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Research Article

In vitro Effects of Nitazoxanide on the Morphology of Nascent and Immature Adult Stages of *Taenia solium*

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Abstract

The broad-spectrum antiparasitic drug nitazoxanide (NTZ) is one of the most successful parasitological treatments for humans with the diseases caused by the tapeworm *Taenia solium*: cysticercosis, caused by the larval muscular stage, and taeniasis, caused by the intestinal adult stage. NTZ is a 5-nitrothiazolyl derivative indicated as an alternative drug when an infection is resistant to other traditional drugs. In protozoans, it inhibits central physiological enzymes and produces lesions in the cell membrane and vacuolization. However, it is necessary to determine if these effects are also produced in helminths. Here, we present morphological evidence at the structural and ultrastructural levels of the *in vitro* effects of NTZ on trypsin-induced evaginated cysticerci of *T. solium* and the intestinal adult parasite stage. NTZ clearly produced important changes on the surfaces of the treated parasites, which affected the morphology of important structures, such as the pore of the invaginated scolex, the initial evagination process, the cephalic, neck, and the strobilar chains of the intestinal tapeworm. These effects may be related to the impairment of glucose metabolism and the consequent loss of the capacity of *T. solium* taenias to become established in their hosts and to establish a successful infection.

ABBREVIATIONS

ABZ: Albendazole; DMSO: Dimethyl Sulfoxide; NTZ: Nitazoxanide; PZQ: Prazicuantel; LM: Light Microscopy; TZ: Tizoxanide; TEM: Transmission Electron Microscopy; SEM: Scanning Electron Microscopy; PFOR: Pyruvate Ferredoxin Oxidoreductase System

INTRODUCTION

Cysticercosis and taeniasis, caused by the cestodes of *Taenia solium*, are important human diseases and public health concerns, predominantly in developing countries (https://www.cdc.gov/parasites/taeniasis/biology.html). These parasitic diseases are not only important in their endemic countries, because highly developed countries can be exposed to the diseases after human migration from endemic countries. Carriers that harbor the adult intestinal parasitic stages can travel without any apparent clinical symptoms and are the main vectors of these diseases [1,2]. Cysticercosis, in which the larval stage infects the tissues of humans, is the most aggressive form because the parasite can localize in the nervous central system, producing neurocysticercosis [3]. Taeniasis, which occurs when the adult tapeworm establishes in the host intestine, is a disease without clear clinical symptoms [4]. To achieve more successful eradication or control of the diseases produced by the *T. solium* parasite, it is necessary to completely remove the intestinal parasite from asymptomatic carriers through effective diagnosis and the use of antiparasitic chemotherapy [5]. However, although the antiparasitic drugs used, praziquantel (PZQ) and albendazole (ABZ), are quite effective, they can have adverse effects or display low efficacy [6]. Because PZQ is expensive, it is not always an available antiparasitic treatment for many infected people. Therefore, alternative options, including combinations of drugs, have been investigated to optimize treatment and reduce its price [7]. However, despite the intensive search for other drugs that act against tapeworms, there are still only a small number of antiparasitic drugs, and many have gaps in their pharmacological activities.

NTZ, a 5-nitrothiazolyl-salicylamide derivative, has a broad-spectrum of action and has been used as an anthelmintic prodrug, the active metabolite of which is tizoxanide (TZ) [8]. The cysticide action of this antihelminthic drug is reportedly less effective than those of other antihelminthic drugs against *T. solium* cysticerci in pigs [8]. Therefore, it has been suggested that it should be combined with other antiparasitic drugs, such as ABZ, to increase its parasiticidal action [8]. Another strategy is to

conjugate the main reactive chemical entity with other moieties, such as parts of benzymidazole derivatives, to produce successful chemical hybrids [9]. However, NTZ is today considered an effective drug for the treatment of helminthic infections and its *in vitro* activity against parasites is well documented [10]. There is a consensus that its mechanism of action in protozoans is related to changes in the physiological reductase enzymes, which induce modifications to the cell membrane and vacuolization [11]. The *in vivo* action of NTZ combined with ABZ was evaluated in a murine model of cysticercosis, infected with *T. crassiceps* cysticerci, and showed that the cysticidal action of NTZ in helminths is mainly caused by tissue damage within the tapeworm. However, the changes that occur during treatment with NTZ are unclear [8]. Biochemically, NTZ has been shown to alter the parasite's anaerobic energy metabolism because it is a noncompetitive inhibitor of the pyruvate ferredoxin oxidoreductase system (PFOR) in protozoans [10]. Different in vitro concentrations of NTZ caused the death of cysticerci by inducing metabolic acidosis, altering their glucose uptake and consequently their lactate production [12]. Based on these results, some authors consider that *T. crassiceps* cysticerci suffer metabolic stress in response to the activity of the NTZ metabolite TZ, which may result in the death of this type of parasite [12]. Again, these metabolic changes could be associated with changes in the cysticerci tissues caused by treatment with NTZ and/or TZ, as demonstrated with light microscopy (LM) and transmission electron microscopy (TEM) [8]. In other tapeworms, such as *Echinococcus multilocularis* [13] and *E. granulosus* [14], important morphological changes have been produced in vitro in different developmental stages, including protoscoleces and metacestodes, by NTZ treatment, which were observed with scanning electron microscopy (SEM).

Taeniasis caused by *T. solium* is considered an intestinal parasitic disease that can be treated successfully with NTZ. The aim of this study was to use LM and SEM to show the effects of *in vitro* NTZ treatment on evaginated cysticerci (nascent taenias) recovered from naturally infected pigs and on adult parasites (immature taenias) recovered from the intestines of experimentally immunosuppressed and infected golden hamsters [15]. The morphological changes in these taenias caused by in vitro treatment with NTZ may be one of the main mechanisms underlying its successful medical use as an antihelminthic drug. The severity of the observed tissue changes could explain why NTZ-treated taenias are unable to establish in the intestinal tissues of the infected hosts.

MATERIALS AND METHODS

Parasites

Evaginated cysticerci: Fresh cysticerci were recovered during the necropsy of a naturally infected pig. After their recovery, each parasite was checked for completeness, the lack of an inflammatory capsule, and the presence of a translucent and undamaged vesicle. The cysticerci were then divided into four groups; one group was processed as complete cysts; the second group was experimentally induced to evaginate with 0.1% trypsin; the third group was used to infect immunosuppressed golden hamsters; and the fourth group, the control group, was treated with parasites incubated with 0.25% dimethyl sulfoxide (DMSO), the solvent for NTZ. Several subgroups were established within these groups of cysticerci and evaginated cysticerci to evaluate the parasites under in vitro conditions in the presence of NTZ (2.5 µg/mL) during 48h in culture. To evaluate the action of NTZ, the taenias were axenized in the presence of antibiotics, maintained under in vitro conditions in RPMI-1640 culture medium under previously established conditions (37°C, 5% CO2, 95% relative humidity). Similar conditions were used to induce the evagination of 90% of the cysticerci. The best time to induce evagination with trypsin was determined to be 60 min. Spontaneous evagination was noted in some parasites only 2h after their dissection from the pig carcass.

Intestinal adult parasites

After the cysticerci were selected, they were washed several times with phosphate-buffered saline (PBS, pH 7.2). Golden hamsters, previously deparasitized with ABZ and immunosuppressed with the subcutaneous administration of 2 mg of methylprednisolone acetate (Depomedrol, Pharmacia & Upjohn Co. Division of Pfizer Inc. New York, NY 10017), were infected by the oral administration of eight cysticerci, as previously described by us [15]. Fifteen days after the administration of cysts, a second dose of the immunosuppressive drug was administered to the animals. At 30 days after infection, the infected immunosuppressed hamsters were killed under ether anesthesia, and the adult parasites were recovered from their small intestines. To evaluate the *in vivo* effects of NTZ, after the second administration of the methylprednisolone, the infected golden hamsters were treated with 200 mg/kg/day NTZ dissolved in 5% DMSO, according to the treatment of immunosuppressed rats infected with *Cryptosporidium parvum* [10].

Reagents

NTZ was synthetized by the group of Dr. R. Castillo-Bocanegra at the School of Chemistry, UNAM, with the following strategy. An intermediate reagent, 2-hydroxy-N-(1,3-thiazol-2-yl) benzamide, was obtained from 2-(chlorocarbonyl) phenyl acetate o-chloride of commercial acetylsalicylic acid, reacted to 2-aminothiazole in acetone in the presence of sodium bicarbonate with refluxing to generate the carboxamide. The carboxamide was then reacted with acetic anhydride at 78°C. The product was then cooled to 3°C and reacted with fuming nitric acid to produce NZT. Before the *in vitro* assays, NTZ was dissolved in a solution containing 0.25% DMSO to improve its dissolution. This compound was completely characterized, and a quality control assessment to evaluate their activities against several protozoa [16].

Macroscopic and microscopic observations of the effects of NTZ on parasites

Two levels of observations were undertaken. For macroscopic observations, a Nikon inverted light microscope was used (Diaphot-TMD; objective Plan 4, 4×, NA 0.13, PhL). For the ultrastructural observations, a Zeiss microscope (DSM 950; 25 kW; WD: 16 mm; magnification 100–1000×) was used. All the images were recorded in JPEG and TIFF formats and processed, for contrast, under Microsoft Power Point conditions. To observe the parasites with SEM, they were processed as described previously by us [15].

RESULTS AND DISCUSSION

Untreated parasites

LM observations: After evagination, the cysticerci were observed under LM and, to better observe them and record the live parasites, images were also obtained with phase-contrast microscopy, as shown in Figure 1. In selected images (A–F), it is possible to follow the dynamics of the evaginated cysticerci. In the figure, only the cephalic portion of a nascent taenia is visible, and the vesicular bladder wall is absent (although in Figure 3, SEM shows a complete cysticercus). Images A–E clearly shows the presence of the untreated crown, in which the rostellar hooks are visible. In all the images, the suckers are clearly evident and the sequence of the images shows how these parasite structures are in constant movement, between extrusion (A,E), and intrusion (B,C,F), and the opening/closing actions of the ventral suckers (A, B, D, E). While the parasites were observed under these conditions, the neck below the cephalic portion was continuously moving, implying that the evaginated cysticerci were looking for any possible place for attachment. Immature tapeworms were recovered from the third portion of the small intestines of the infected hamsters. As shown in Figure 2, they were long, whitish, smooth, and occasionally translucent in some regions at the level of the scolex (A). To remove the attached adult parasites from the hamster intestines, which were strongly anchored to the intestine, the temperature was reduced with an ice bath and tension was applied with forceps. After their recovery, the tapeworms became very active, displaying intense dynamic movements of their strobilar chains (A). These constantly showed changing aspects with the contraction and expansion of their strobilae. At the level of the cephalic region at the scolex (B), the live parasites were continuously opening and closing their suckers or protruding and retracting the rostellum. The hooks were retracted or extended, depending on the movements of the parasites, as seen in panel B, in an apparent search for an intestinal place of attachment. Because these parasites were immature taenias, their terminal proglottids did not contain eggs.

SEM observations: After the induction of evagination, some parasites were selected for ultrastructural observation, as shown in Figure 3. The integrity of the surfaces of the untreated evaginated cysticerci was similar in all the parasites observed, as shown in the figure. The scolex emerging in the cephalic region (A) contained four open and contracted suckers, an open apical organ in the center of the scolex, and the neck (B,D). Inside the apical organ (C), the invaginated tissue that would ultimately emerge as the rostellar hooks could be seen (Figure 3B). Closer observation of image A, shown in image B, suggested that the four suckers were ready for suction, which would permit the attachment of the nascent taenia to the intestinal tissues of the host. The neck was prepared for the necessary extension of this region of the parasite, so the nascent tapeworm was ready to search for an attachment site. Closer observation of the neck (D) showed that the surface of the nascent taenia is a brush border covered in microvilli. The disposition of this tissue region allows the complete invagination or extension of the region, so the tapeworm is prepared for its complete integration into the host's intestinal tissues.

Importantly, the parasites in the control group, incubated in the presence of 0.25% DMSO only, were not affected during the time of culture (48h), and were dynamic and well preserved.

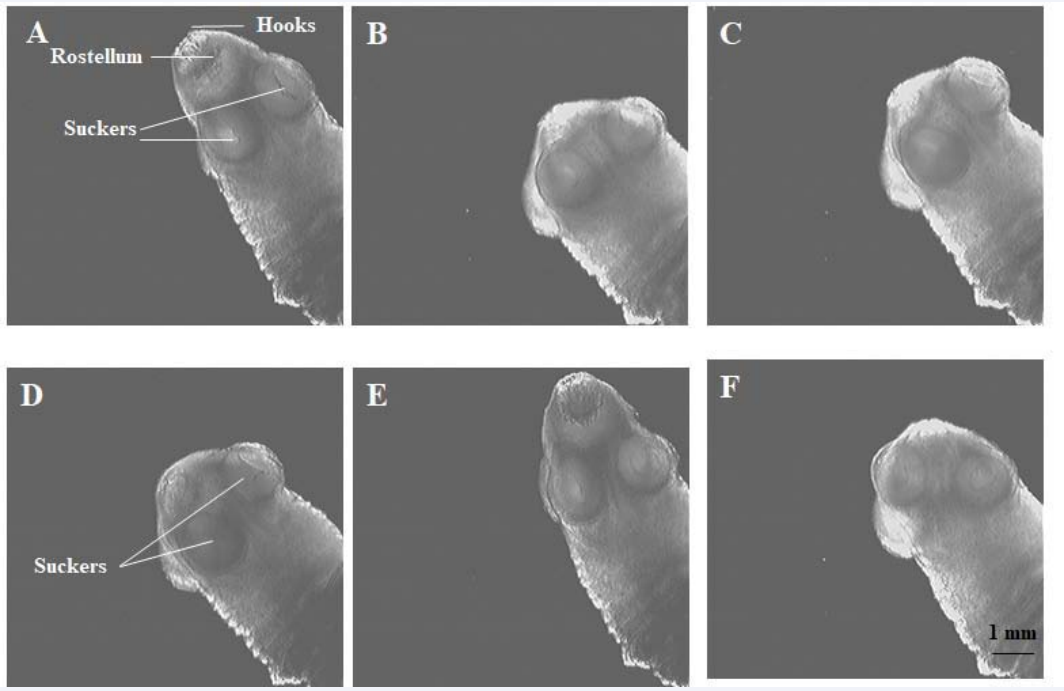

Figure 1 Behavior of the cephalic portion of the untreated evaginated *T. solium* cysticercus. After the evagination of the cysticercus was induced with 0.1% trypsin, the parasite was observed and recorded with phase-contrast light stereomicroscopy. Observations were made directly at 2× magnification in a culture dish. A–F are selected images of a video sequence obtained from 32 images recorded during a 5min period using a stereoscopic microscope equipped with a conventional video camera.

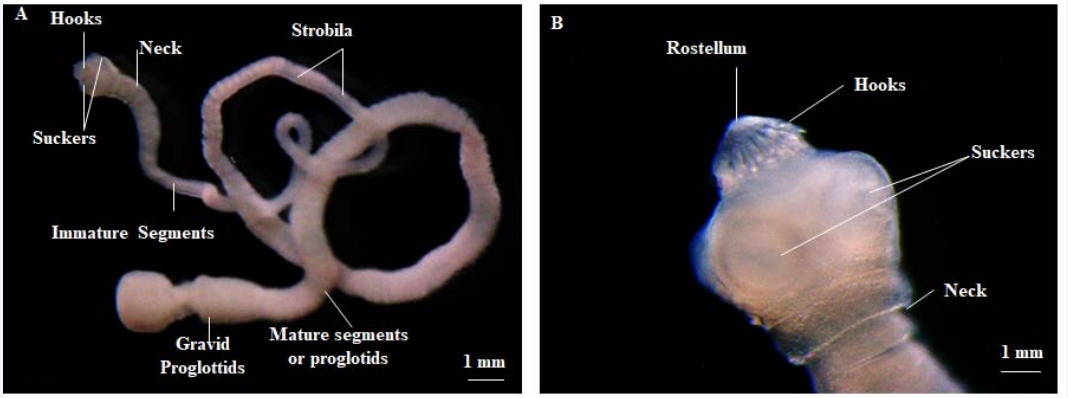

Figure 2 Immature intestinal *T. solium* tapeworm recovered from an experimentally infected golden hamster. After immunosuppression for 30 days, the infected hamsters were killed and the adult parasites were recovered from the first third of their intestinal segments, after extensive washing with cold PBS (pH 7.2). The parasites were observed and images were recorded as indicated in Figure 1. The tapeworms measured almost 5–8 cm in length.

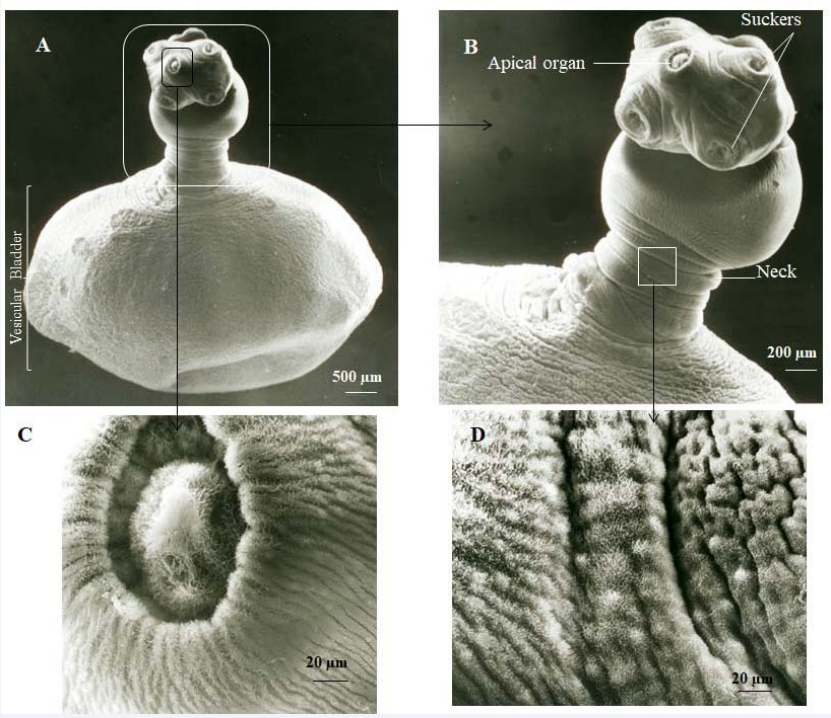

Figure 3 Recently evaginated cysticercus observed with SEM. After the parasites were processed, different regions of the cysticerci were observed at different magnifications and recorded as shown in the images. A, During the initial evagination process, the scolex had no central opening. C, Apical organ, with non-emerged rostellar hooks. B, Four suckers and the neck. Panel A shows the complete vesicular bladder, from which the scolex is protruding. Images C and D are closer views of the tissue surface of the central opening in the apical organ shown in B.

NTZ-treated tapeworms

LM observations: After exposure to NTZ, no movement of the parasites was detected with LM. They were clearly damaged because their tissues were roughish and yellowish, and their appearance in Figure 2 had been lost. In Figure 4, the rostellar hook is evident in both the untreated (Figure 4A) and NTZ-treated taenias (Figure 4B), whereas the suckers and the neck tissues displayed a different consistency after the NTZ treatment, and the parasites appeared unhealthy, with no active movement. A comparison of the displayed hooks in Figure 4B with those in Figure 4A showed them to be atrophied, dispersed, and nontranslucent, so they were unable to attach the nascent taenia to the host's intestinal tissues. A similar comparison of the suckers showed that they differed in consistency from those in the untreated taenias. Again, the altered suckers were unable to perform any opening or closing movements, which attach the taenias to the intestinal tissues. The neck of the NTZ-treated tapeworm was also altered, appearing rigid, so that the

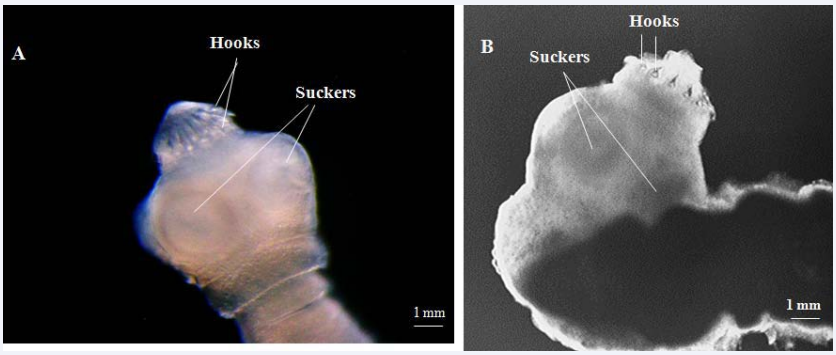

Figure 4 Scolices of immature taenias in the absence and presence of NTZ. Image A (no treatment) corresponds to panel B of Figure 2, whereas image B (NTZ treatment) was taken after continuous microscopic observations for 10s and after the behavior of the parasite was recorded. In image B, the nascent taenia shows damage to the rostellar hooks, the suckers, the neck, and the associated tissues, including the short strobilar chain, where the tissue appears black in the image.

worm was unable to perform the classical movements (shown in Figure B) and the parasite had not changed its position for 10s. The untreated tapeworms moved continuously in all directions, looking for a possible site of attachment. These observations indicate that treatment with NTZ produced important changes in the morphology of the immature taenias. LM observations of cysticerci in the presence or absence of NTZ (data not shown) showed that NTZ had little effect on the vesicular bladders of the cysticerci, as shown in Figure 3.

SEM observations: Defective evagination of the cysticerci was observed after treatment of the parasite with NTZ (Figure 5). The organs of the cephalic section were not clearly visible in the external opening (Figure 5A,B) because the associated tissues were destroyed, and the egress of the invaginated structures was disrupted. Closer observation of the surrounding region, which limits the apical organ, (Figure 4C) showed that the continuity of the tissue surface was destroyed. No suckers were seen in the remains of the nascent taenia. In contrast to these changes, the vesicular bladder of the cysticercus was apparently undamaged, maintaining its classical tegumentary surface (Figure 4D).

The effects of NTZ on the nascent tapeworms recovered from the hamster's intestines are shown in Figures 6-8. As can be seen in the representative intestinal parasite in Figure 6, the tissues were damaged along the length of the tapeworm (A), and significant destruction of the tissues in the cephalic region was detected. The surface of the tapeworm was frequently peeled off, and the destruction of important organs of the scolex was also observed. In the rostellum, the tissue surrounding the hooks was partly lost, so that the hooks could not adequately anchor the parasite to the intestinal host tissues (B,C). The suckers were also completely destroyed (C), so were unfit to provide suction for the attachment of the cephalic portion of the taenia to the host tissues.

Higher magnification of the scolex

The gross effects of NTZ were observed at the level of the scolex, as shown in Figure 7. In panel A, the tissue that supports the crown was destroyed, exposing the basement anchorage of the hooks. The suckers were completely closed and were clearly incapable of performing their classical relaxation/contraction, which produces the necessary suction to allow adherence to the host intestinal tissues. In panels B and C, the surface of the treated parasite, and the basement of the hooks have been destroyed, so that they are not adequately attached to the cephalic section of the taenia [17].

Higher magnification of the strobilar chain

Closer observation of the strobila (Figure 8) showed that NTZ treatment caused the peeling of the surface of the tissue (A,B), and in some regions of the strobilar chain, the internal layers were exposed (C,D). This exposure of the internal tissues is indicative of the damage to the functions of the strobilar chain. With the exposure of the deep tissue (C,D) caused by the rupture of the brush border, the tissue components in this region (extracellular matrix, ducts of the protonephridial system, and some possible cell bodies) were exposed, demonstrating the irreparable destruction of the external and internal layers of the tapeworm tissues.

Host infections produced by the developmental stages of *T. solium*, including metacestodes and adult intestinal parasites, require successful establishment, development, and the preservation of their tissue structures. Because cestodes lack an alimentary tract, the continuous internalization of nutrients and the excretion/secretion of wastes mainly occur at the host–parasite interface. Therefore, the integrity of the tapeworm body surface is crucial for the survival of the parasite inside its host [18] and any irreparable rupture of the surface layer of the tapeworm will affect its ability to survive inside its host. In this study, the main *in vitro* and *in vivo* effects of NTZ were the destruction of the surface tissue layer of the nascent tapeworm (Figure 5B,C) and the recently formed adult intestinal parasite (Figures 6-8). Our microscopic observations of the effects of NTZ have allowed us to show how this type of drug acts against cestodes, especially those that affect humans, such as *T. solium*. Therefore, these findings extend our previously limited understanding of the action of this antiparasitic drug.

According to the life cycle of *T. solium* (https://www.cdc.gov/parasites/taeniasis/biology.html and https://www.cdc.gov/media/DPK/2014/docs/npi/Cysticercosis_LifeCycle.pdf), the developmental stages of the tapeworm must maintain their

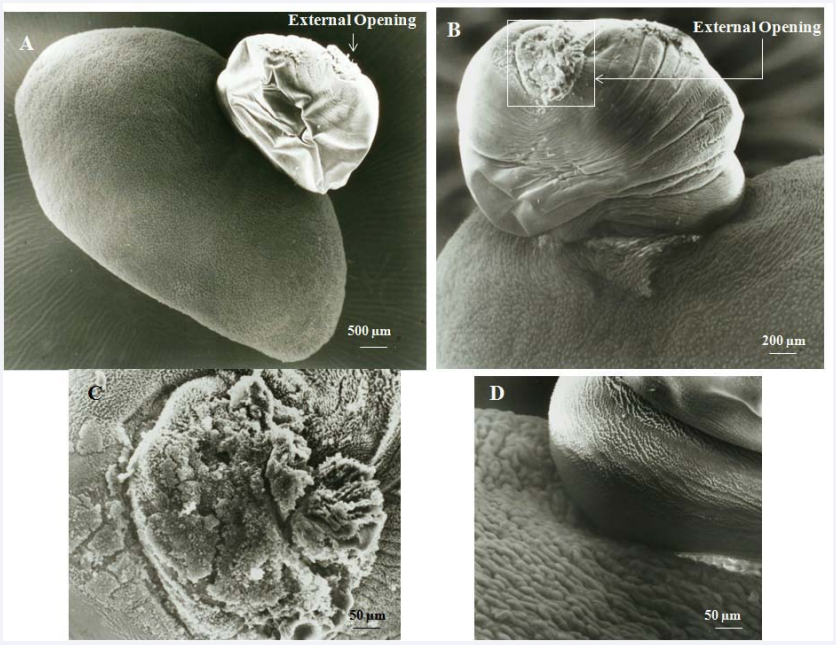

Figure 5 SEM images of the evagination of a cysticercus affected by NTZ treatment. During its evagination, the parasite was exposed to 2.5 µg/mL NTZ for 28h. After the parasite was processed, different regions of the cysticercus were observed and recorded at different magnifications, and show a similar presentation to that in Figure 3. In A, the evagination of the cysticercus was unsucessful. The organs of the cephalic section are not correctly displayed and because the apical organ is damaged, the scolex has not emerged (C). Only the remains of the suckers are visible and there are changes to the neck (A,B). The treatment did not produce any obvious changes in the vesicular bladder surface (A,D).

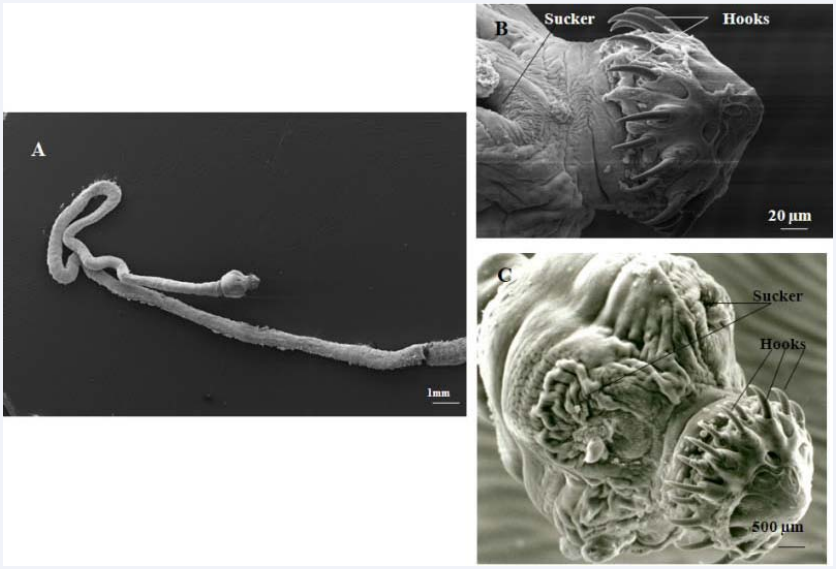

Figure 6 SEM images of an immature taenia after treatment with NTZ. The effect of the NTZ treatment is clearly visible along the length of the tapeworm (A), and the strobilar chain shows peeling of the superficial tissue. High magnification of the cephalic region (B,C) shows the destruction of the superficial tissue and the complete alteration of the suckers (C).

integrity to effectively their establish an infection in the host's tissues, to undergo complete development, and to adapt to their microenvironment (the tissue of the host). The larval stage, or cysticercus, is well adapted to survive inside the host's tissues (muscle, brain, eyes, and heart), and the intestinal tapeworm attaches by its scolex to the host's intestine. In both stages of development, the parasite is in direct contact with the host through its external surface, which is composed of a brush border bearing surface structures and the tegumentary syncytium, which surrounds the whole tapeworm. Of these structures, the villi-like microtrichia are characteristic of the cestode [19].

Metacestodes

Morphology and development: The morphology and

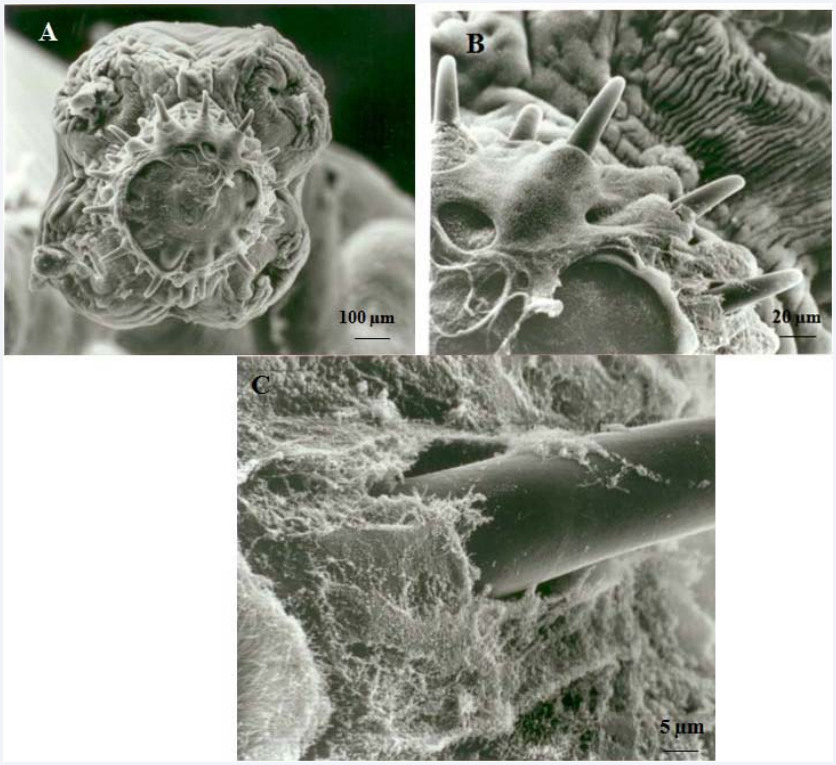

Figure 7 SEM images of the scolex of an immature taenia after NTZ treatment. Closer observations of the cephalic portion of the scolex seen in Figure 6 shows the effects of NTZ in this region; A. Overall view of the scolex, clearly showing the crown containing the hooks, and below it, four severely damaged suckers. In B and C, the surrounding tissue at the basement of the hooks is completely destroyed, and the peeling of the surface tissue has exposed the internal tissues of the parasite.

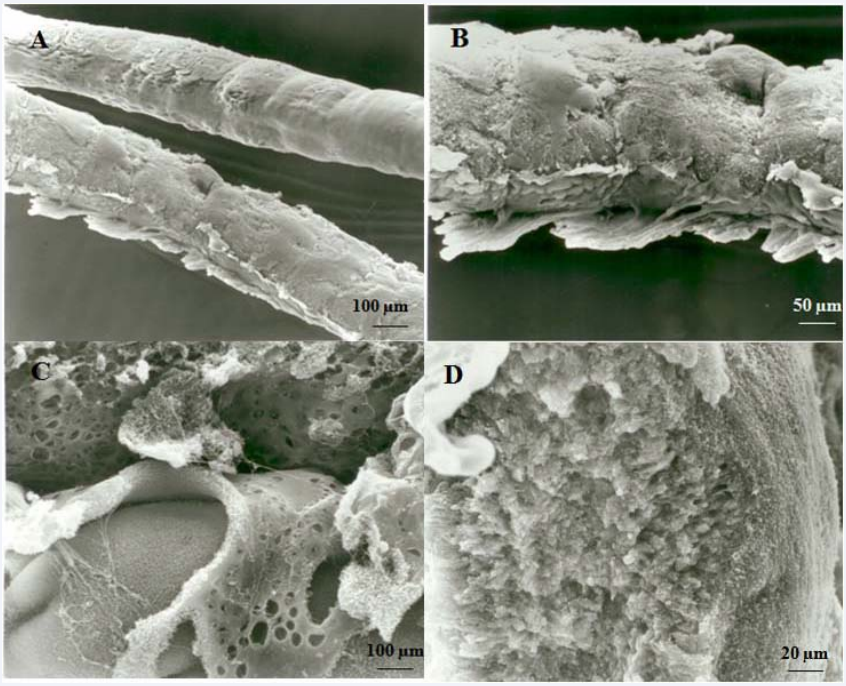

Figure 8 SEM images of the strobila of an immature taenia after treatment with NTZ. Closer observation of the strobilar chain seen in panel A of Figure 6 shows that the superficial tissue is destroyed (A,B), whereas in panel C, the germinal layer is exposed in the upper part of the image. Exposure of the internal tissue layer (image D), which is typically composed of the extracellular matrix, the ducts of the protonephridial system, and some body cells.

development of these organisms are important because they increase their surface area for the absorption and exchange of the nutrients and substances required for their survival. The active and dynamic internalization of proteins by the cysticerci of *T. crassiceps* strain ORF has been demonstrated [20]. These ultrastructural projections, together with the tegument, are essential for the constitution of their parenchymal tissue and to support the physiological survival of the worms inside their hosts because these organisms do not have a digestive system. For intestinal worms to develop their long dorsoventrally flattened and ribbon-like structures, they must be completely attached to the intestine through their scolices. This attachment is sufficiently strong to permit the growth of the tapeworm until it reaches 2–3 meters long. As previously suggested [21], the complete disposition of the organs in the cephalic section of the taenia (the scolex, including four cup-like suckers, and the rostellum, with 20–30 curved chitinous hooks) is crucial for its attachment. According to this suggestion, specific sequential events are involved in the insertion of the rostellar hooks and the adhesion of the suckers to the host's intestinal tissues and the parasite's ultimate development into a mature worm. Under these circumstances, the tapeworm extends its long strobilar chain, which is composed of 800–900 proglottids, and its firm attachment and the resistance of its neck allow the flatworm to withstand the hostile environment of the host intestines until its fully developed gravid proglottids are excreted with the human feces. These events maintain the life cycle of *T. solium*. Any effect of NTZ that disrupts the tapeworm's ability to attach to the intestine of its host will disrupt its retention, growth, and development.

In this study, NTZ affected the tapeworm on two levels: in the cephalic section and at the parasite surface. On the first level, the failure of the parasite to establish adequately was related to the changes induced in the organs essential for suction and the attachment of the tapeworm to the tissues of its host (Figures 4B, 5C,6,7). On the second level, the integrity of the tapeworm surface was clearly destroyed (Figures 5-8). The preferential action of NTZ on these body regions of *T. solium* may be related to the importance of these regions in the metabolism of energy derived from the transformation of glucose. Our previous *in vitro* investigation identified the metabolic changes that occur in response to the treatment of *T. crassiceps* cysticerci with NTZ [12]. It is irrelevant that in the present study, the NTZ concentration used was higher than that used previously (2.5 μg/mL vs 1.2 μg/mL, respectively), because the main effect of NTZ was the induction of metabolic acidosis, which caused the death of the parasites. The surfaces of the cephalic regions of the tapeworms capture and utilize glucose most efficiently to meet the physiological requirements of the growing tapeworms and it has been demonstrated (using TEM) at the ultrastructural level that glycogen accumulates in large cytoplasmic sacs in the scolex and neck tissues [22]. Interestingly, the microscopic observations in the present study suggest that the effects of NTZ on the cephalic and scolex tissues of the tapeworm correspond to the glucose gradient found in the *Hymenolepis diminuta* tapeworm [23]. According to the images shown in Figures (4-7), the action of NTZ has a major impact at the level of the taenial scolices during their emergence from the bladder. It has been demonstrated that the invaginated structures (the rostellar hooks, the suckers, and the neck, and the microtrichia at their basement) are wholly assembled in the invaginated canal in a well-developed cysticercus [19].

Metacestodes

Morphology and development: In the immature taenias recovered from the infected hamster, the greatest effect of NTZ was seen in the cephalic section of the parasites (Figures 6,7). Similar to our *in vitro* observations, treatment with NTZ did not affect the morphology of the cysticerci or the outer surface of the bladder, as has also been reported after the NTZ treatment of *T. crassiceps* cysticerci [8,12]. The damage to the strobilae was intense at the tegumental surface, where the tegument peeled away to expose the parenchyma. Therefore, the tapeworms lost their capacity to absorb nutrients through the strobilar tegumentary surface (Figures 6-8), reducing their ability to obtain glucose along their length [23]. To clarify whether the effects of NTZ are associated with the tapeworm's use of glucose, it will be interesting to undertake a proteomic analysis of the expression of relevant glucolytic enzymes or glucose transporters, and the enzymes reported to be associated with the action of NTZ (pyruvate ferredoxin oxidoreductase, nitroreductase 1, and quinone reductase), to determine their expression in the intestinal tapeworm body compartments.

It is possible that for the complete establishment, development, and survival of intestinal taenias, the nascent and immature taenias require the active absorption of glucose through their tegumentary surface, as has been demonstrated *in vitro* [22]. Our results demonstrate visually that the death of these tapeworms in response to NTZ treatment is associated with changes in the glucose uptake by the tapeworm and the production of metabolic stress [12]. This may result in the death of the parasite because its survival depends on its acquisition of glucose from its host, which allows the development of its gravid proglottids, as reported by Willms in 2005.

CONCLUSIONS

In conclusion, we have demonstrated that the intestinal tapeworm *T. solium* is very susceptible to treatment with NTZ, and that these effects occur in the nascent and immature taenias. Therefore, when used clinically, NTZ alters the tapeworm in such a way that it cannot attach or adapt to the intestine of its host. The action of the drug acts mainly at the level of the tapeworm scolex and causes tissue changes at the strobilar tegumentary surface, which cause the parasite to lose its ability to absorb nutrients from its host.

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