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.

2.7. Microscopic analysis

Differential interference contrast (DIC) imaging and fluorescence CLSM analyses were made in LSM-510 NLO laser scanning microscope (Carl Zeiss). Inverted and light microscopy analyses were made in Axiovert 25 and Axiolab HBO 5A/ac microscopes (Carl Zeiss), respectively.

3. Results

As previously shown [22], dramatic changes take place during the *M. corti* strobilization process, which were evidenced by HE staining and light microscopy (Fig. 1). The significant morphological alterations led us to investigate the damaging action of two broad spectrum anthelmintic drugs

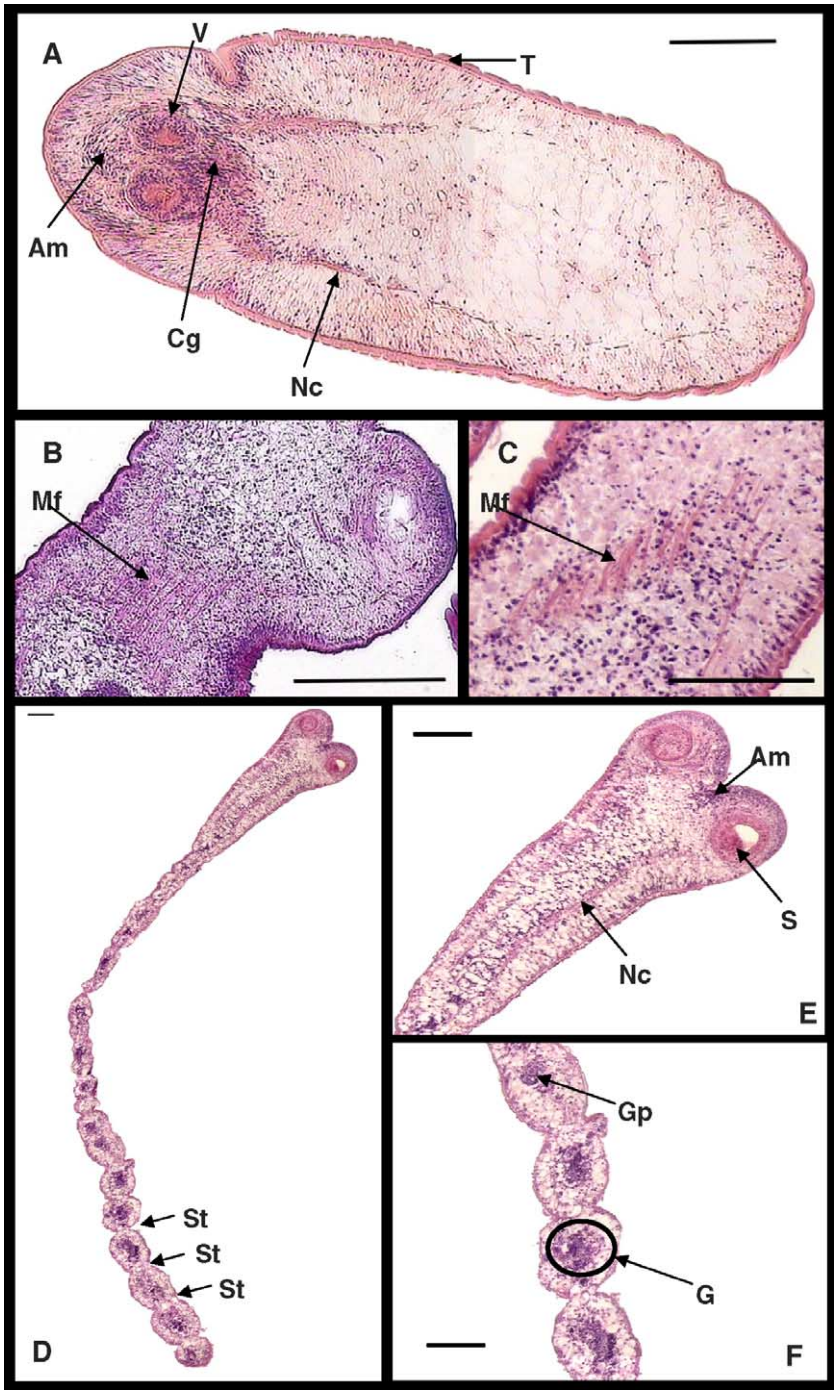

Fig. 1. Photomicrography of *M. corti* at different developmental stages. Histological sections were stained with haematoxylin-eosin. (A) Cultured tetrathyridium. (B) Young adult worm (days 3–5 after strobilization induction) scolex. (C) Young adult worm (days 6–11 after strobilization induction) proximal body. (D) Adult worm. (E) Adult worm scolex. (F) Adult worm distal proglottides. Am, apical massif; Cg, cerebral ganglions; Mf, muscle fibers; Nc, nervous cords; T, tegument; S, suckers; G, genitals; Gp, genital pore; St, strobilization. Bars, 100 μm.

(PZQ or ABZ) throughout the in vitro strobilization process of *M. corti*, in order to identify more susceptible stages or body structures. Cultured worms were then exposed to PZQ or ABZ in different developmental stages, to comparatively assess the drug effects upon tegument structures, muscle fibers, and cell organization after drug action. Light microscopy, and WGA lectin, phalloidin and DAPI staining analyses by CLSM and DIC imaging revealed that exposure to both PZQ and ABZ caused progressive damages. Such damages were better evidenced when comparing worms exposed for 1 or 24 h to each drug, with differences being not very significant when comparing samples taken at shorter time intervals. In all stages and exposure times, worm motility, usually characterized by rhythmic longitudinal contractions, was severely impaired immediately after the exposure to PZQ, which also caused paralysis and formation of surface vesicles. Eventually, ABZ also led to a paralysis state, but it occurred only after 72 h of drug treatment. Real time images of cultured *M. corti* tetrathyridia exposed to PZQ can be viewed at http://www.cbiot.ufrgs.br/~wwwlbmc/misc/videos/.

WGA lectin analysis confirmed that the *M. corti* tegument is very rich in carbohydrates. The tegument covers the body parenchyma in all its extension, with its glycocalyx layer thinner in the posterior region. Muscle tissue, evidenced under

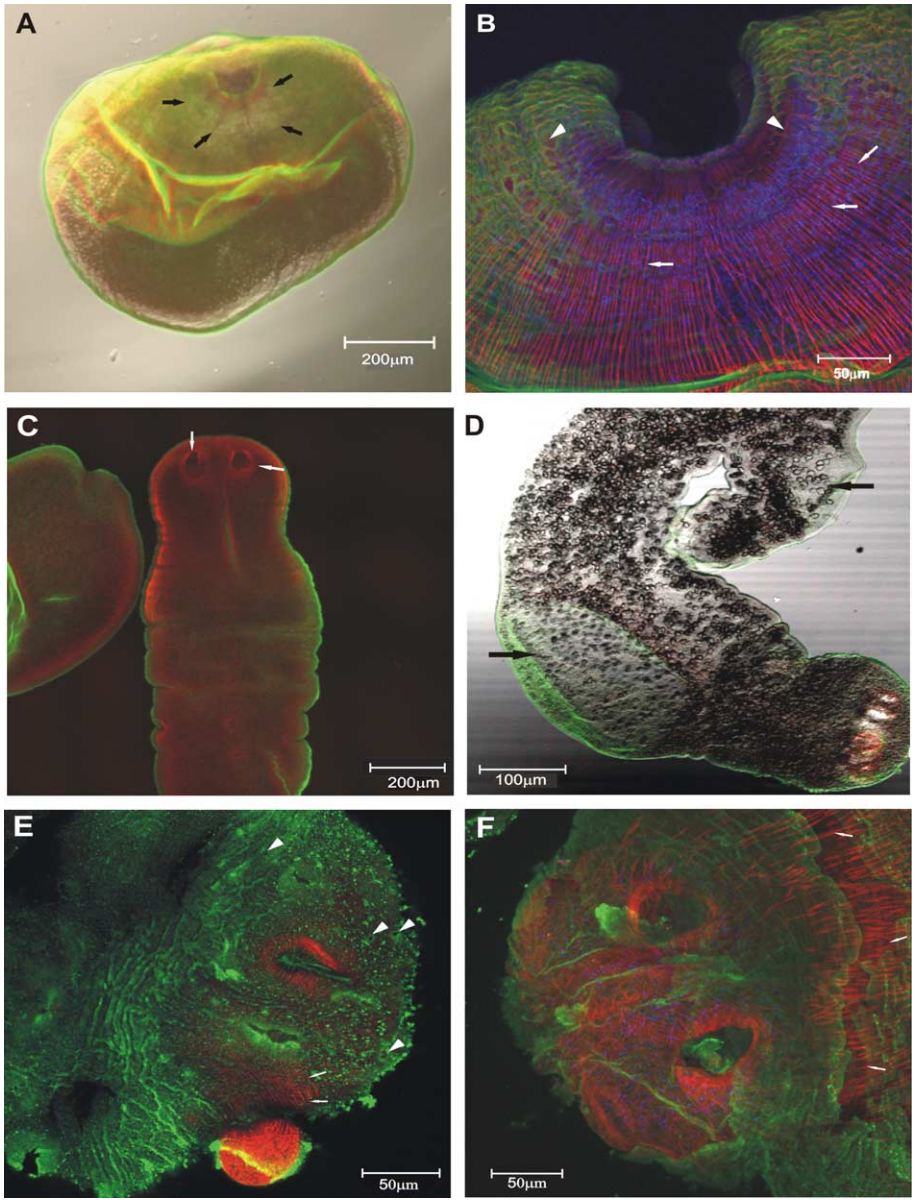

Fig. 2. CLSM images of *M. corti* tetrathyridia cultured in the presence or absence of PZQ or ABZ stained with phalloidin (red), lectin (green) and DAPI (blue). (A) Overlay of images after DIC and CLSM analyses of standard cultured invaginated tetrathyridium. Arrows indicate internal suckers. (B) CLSM analysis of the scolex chamber of a standard cultured invaginated tetrathyridium. Arrows indicate longitudinal fibers and arrowheads circular fibers. (C) CLSM analysis of a worm at day 1 after strobilization induction. Arrows indicate evaginated suckers. (D) DIC and confocal analysis of a 24 h PZQ incubated tetrathyridium. Arrows point to forming vesicles. (E) Scolex of a 24 h PZQ incubated tetrathyridium. Arrows point to fibers; arrowheads point to holes in the tegument. (F) Scolex of a 24 h ABZ incubated tetrathyridium. Arrows point to longitudinal fibers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the tegument by phalloidin staining, appeared as an outer layer of circular fibers enclosing inner longitudinal fibers, which extend from the suckers to the posterior end. In segmented worms, muscle fibers were constricted but not interrupted in proglottid boundaries. Interrupted longitudinal fibers appeared only in the genital pore region of sexually mature proglottides. A comparative analysis between drug damaged and normal tissues at different developmental stages is provided below.

3.1. Tetrathyridia before and after strobilization induction at day 1

Cultured tetrathyridia presented the scolex usually in an invaginated state (Fig. 2A, B). Opening of the scolex chamber for scolex and neck emergence occurred within 24 h after induction (Fig. 2C). The physiological mechanism involved is unknown, but there was significant muscle fiber reorganization

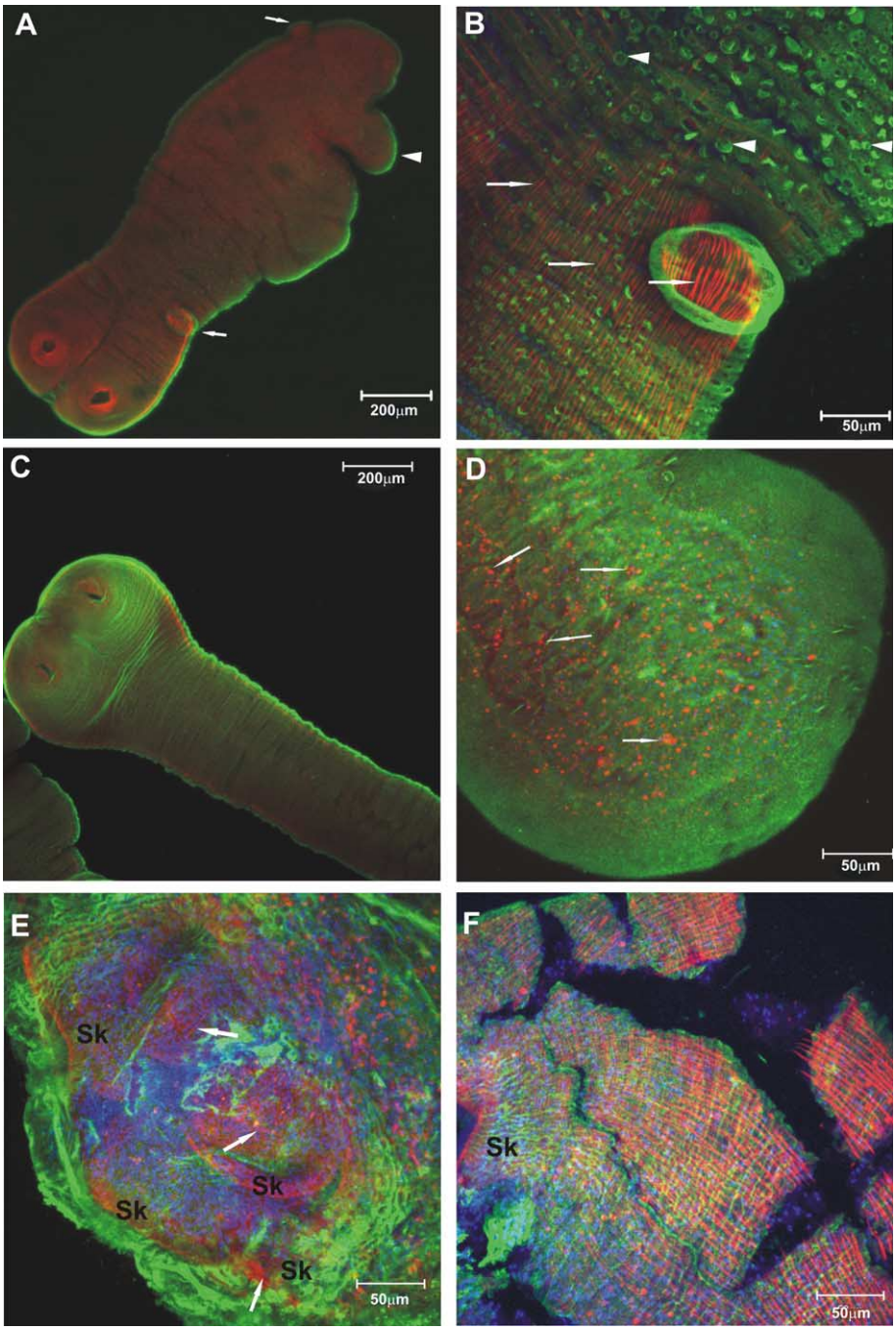

Fig. 3. CLSM images of *M. corti* young adult worms cultured in the presence or absence of PZQ or ABZ stained with phalloidin (red), lectin (green) and DAPI (blue). (A, B) Day 3 strobilization induced worm: view of the whole body (A, arrows point to buds; arrowhead points to a stump resulting of an asexual daughter splitting) and detail of the detaching tegument and an attached bud (B, arrows point to longitudinal fibers; arrowheads show expelled calcareous bodies). (C–F) Day 5 strobilization induced worms. Detail of the anterior (C) and posterior (D) regions (arrows point to flame cells). (E) Scolex of a worm after 24 h of PZQ exposure (arrows point to sucker (Sk) fibers). (F) Anterior region with sucker (Sk) of a worm after 24 h of ABZ exposure. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

in the scolex chamber (Fig. 2B,C). A great number of cells corresponding to suckers could be distinguished in this cavity. PZQ and ABZ treatment did not lead to death of the tetrathyridia, either before or after strobilization induction (Fig. 2D–F). Once PZQ was added to cultures, scolex emergence occurred even without the strobilization induction treatment (Fig. 2D). PZQ exposure induced tegument perforations and detachment (Fig. 2E) and the formation of body vesicles (Fig. 2D) in tetrathyridia and also in later developmental stages. The standard arrangement of outer circular and inner longitudinal muscle fibers was

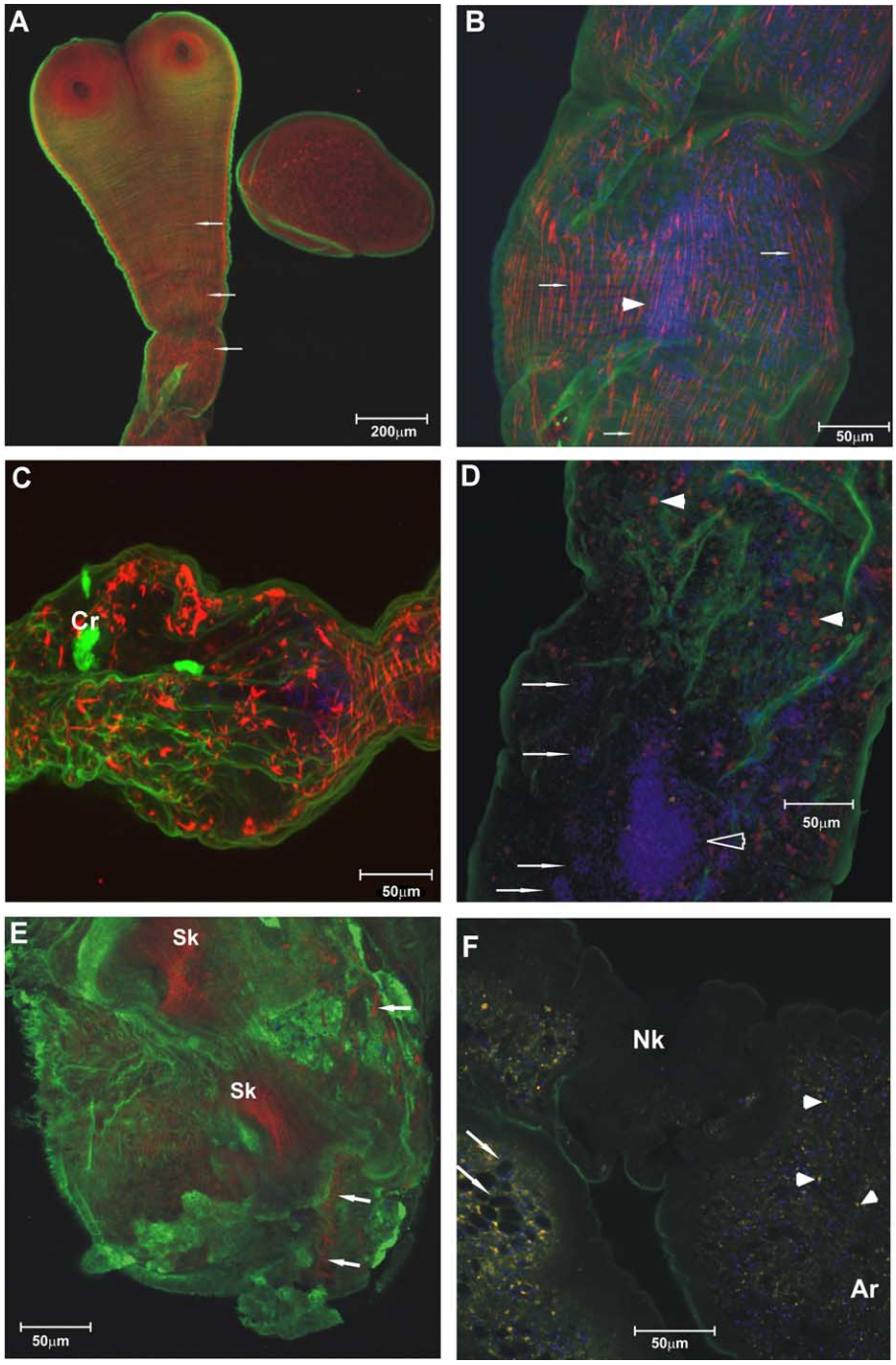

Fig. 4. CLSM images of *M. corti* adult worms cultured in the presence or absence of PZQ or ABZ stained with phalloidin (red), lectin (green) and DAPI (blue). (A–E) Adult at day 6 of strobilization induction. (A) Scolex of a standard cultured induced worm. Arrows point to longitudinal fibers (also visible, but not indicated, in B–E). (B) Standard developing proglottid. Arrowhead points to the central group of cells. (C) Detail of a proglottid still linked to the proximal segment in an adult of day 11 after strobilization induction. Strong WGA staining may be indicating the cirrus (Cr). (D) PZQ damaged proglottid. An open arrowhead points to the central mass of cells; arrows point to cells separated from that mass; arrowheads show flame cells. (E) ABZ damaged scolex; fibers are only visible in the suckers (Sk). (F) Strangled neck (Nk) after ABZ damaged of day 11 strobilization-induced worm. Arrows indicate tegument holes; arrowheads point to lectin and phalloidin co-localization, which is related to flame cells. The worm anterior region (Ar) is indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

partially lost after PZQ treatment (Fig. 2E). ABZ exposure presented less obvious effects (Fig. 2F), but it eventually induced tegument perforations.

3.2. Young adults at culture days 3 and 5 after strobilization induction

Worms at day 3 after strobilization induction were characterized by a better definition of the scolex due to shaping of the neck (Fig. 3A). After strobila elongation, which became more evident at day 5, there was a progressive loss of muscle staining in the posterior region, associated with increased staining of flame cells and muscle fiber rearrangements (Fig. 3C,D). Budding occurred preferentially at day 3 after strobilization induction and the forming buds presented internal muscle fibers in an orientation that followed their external contour and were discontinued and distinct from those in the adjacent body tissue (Fig. 3B).

Interestingly, at day 3 of culture, there was a small degree of tegument detachment even in worms not exposed to drugs (Fig. 3B), although these alterations were quite different from perforations caused by PZQ or ABZ action (Fig. 3E,F). Overall, damages caused by both drugs in day 3 worms were not significantly different from those previously described for tetrathyridia (Fig. 2D–F). However, in a few cases, longer ABZ exposure times caused body rupture and increased tegument perforations (Fig. 3F). The longer and thinner worms at day 5 were more sensitive to both drugs. PZQ often induced muscle fibers disruption (Fig. 3E), and, in 24 h exposed worms, both PZQ and ABZ caused the detachment of body segments.

3.3. Adult worms in culture days 6 and 11 after strobilization induction

In standard cultures, worms at day 6 were fully segmented, with clearly defined scolex and proglottides (Fig. 4A,B). Longitudinal muscle fibers follow the entire strobila length, but became constricted, although not interrupted, between proglottides. The tegument became thinner and, within each proglottid, cells corresponding to the forming sexual organs became apparent, as a central, dense mass of nuclei (Fig. 4B). At days 6 and 11 worms were typically segmented in 22–25

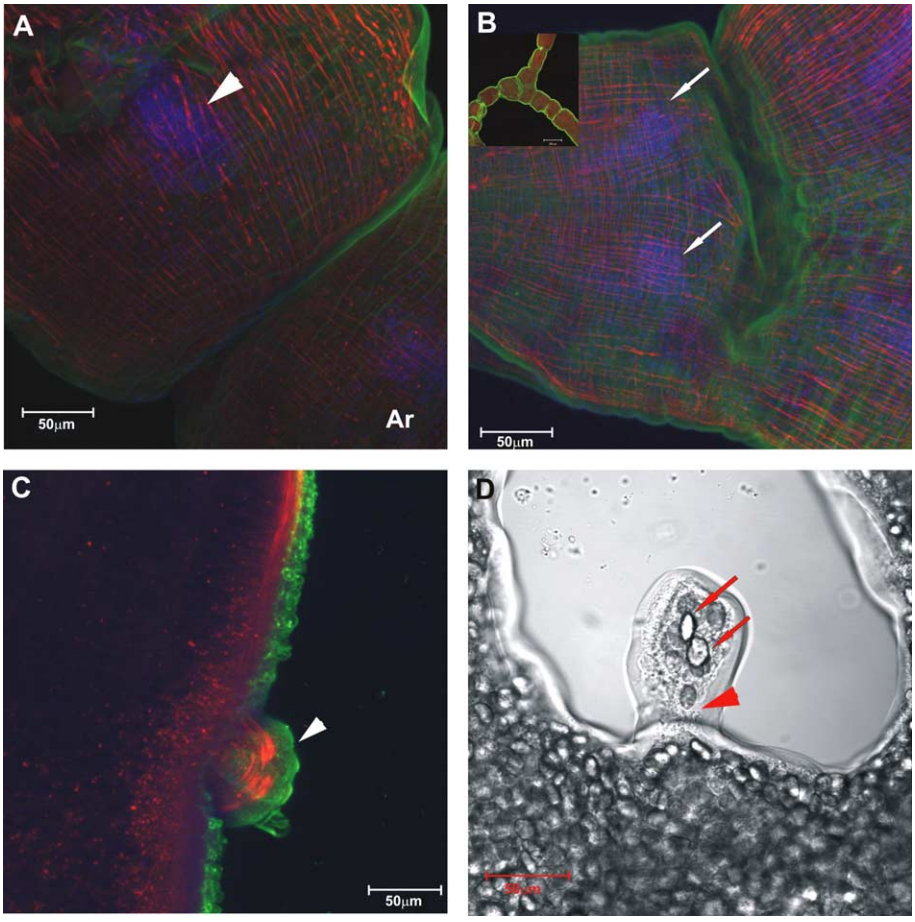

Fig. 5. CLSM images of *M. corti* cultured worms stained with phalloidin (red), lectin (green) and DAPI (blue) showing morphological aspects and structures related to reproduction. (A) Adult worm at day 6 after strobilization induction. The worm anterior region (Ar) is indicated; the arrowhead points to the central mass of cells in the last proglottid, corresponding to the egg pouch. (B) Adult at day 6 after strobilization induction carrying out fissiparity (visible in the small inset). The main enlarged image shows two different masses of cells (arrows) in a single proglottid not yet splitted. (C) Worm at day 3 after strobilization induction. Arrowhead shows an attached bud. (D) DIC of worm at day 3 after strobilization induction after 1 h exposure to PZQ. Arrows point to calcareous bodies inside the folding; arrowhead indicates the parenchyma link between stump and the worm body. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

proglottides. Starting at day 11, worms underwent a progressive degeneration, characterized by overall tegument disintegration, muscle fibers fragmentation and necrosis (Fig. 4C). Up to 90% of these worms presented segment detachment upon both PZQ and ABZ exposure, showing significant decrease in the number of proglottides. Fully segmented worms were readily and thoroughly damaged by PZQ and ABZ treatment even after 1 h exposure, showing both tegument and muscle fibers disruption (Fig. 4D–F). PZQ also affected the architecture of the central mass of cells in each proglottid, which became diffuse and suggestive of inner organs disruption (Fig. 4D).

As any other developmental stage (data not shown), adult worms often underwent asexual reproduction by antero-posterior binary fission. The putative parauterin organ usually became apparent in the last proglottid around day 6 (Fig. 5A). Interestingly, asexual reproduction involving sexually differentiated proglottides occurred with duplication of internal sexual organs prior to longitudinal splitting (Fig. 5B). Buds were clearly distinguishable from tegument folds, whose muscle fibers and parenchyma organization were contiguous to those from adjacent tissues and included calcareous corpuscles (Fig 5C). No budding was observed after drug exposure, while tegument folds were still present in these worms (Fig. 5D).

4. Discussion

The *M. corti* strobilization process involves rearrangements in body architecture, with evident modifications on tegument and inner structures. *N*-acetyl-β-D-glucosamine staining using WGA lectin showed that carbohydrate networks in the tegument vary during strobilization, stretching and becoming thinner as the worm progresses from tetrathyridium to adult stage. Tegument detachments might have been consequence of calcareous corpuscles excretion [23], in addition to a trypsin treatment effect, as recently described by Espinoza et al. [24]. This in vitro situation may represent, at least in part, what happens in vivo, upon ingestion of *M. corti* larvae by a definitive carnivore host, when glycoconjugates would play an important role to insulate parasite from proteases and lipases in the host gut lumen [25]. In early infection steps, the thicker larval tegument would help to survive the passage through the stomach, and, later, a thinner tegument would be enough to endure the relatively less hostile gut environment.

Muscle arrangement of longitudinal and circular fiber follows body contour all over development, which is characteristic of the Cyclophyllidea order [8,19]. However, fiber rearrangement, possibly representing some degree of muscle specialization, was observed in the suckers, as well as in tetrathyridia [19]. In this work, we observed general muscle fiber reorganization within the buds and in the genital pore formation. Neck formation and proglottid definition was followed only by localized fiber constriction.

The existence of actual buds, as described by Specht and Voge [26], is a controversial matter. Hart [27] and Novak [28] proposed that buds would be merely stumps, remaining on the parental body after the separation of split off tetrathyridia. Although stumps also occur in our culture conditions, in the form of lateral outgrowths that may eventually bud off, we were able to clearly distinguish them from what we believe are actual buds, with a differentiated inner structure, at least in what concerns to muscle fibers. Budding was observed in all body portions, which supports the idea [29,30] of localized developmental signals that activate stem cells spread all over the body. As previously reported [22,27,28], there was no evidence of further bud development into tetrathyridia in vitro. However, in vivo, this might occur, accounting for the huge number of asexually generated worms in oral dog infections, not explainable only by fissiparity [31].

Binary fission starts from the anterior region, probably in the apical massif, with the generation of daughter suckers [30]. In later stages (from days 5 to 11), it involves three sequential steps: (i) lateral duplication of inner structures (e.g. sexual organs); (ii) proglottid definition by constriction; and (iii) lateral splitting. This suggests that antero-posterior and lateral developmental signals act in concert to define the worm longitudinal fission. The nature of these signals, however, remains to be elucidated.

There is a general lack of knowledge about anthelmintic drug effects upon different developmental stages of cestode parasites, especially due to difficulties to deal with sexually maturing stages from species infective to humans. *M. corti* tetrathyridia have been commonly used for the evaluation of anthelmintic effects [20,21,32,33], but the recent establishment of an inducible in vitro strobilization system [22] now allowed the study of the differential drug susceptibility of distinct developmental forms. As far as we know, this is the first time that anthelmintic drug effects upon *M. corti* are assessed from tetrathyridia to adult worms.

Two commonly used anthelmintic drugs, PZQ and ABZ, were used in doses equivalent or below those recommended for humans or animals by the World Health Organization for Tropical Diseases Research [34]. Drug dosages were defined empirically, as those allowing the follow up of their progressive effects for up to 24 h; death eventually occurred, usually after 48 h. Both drugs caused severe damages to all developmental stages analyzed. However, damages were more significant in fully segmented worms, usually not addressed in studies to evaluate their effects on cestodes of medical or veterinary importance, such as those from genera *Taenia* and *Echinococcus*. The observed effects are probably, at least in part, representative of those happening to intestine living adult cestode worms upon host oral treatment with PZQ or ABZ. This assumption, however, should be experimentally addressed, applying the same confocal microscopy-based approach to evaluate, for instance, *Echinococcus granulosus* adults obtained from PZQ treated dogs.

Although qualitatively similar in all developmental stages analyzed, damages caused by exposure to PZQ or ABZ were progressively more severe in the later ones. Basically, PZQ caused strong vacuolization, rupture of muscle fibers and disorganization of inner cell masses corresponding to developing organs. ABZ caused less severe damages than PZQ, with its effects restricted to tegument perforations and muscle fiber ruptures.

In *Schistosoma mansoni*, PZQ-sensitive sites are present in the tegument as well as in muscle cells [35,36]. Probably, this

is also the case for *M. corti*, since these tissues were severely, although locally, affected by this drug. The rapid PZQ-induced Ca^{2+} influx [13] could account for both the body contraction observed immediately after drug exposure and the vacuolization [37,38].

The mechanisms of PZQ action upon the platyhelminth tegument barrier have been studied [3,13,39,40]. As demonstrated for *S. mansoni*, PZQ deregulates voltage depending gates activation or inactivation [41]. The drug interferes with a key protein kinase C (PKC)-mediated phosphorylation event, necessary for the regulatory interaction between α and β channel subunits domains and therefore activity inhibition [40,42]. PZQ activates a glycosylphosphatidylinositol-specific phospholipase D (GPIPLD) pathway, which produces phorbol esters or diacylglycerol that activate PKC [43]. The PKC-mediated phosphorylation of proteins destabilizes tegument structure, also resulting in vacuolization [37]. The occurrence of such PZQ-induced and PKC-mediated effect leading to vacuolization in *M. corti*, however, remains to be confirmed.

PZQ-induced Ca^{2+} entry through the tegument also interferes with the electrically coupled muscle cells [13]. Upon reaching the sarcoplasmic reticulum of subtegumental muscle cells Ca^{2+} would induce the observed contraction, and, later, progressive muscle fibers disruption. These effects were more prominent in segmented worms, which is consistent with their thinner and more vulnerable tegument.

Muscle actin fibers are also targets for PZQ action. Upon PZQ exposure, fibers were progressively shattered, which could be easily followed through phalloidin staining. This effect would be accounted for PZQ activation of gelsolin, an actin filament fragmenting protein [44]. Furthermore, Apinhasmit and Sobhon [39] described that, in *Opisthorchis viverrini*, PZQ-induced Ca^{2+} influx could cause depolymerization of the microtrabecular network, which would lead to vacuolization, swelling, blobbing, disruption and detachment of the tegument, and myofilaments breakdown in muscle cells, effects at least in part observed for *M. corti*.

Phalloidin and lectin staining of flame cells was already shown in *M. corti* and other platyhelminthes, due to the presence of both actin and carbohydrate residues within these cells [8,19,45]. Intact muscle fibers preferentially stained with phalloidin, in detriment of the corresponding staining in flame cells. However, upon PZQ exposure, the fluorescence was progressively transferred to the excretory cells. This may be interpreted as a greater susceptibility of muscle cells to PZQ in comparison to flame cells. Furthermore, PZQ-induced tegument damage could make flame cells more accessible to WGA.

Besides the damaging effect on tegument and muscle fibers, PZQ caused ungrouping of cells corresponding to sexual organs within proglottides. Effects of in vitro exposure to PZQ on internal organs, such as severe drug concentration-dependent vacuolization in intestine, ovary, testis, Mehlis' gland and excretory bladder and disintegration of reproductive organs, was described for *Paragonimus westermani*[12].

The ABZ mode of action is through its binding to β tubulin and blocking of microtubule formation, leading to parasite paralysis and death [46]. ABZ exhibits low water solubility, but once orally administered to a host, it is metabolized into the slightly soluble sulfoxide (SOABZ) and sulfone [34]. Both ABZ and SOABZ induce tubulin alterations in *E. granulosus* protoscolices [47]. Although fully soluble in the concentration used, ABZ did not significantly damage muscle fibers in exposure times of up to 24 h. However, for adult worms, longer exposure times caused limited disruption of muscle fibers. Worms of day 11 had muscle fibers affected even after 1 h of drug exposure, possibly due to their increased body fragility in comparison to tetrathyridia and youngsters. Paralysis followed by death was only observed after 72 h of incubation (data not shown). In contrast, ABZ was markedly harmful to the tegument in longer (24 h) exposure times. This is probably consequence of the loss of cytoplasmic tubules of tegument cells caused by benzimidazoles [48].

In this work, we have used an in vitro *M. corti* strobilization inducible system developed by our group [22] to assess drug effects upon different developmental stages of a parasite cestode. We were able to show that fully segmented worms are more susceptible to the cestode broad-spectrum drugs PZQ and ABZ and opened the possibility to study, at the molecular level, the parasite mechanisms of drug response. It will be important to know when and why the parasite is more susceptible to drug action, identifying specific drug receptors and understanding mechanisms of drug delivery. These are fundamental aspects to be considered when defining effective therapeutic protocols. Our model system can also be useful to investigate such aspects, as well as to the study of differentially expressed genes during development [49] or in response to drugs.

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