, to stain flame cells [8,9]. *Triticum vulgaris* wheat germ agglutinin (WGA) lectin-conjugated markers bind specifically to *N*-acetyl glucosamine, an abundant carbohydrate in the tegument glycocalyx of platyhelminthes [10,11]. This and other carbohydrate moieties on parasite glycocalyx play important roles in the host–parasite relationship, and form some of the major epitopes that elicit host immune responses [11].

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Praziquantel (PZQ) and albendazole (ABZ) are largely used to treat human and animal cestode infections, and tegument and muscle tissues are major targets to these anthelmintic drugs. PZQ acts specifically on tegument and muscle cells ion channels, altering Ca^{2+} flux and leading to immediate muscle contraction [12–14]. ABZ, a benzimidazole carbamate derived drug, binds to β-tubulin and blocks microtubule formation, which causes a late paralysis [15,16].

Mesocestoides corti, belonging to the Cestoda class [17], represents a good model for the study of several aspects of cestode biology, from drug action effects to parasite interactions with the host immune system [18–21]. Its larval stage (tetrathyridium) is capable of asexual reproduction both in vivo (including in experimental hosts) and in vitro, and its strobilization (segmentation and sexual differentiation) can be induced in controlled culture conditions [22].

The analysis of the three-dimensional rearrangement of *M. corti* tissues and cells under the effect of anthelmintic drugs throughout the in vitro strobilization process is important for the identification of drug targets for future drug delivery studies. In the present work, we have used CLSM to follow muscle and tegument development during strobilization and to assess the effects of PZQ and ABZ on these tissues in different developmental stages.

2. Material and methods

2.1. Collection of parasites and culture conditions

The collection and culture procedures were performed as previously described [22]. Briefly, after 5 months of intraperitoneal infection, tetrathyridia were collected from an euthanized mouse using sterile Pasteur pipette and washed prior to culture. Around 15,000 tetrathyridia were collected from an infected mouse. Tetrathyridia were individually selected (to separate from buds and acephalic structures) and initially cultured in starvation McRPMI medium (Life Technologies, USA), in 25 cm^2 culture flasks incubated in 5% CO_2 at 37 °C, for to 2 days prior to segmentation induction.

2.2. Segmentation induction

Tetrathyridia segmentation was induced by incubation of starved cultured larvae in McRPMI medium containing 0.662% (w/v) trypsin (Sigma-Aldrich, USA), equivalent to $10^5 N_\alpha$-benzoyl-L-arginine ethyl ester (BAEE) units/ml, at 39 °C for 24 h [22]. After induction, cultures were transferred to 24 well plates (Corning, USA) supplied with 2 ml/well of McRPMI medium supplemented with 20% fetal bovine serum (FBS, Cultilab, Brazil) and maintained at 39 °C under 5% CO_2 for up to 11 days.

In order to follow the major changes during strobilization, worms were fixed after established culture intervals according to their different developmental stages, following evagination of scolex (day 1), neck shaping (day 3), strobila elongation and genital pore formation (day 5), segmentation and sexual maturation (day 6) and body necrosis (day 11). For each culture interval (tetrathyridia and developing worms at days 1, 3, 5, 6 or 11) four replicates of 50 individuals/well were analyzed.

2.3. Drug treatment

Tetrathyridia and cultured worms, after strobilization induction at days 1, 3, 5, 6 and 11 were submitted to ABZ or PZQ treatment. The treatments consisted of culture in medium containing 200 ng/ml of PZQ active principle (Sigma-Aldrich) or 200 ng/ml of ABZ (Sigma-Aldrich) for 1, 3, 6 or 24 h. ABZ and PZQ were solubilized in 50 μl of methanol (Merck) or ethanol (Merck), respectively, prior to addition to culture medium. The same volume of vehicle (methanol or ethanol), without PZQ or ABZ, was added to untreated control cultures. Four replicates of 50 individuals/well were treated or not (controls) with PZQ or ABZ for each drug exposure time. After drug exposure, the worms were washed to remove medium and fixed.

2.4. Whole mount fixation and permeabilization

Cultured worms (strobilization-induced or not, and treated or not with PZQ or ABZ) were collected at different culture/drug exposure time intervals as indicated, and washed 5 times in 0.1 M PBS (137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, 15.22 mM Na_2HPO_4, pH 7.4). Samples were fixed in 2% (v/v) paraformaldehyde (Electron Microscopy Sciences)/PBS for 24 h. After fixation, samples were rinsed twice in PBS, once in PBS/0.1 M glycine, and twice in PBS, and permeabilized to fluorophores by incubation in 0.1% (w/v) saponin (Sigma-Aldrich) for 24 h.

2.5. Sectioning and eosin (HE) staining and analysis

Prior to sectioning, tetrathyridia and segmented worms were fixed in 1% (w/v) paraformaldehyde in PBS pH 7.4 at 4 °C for 24 hr, and then embedded in paraffin (all reagents from Merck). Blocks were cut in 5-μm-thick sections and HE stained following standard methods.

2.6. Lectin WGA, phalloidin, and DAPI staining

After permeabilization, whole mounted worms were incubated in blocking solution (1% BSA in PBS containing 0.1% saponin) for 10 min. Staining was performed in the same solution with the addition of 4 μg/ml FITC-conjugated WGA lectin (Sigma-Aldrich), 13.2 nM/ml Phalloidin Alexa Fluor 594 (Molecular Probes), and 10 nM/ml 4′,6′-diamidino-2-phenylindole (DAPI, Molecular Probes) for 60 min at room temperature. After staining, worms were washed once in PBS, and suspended in 100 μl of Fluormount (Molecular Probes)/PBS (2:1) prior to slide assembly. As negative controls, worm samples were incubated with the fluorophores and taken to the microscope to analyze autofluorescence background.